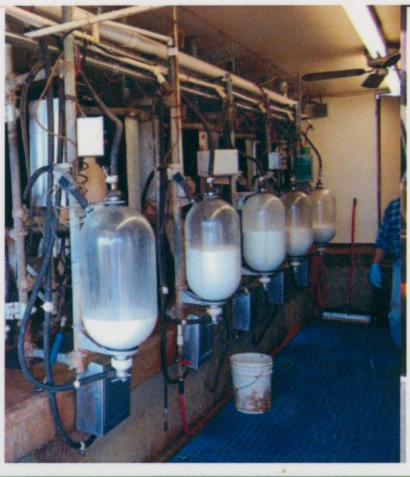
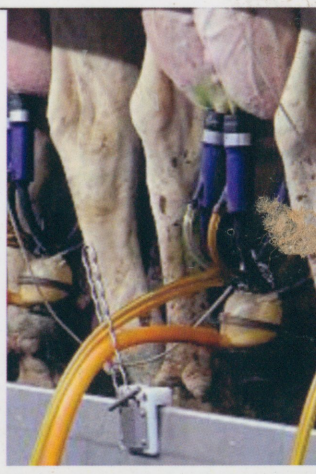
# ADVANCES IN DAIRY SCIENCE TECHNOLOGY

PRAMOD KUMAR SINGH









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# ADVANCES IN DAIRY SCIENCE TECHNOLOGY

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Educationist Press
*An Imprint of*
**Write & Print Publications**
**New Delhi-110 052**

**Cataloging in Publication Data—DK**
Courtesy: D.K. Agencies (P) Ltd. <docinfo@dkagencies.com>

**Singh, P. K.** *(Associate professor of livestock products technology)*, **author.**
**Advances in dairy science technology / P.K. Singh.**

pages cm
Running title: Dairy technology.
Includes bibliographical references and index.
ISBN 9789386283474

1. Dairy processing. 2. Dairying. I. Title. II. Title: Dairy technology.

SF250.5.S56 2017 DDC 637.1 23

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ISBN: 978-93-86283-47-4

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*Published by* : Hitesh Mittal
**For Write & Print Publications**
A-2, Mittal Tower,
Nimri Commercial Complex,
Ashok Vihar, Phase-4,
New Delhi-110 052
Phone: 011-45635684
E-mail: info@writeandprint.com
Website: www.writeandprint.com

*Printed in India.*

# Preface

Dairy Technology deals with all methods of handling milk from production and consumption and includes processing, packaging, storage, transport and physical distribution. Based on the sciences of biochemistry, bacteriology, and nutrition. Dairy Technology employs the principles of engineering. Its objectives are to prevent spoilage, improve quality, increase shelf-life, and make milk palatable and safe for human consumption.

The primary theme of this book is the efficient transformation of milk into high quality products. This needs a thorough understanding of the composition and properties of milk, and of the changes occurring in milk and its products during processing and storage.

Aimed at university food science and technology majors, the book is written as a text, though it will also be useful as a work of reference. It is assumed that the reader is familiar with the rudiments of food chemistry, microbiology, and engineering. Nevertheless, several basic aspects are discussed for the benefit of readers who may be insufficiently acquainted with these aspects. The book contains no references to the literature, but suggestions for further reading are given. The book is made up of four main parts. The book discusses the chemistry, physics, and microbiology of milk. Besides providing knowledge of the properties of milk itself, it forms the basis for understanding what happens during processing, handling and storage, and treats the main unit operations applied in the manufacture of milk products. These are discussed in some detail, especially the influence of product and process variables on the (intermediate) product resulting. A few highly specific processes, such as churning, are discussed in product chapters.

There is no doubt that the book will have recognition by dairy scientists, students, researchers and dairy operatives and technologists.

**P.K. Singh**

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# Abbreviations

| | |
|---|---|
| BSA | Bovine serum albumin |
| CCP | Colloidal calcium phosphate; Carboxymethyl cellulose |
| CFU | Colony-forming units |
| CMC | Critical micellization concentration |
| CMP | Caseinomacropeptide |
| DF | Diafiltration |
| D (-) | Levorotatory |
| DSC | Differential scanning calorimetry |
| DVI | Direct vat inoculation |
| FDM | Fat content in dry matter |
| FFA | Free fatty acids |
| HACCP | Hazard analysis/critical control points |
| HMF | Hydroxymethyl furfural |
| IEP | Isoelectric pH |
| L (+) | Dextrorotatory |
| MF | Microfiltration |
| NANA | N-acetylneuraminic acid (residue) |
| NPN | Nonprotein nitrogen (compounds) |
| PCBs | Polychlorinated biphenyls |
| PP3 | Proteose peptone, fraction 3 |
| R | Residue (in chemical formulas) |
| RH | Relative humidity (air) |

| | |
|---|---|
| r.p.m. | Number of revolutions per minute |
| SDS | Sodium dodecyl sulfate |
| sn | Stereospecific numbering |
| snf | Solids-not-fat |
| UF | Ultrafiltration |
| UHT | Ultra-high temperature short time (heating) |
| WP | Whey protein |
| WPC | Whey protein concentrate |
| WPI | Whey protein isolate |

# Symbols

| | |
|---|---|
| $A$ | Specific surface area of particles ($m^{-1}$) |
| $a$ | Thermodynamic activity |
| $a_w$ | Water activity (-) |
| $B$ | Permeability ($m^2$) |
| $C$ | Constant |
| $c$ | Concentration |
| $cs$ | Relative standard deviation of a size distribution (-) |
| $D$ | Diffusion coefficient ($m^2.s^{-1}$) |
| | Fractal dimensionality (-) |
| $d$ | Diameter (m) |
| $d_{vs}$ | Volume/surface average diameter (m) |
| $E_h$ | Redox potential (V) |
| $G$ | Elastic shear modulus (Pa) |
| $g$ | Acceleration due to gravity (9.807 $m.s^{-2}$) |
| | Generation time (s) |
| $H$ | Sedimentation parameter ($m^2$) |
| $I$ | Total ionic strength (molar) |
| $M$ | Molar mass ($g.mol^{-1}$ = Da) |
| $N$ | Number concentration ($m^{-3}$) |
| $n$ | Refractive index (-) |
| $pK$ | pH of 50% dissociation |

| | |
|---|---|
| $p$ | Pressure (Pa) |
| $Q$ | Degree of concentration (e.g., of milk) (–) |
| $R$ | Gas constant (8.315 $J.K^{-1}.mol^{-1}$) |
| $r$ | Radius (m) |
| $S_n$ | $n$-th moment of a (size) distribution |
| $T$ | (Absolute) temperature (K) |
| $t$ | Time (s) |
| $t_{0.5}$ | Time (s) needed to halve the value of a parameter |
| $v$ | Linear velocity ($m.s^{-1}$) |
| | Voluminosity ($ml.g^{-1}$) |
| x, y, z | Linear coordinates (m) |
| $\Gamma$ | Surface load (surface excess) ($kg.m^{-2}$; $mol.m^{-2}$) |
| $\gamma$ | Activity coefficient (-) |
| | Surface tension ($N.m^{-1}$) |
| $\varepsilon$ | Relative dielectric constant (-) |
| | Relative deformation (-) |
| $\eta$ | Viscosity (Pa.s) |
| $\eta_a$ | Apparent viscosity (Pa.s) |
| $\eta_{rel}$ | Relative viscosity (-) |
| $\Pi$ | Osmotic pressure (Pa) |
| $\rho$ | Mass density ($kg.m^{-3}$) |
| $\sigma$ | Stress (Pa) |
| $\sigma_{fr}$ | Fracture stress (Pa) |
| $\sigma_y$ | Yield stress (Pa) |
| $\varphi$ | Volume fraction (-) |

# 1

# Introduction

**Topics**

*• Milk Grading and Defects • Composition and Structure • Formation of Milk • Changes in Milk*

Milk is defined as the secretion of the mammary glands of mammals, its primary natural function being nutrition of the young. Milk of some animals, especially cows, buffaloes, goats and sheep, is also used for human consumption, either as such or in the form of a range of dairy products. In this book, the word milk will be used for the 'normal' milk of healthy cows, unless stated otherwise. Occasionally, a comparison will be made with human milk.

This book is about the study of milk and milkprocessing from a food science perspective. It focuses on the biological, chemical, physical, and microbiological aspects of milk itself, and on the technological (processing) aspects of the transformation of milk into its various consumer products, including beverages, fermented products, concentrated and dried products, butter and ice cream.

Milk is as ancient as mankind itself, as it is the substance created to feed the mammalian infant. All species of mammals, from man to whales, produce milk for this purpose. Many centuries ago, perhaps as early as 6000-8000 BC, ancient man learned to domesticate species of animals for the provision of milk to be consumed by them. These included cows (genus Bos), buffaloes, sheep, goats, and camels, all of which are still used in various parts of the world for the production of milk for human consumption.

Fermented products such as cheeses were discovered by accident, but their history has also been documented for many centuries, as has the production of concentrated milks, butter, and even ice cream.

Technological advances have only come about very recently in the history of milk consumption, and our generations will be the ones credited for having turned milk processing from an art to a science. The availability and distribution of milk

and milk products today in the modern world is a blend of the centuries old knowledge of traditional milk products with the application of modern science and technology.

The role of milk in the traditional diet has varied greatly in different regions of the world. The tropical countries have not been traditional milk consumers, whereas the more regions of the world, have traditionally consumed far more milk and milk products in their diet. In tropical countries where high temperatures and lack of refrigeration has led to the inability to produce and store fresh milk, milk has traditionally been preserved through means other than refrigeration, including immediate consumption of warm milk after milking, by boiling milk, or by conversion into more stable products such as fermented milks.

## MILK GRADING AND DEFECTS

The importance of milk grading lies in the fact that dairy products are only as good as the raw materials from which they were made. It is important that dairy personnel have a knowledge of sensory perception and evaluation techniques. The identification of off-flavours and desirable flavours, as well as knowledge of their likely cause, should enable the production of high quality milk, and subsequently, high quality dairy products.

An understanding of the principles of sensory evaluation are neccessary for grading. All five primary senses are used in the sensory evaluation of dairy products: sight, taste, smell, touch and sound. The greatest emphasis, however, is placed on taste and smell.

### Sense of Taste

Taste buds, or receptors, are chiefly on the upper surface of the tongue, but may also be present in the cheek and soft palates of young people. These buds, about 900 in number, must make contact with the flavouring agent before a taste sensation occurs. Saliva, of course, is essential in aiding this contact. There are four different types of nerve endings on the tongue which detect the four basic "mouth" flavours - sweet, salt, sour, and bitter. Samples must, therefore, be spread around in the mouth in order to make positive flavour identification. In addition to these basic tastes, the mouth also allows us to get such reactions as coolness, warmth, sweetness, astringency, etc.

### Sense of Smell

We are much more perceptive to the sense of smell than we are to taste. For instance, it is possible for an odouriferous material such as mercaptain to be detected in 20 billion parts of air. The centres of olfaction are located chiefly in the uppermost part of the nasal cavity. To be detectable by smell, a substance must dissolve at body temperature and be soluble in fat solvents.

**Note**: The sense of both taste and smell may become fatigued during steady use.

A good judge does not try to examine more than one sample per minute. Rinsing the mouth with water between samples may help to restore sensitivity.

## COMPOSITION AND STRUCTURE

### Principal Components

The principal chemical components or groups of chemical components are those present in the largest quantities. Of course, the quantity (in grams) is not paramount in all respects. For example, vitamins are important with respect to nutritive value; enzymes are catalysts of reactions; and some minor components contribute markedly to the taste of milk.

***Lactose or milk sugar*** is the distinctive carbohydrate of milk. It is a disaccharide composed of glucose and galactose. Lactose is a reducing sugar. The fat is largely made up of triglycerides, constituting a very complicated mixture. The component fatty acids vary widely in chain length (2 to 20 carbon atoms) and in saturation (0 to 4 double bonds). Other lipids that are present include phospholipids, cholesterol, free fatty acids, monoglycerides, and diglycerides.

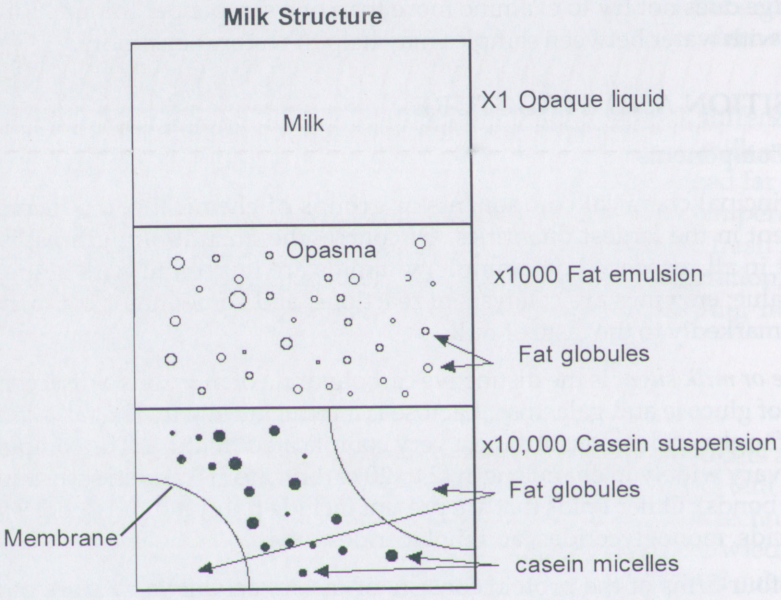
About four fifths of the protein consists of casein, actually a mixture of four proteins: $\alpha_{S1}$-, $\alpha_{S2}$-, β-, and κ-casein. The caseins are typical for milk and have some rather specific properties: They are to some extent phosphorylated and have little or no secondary structure. The remainder consists, for the most part, of the milk serum proteins, the main one being β-lactoglobulin. Moreover, milk contains numerous minor proteins, including a wide range of enzymes.

S2

***The mineral substances*** - primarily K, Na, Ca, Mg, Cl, and phosphate - are not equivalent to the salts. Milk contains numerous other elements in trace quantities. The salts are only partly ionized. The organic acids occur largely as ions or as salts; citrate is the principle one.

Furthermore, milk has many miscellaneous components, often in trace amounts. The total content of all substances except water is called the content of dry matter. Furthermore, one distinguishes solids-not-fat and the content of fat in the dry matter. The chemical composition of milk largely determines its nutritional value; the extent to which microorganisms can grow in it; its flavor; and the chemical reactions that can occur in milk. The latter include reactions that cause off-flavours.

The role of milk in nature is to nourish and provide immunological protection for the mammalian young. Milk has been a food source for humans since prehistoric times; from human, goat, buffalo, sheep, yak, to the focus of this section - domesticated cow milk (genus Bos). Milk and honey are the only articles of diet whose sole function in nature is food. It is not surprising, therefore, that the nutritional value of milk is high. Milk is also a very complex food with over 100,000 different molecular species found. There are many factors that can affect milk composition such as breed variations, cow to cow variations, herd to herd variations




- including management and feed considerations, seasonal variations, and geographic variations. With all this in mind, only an approximate composition of milk can be given:

- 87.3% water (range of 85.5% - 88.7%)
- 3.9 % milkfat (range of 2.4% - 5.5%)
- 8.8% solids-not-fat (range of 7.9 - 10.0%):
  - protein 3.25% (3/4 casein)
  - lactose 4.6%
  - minerals 0.65% - Ca, P, citrate, Mg, K, Na, Zn, Cl, Fe, Cu, sulfate, bicarbonate, many others
  - acids 0.18% - citrate, formate, acetate, lactate, oxalate
  - enzymes - peroxidase, catalase, phosphatase, lipase
  - gases - oxygen, nitrogen
  - vitamins - A, C, D, thiamine, riboflavin, others

The following terms are used to describe milk fractions:

- **Plasma** = milk - fat (skim milk)
- **Serum** = plasma - casein micelles (whey)

- **solids-not-fat (SNF)** = proteins, lactose, minerals, acids, enzymes, vitamins
- **Total Milk Solids** = fat + SNF

Not only is the composition important in determining the properties of milk, but the physical structure must also be examined. Due to its role in nature, milk is in a liquid form. This may seem curious if one takes into consideration the fact that milk has less water than most fruits and vegetables. Milk can be described as:

- an **oil-in-water emulsion** with the fat globules dispersed in the continuous serum phase
- a **colloid suspension** of casein micelles, globular proteins and lipoprotein partilcles
- a **solution** of lactose, soluble proteins, minerals, vitamins other components.

Looking at milk under a microscope, at low magnification (5X) a uniform but turbid liquid is observed. At 500X magnification, spherical droplets of fat, known as fat globules, can be seen. At even higher magnification (50,000X), the casein micelles can be observed. The main structural components of milk, fat globules and casein micelles, will be examined in more detail later.

## FORMATION OF MILK

Milk components are for the most part formed in the mammary gland (the udder) of a cow, from precursors that are the results of digestion. Digestion. Mammals digest their food by the use of enzymes to obtain simple, soluble, low-molar-mass components, especially monosaccharides; small peptides and amino acids; and fatty acids and monoglycerides.

These are taken up in the blood, together with other nutrients, such as various salts, glycerol, organic acids, etc. The substances are transported to all the organs in the body, including the mammary gland, to provide energy and building blocks (precursors) for metabolism, including the synthesis of proteins, lipids, etc.

In ruminants like the cow, considerable predigestion occurs by means of microbial fermentation, which occurs for the most part in the first stomach or rumen. The latter may be considered as a large and very complex bio-fermenter. It contains numerous bacteria that can digest cellulose, thereby breaking down plant cell walls, providing energy and liberating the cell contents. From cellulose and other carbohydrates, acetic, propionic, butyric and lactic acid are formed, which are taken up in the blood. The composition of the organic acid mixture depends on the composition of the feed. Proteins are broken down into amino acids. The rumen flora uses these to make proteins but can also synthesize amino acids from low-molar-mass nitrogenous components. Further on in the digestive tract the microbes are digested, liberating amino acids. Also, food lipids are hydrolyzed in the rumen and partly metabolized by the microorganisms. All these precursors can reach the mammary gland.

### Milk Synthesis

The synthesis of milk components occurs for the greater part in the secretory cells of the mammary gland.

At the basal end precursors of milk components are taken up from the blood, and at the apical end milk components are secreted into the lumen. Proteins are formed in the endoplasmic reticulum and transported to the Golgi vesicles, in which most of the soluble milk components are collected. The vesicles grow in size while being transported through the cell and then open up to release their contents in the lumen. Triglycerides are synthesized in the cytoplasm, forming small globules, which grow while they are transported to the apical end of the cell. They become enrobed by the outer cell membrane (or plasmalemma) while being pinched off into the lumen. This type of secretion is called merocrine, which means that the cell remains intact.

### Fat Globules

To a certain extent, milk is an oil-in-water emulsion. But the fat globules are more complicated than emulsion droplets. In particular, the surface layer or membrane of the fat globule is not an adsorption layer of one single substance but consists of many components; its structure is complicated. The dry mass of the membrane is about 2.5% of that of the fat. A small part of the lipids of milk is found outside the fat globules. At temperatures below 35°C, part of the fat in the globules can crystallize. Milk minus fat globules is called milk plasma, i.e., the liquid in which the fat globules float.

### Casein Micelles

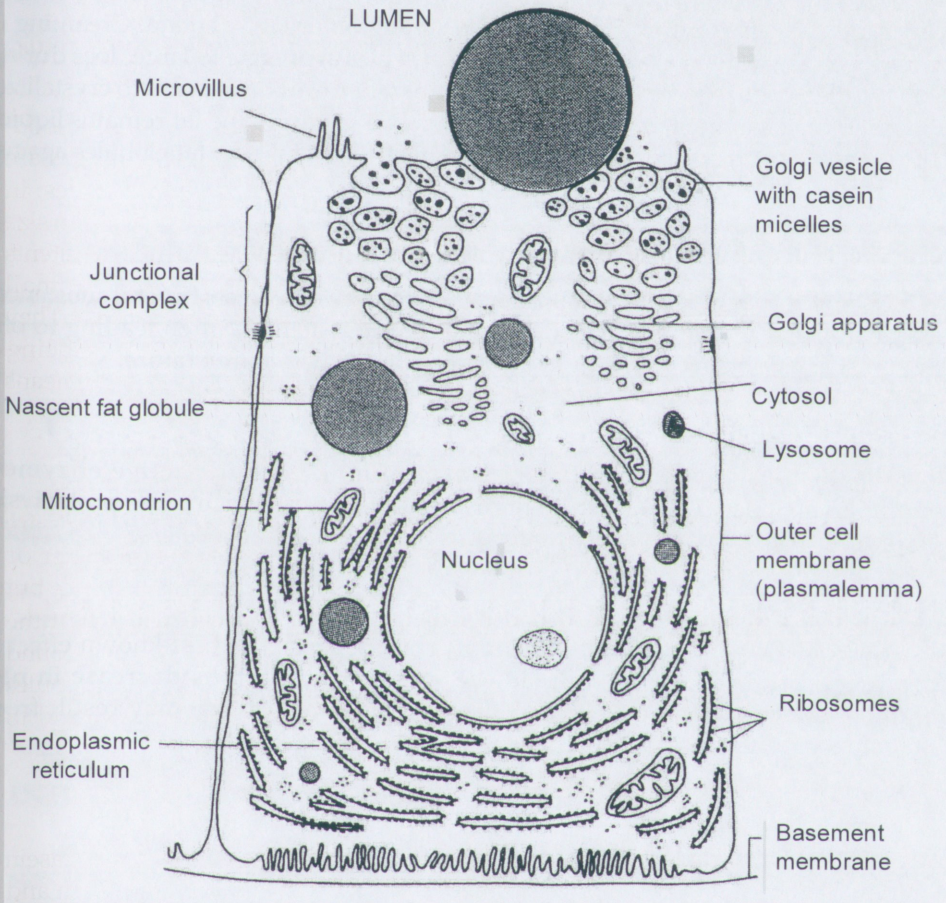
Casein micelles consist of water, protein, and salts. The protein is casein. Casein is present as a caseinate, which means that it binds cations, primarily calcium and magnesium. The other salts in the micelles occur as a calcium phosphate, varying somewhat in composition and also containing a small amount of citrate. This is often called colloidal phosphate. The whole may be called calcium-caseinate/calcium-phosphate complex. The casein micelles are not micelles in the colloid-chemical sense but just 'small particles.' The micelles have an open structure and, accordingly, contain much water, a few grams per gram of casein. Milk serum, i.e., the liquid in which the micelles are dispersed, is milk minus fat globules and casein micelles.

### Serum Proteins

Serum proteins are largely present in milk in molecular form or as very small aggregates.

### Lipoprotein Particles

Lipoprotein particles, sometimes called milk microsomes, vary in quantity and shape. Presumably, they consist of remnants of mammary secretory cell membranes.




**Stylized diagram of a mammary secretory cell. Below is the basal part, on top the apical part of the cell.**

## CHANGES IN MILK

Milk is not a system in equilibrium. It changes even while in the udder. This is partly because different components are formed at various sites in the mammary secretory cell and come into contact with one another after their formation. Furthermore, several changes can occur due to the milking, the subsequent lowering of the temperature, and so on. Changes may be classified as follows:

### Physical Changes Occurring

Physical changes occurring, for instance, when air is incorporated during milking: Because of this, additional dissolution of oxygen and nitrogen occurs in milk. Moreover, a new structural element is formed: air bubbles. Milk contains many surface-active substances, predominantly proteins, which can become attached to the air–water interface formed.

Furthermore, by contact with the air bubbles, fat globules may become damaged, i.e., lose part of their membrane. Fat globules may cream. Creaming is most rapid at low temperature because the globules aggregate to large flocs during the so-called cold agglutination. On cooling, part of the milk fat starts to crystallize the more so at a lower temperature. But even at 0°C part of the fat remains liquid. The presence of fat crystals can strongly diminish the stability of fat globules against clumping.

### Chemical Changes

Chemical changes may be caused by the presence of oxygen: Several substances may be oxidized. In particular, light may induce reactions, often leading to off flavors. Composition of salts can vary, for example, with temperature.

### Biochemical Changes

Biochemical changes can occur because milk contains active enzymes. Examples are lipase, which causes lipolysis; proteinases, which cause proteolysis; and phosphatases, which cause hydrolysis of phosphoric acid esters.

### Microbial Changes

Microbial changes are often the most conspicuous: The best-known effect is production of lactic acid from lactose, causing an obvious decrease in pH. Numerous other changes, such as lipolysis and proteolysis, may result from microbial growth.

# 2

# Structural Elements of Milk: Fat Globules, Casein Micelles, Globular Proteins, Lipoproteins Particles and Their Properties and Grading of Milk

**Topics**

*• Introduction • Structure of Milk • Structural Elements • Test for Grading of Milk • Dairy animals • Variability • Changes*

## INTRODUCTION

The multi cellular organisms organize the cells into tissues and further into specific organs with specific functions. Consequently the secretions of these cells will have specific composition with the arrangement of these components in a specific manner giving unique physical structure to it. Depending up on the requirements , the structure of milk is either maintained or being disturbed in order to prepare certain products. As such a knowledge of these elements will give better understanding of the structure of the milk.

## STRUCTURE OF MILK

Milk being a secretion of the epithelial cells of the mammary gland has specific interactive forces working between its components which help in maintaining the integrity of the whole system under different conditions and thereby maintaining its specific structure. The physical structure i.e., the arrangement of the components in space must also be known. The interactive forces between the components are important; they determine the integrity of the whole system under various conditions. They are microscopicor sub-microscopic in scale. The structure of milk is very has simple and extensive studies are being conducted on this aspect. Since milk constituents exist in different physical state influencing milk properties in addition to the chemical nature of the constituents.

## STRUCTURAL ELEMENTS

### Milk Fat

In milk, fat exists in the form of fat globules. The unique feature of these fat globules is that these fat globules are surrounded with a membrane which is derived from the apicalmembrane of the mammary secretory cell. Milk minus fat globules is called "Milk plasma".

### Proteins

Casein is the major protein of bovine milk and exists mainly as micellar form, where as in human milk major proteins are whey proteins. The Casein micelle consists of water, casein, salts and some minor components including lipase and proteinase. Casein micelles are built of smaller particles called sub-units or sub-micelles. Milk plasma free from caseinmicelles is known as milk serum. The liquid that is obtained on clotting of milk either with rennet or by acidification to pH 4.6 (isoelectric point of casein) is known as whey. This whey differs in its composition from that of serumin having some of the polypeptides cleaved from casein by the action of rennet.The proteins present in whey are termed as whey proteins of milk. The whey proteins are mainly globular proteins. Lipoprotein particles sometimes called microsomes vary in their quantity, composition, and shape. They consists remnants of cell membranes, microvilli, etc.

### Somatic Cells

The somatic cells consist of mainly leukocytes of various types is considered as extraneous particles although they are always present. They are about 10 μ m in diameter; number is about 100,000 per milliliter and accounts for about 0.005% of the volume of milk. They contain all cytoplasmic components notable nucleic acids and enzymes. They are rich in catalase. In mastitic milk the cell counts are much higher

### Physical structure of Milk

If we try to see a drop of milk with only 5 times magnification it appears as uniform liquid, however, it cannot be homogeneous since it is turbid in nature.With an increase in the magnification by another 100 times one can observes pherical droplets of fat floating in plasma. A further increase of magnification by another 100 times the proteinaceous particles (casein micelles)become visible. Fat, casein micelles and other proteins form the major structural elements for milk, and these elements are microscopic or submicroscopic in their size. Although fat is considered to be present in the form of an emulsion but it is not just a simple emulsion, because of the fact that the fat globules are being covered with the fat globule membrane which is derived during its biosynthesis. In addition to the fat globules some of the fat in the milk is found outside the globules while the membrane material includes several enzymes and proteins and polar lipids. As such fat and fat globules are not identical.

### Milk Plasma

Milk gets its structure with the structural elements. The main structural element being the fat, all that portion of milk which is free from the fat is considered as milk plasma. It is often a practice to use cream separators for getting the skim milk, but all the fat present in milk will not be separated as such we cannot consider milk plasma to be synonymous to skim milk. Here we consider fat free -milk to be plasma but not skim milk. Although the words serum and plasma are used for fractionation of blood here in milk these words are denoting the physical fractions of milk.

### Milk Serum

All that portion of milk excluding the casein micelles is referred as milk serum. By using either rennet or dilute acids the casein micelles could be separated from the plasma to obtain milk serum. The globular proteins consists mainly the whey proteins. They also bind some cations and a little water. Lipoprotein particles present in milk are also referred as the milk microsomes.

### Casein Micelles

Water,casein and salts along with some minor components such as lipases and proteinases constitute this structural element of milk.Casein micelles does not completely account for the casein content since some of the casein is also present in a solution form. As casein binds cations especially the $Ca^{++}$ and $Mg^{++}$ ions at the normal pH of milk i.e ~6.6, it is referred as caseinate. Amorphous calcium phosphate and small amounts of citrates are the constituents associated with the casein micelle. Calcium caseinate phosphatecomplex is the term applicable to describe the casein micelles. Phosphatebeing a part of the colloidal particles, the term colloidal phosphate is also used.

### Whey Proteins

The non micellar proteins constitute the whey proteins, but it is often the globular proteins present as such are considered to be the whey proteins

## TEST FOR GRADING OF MILK

The milk procured at the dairy plants is not only sold as fluid milk but also used for the preparation of various dairy products. As such it is necessary to grade the milk procured at the dairy plants by conducting various tests. The tests that are commonly conducted for grading of raw milk are:

### Sensory Tests

- Organoleptic tests

### Physical tests

- Sediment test

- Clot on boiling test
- pH

**Chemical Test**

- Alcohol test
- Alizarin-alcohol test

**Organoleptic Tests**

The first test that is expected to be conducted in all the dairies as soon as mil is received at the milk procurement section is organoleptic test. This test is primarily based on smell (odour) and appearance. An experienced person with an ability to detect the various abnormalities in the milk flavours should conduct this test. This will help in grading the good and bad quality milk. During this examination it is possible to detect the abnormalodours such as garlic, onion, cabbage etc., and sourness of the milk. Similarly,the abnormalities in the colour could be detected in these tests, apart from presence of various extraneous objects in the milk, such as hair, fibres, dung cakes etc.

**Sediment Test**

This test reveals the extent to which visible insoluble matter has gained entry into milk. The sediment test presents a simple rapid and a quantitative measure of indicating the cleanliness of milk with respect to visible dirt. However it is necessary to give the interpretation judiciously since absence of visible dirt never ensures good quality milk

**Clot-On-Boiling (COB) Test**

This is a quick test to determine developed acidity and the suitability of milk for heat processing. Milk giving a positive test has acidity generally above0.17% lactic acid and is not suitable for distribution as liquid milk or for heat processing

**pH**

The hydrogen ion concentration or the pH value is a measure of true acidity of milk. The pH of normal cow milk ranges from 6.6 to 6.8. Due to the developmen of acidity by the conversion of lactose into lactic acid this value would be reduced Milk obtained form the animals suffering from mastitis will have pH above 7.0. The pH test is mainly used for detecting of abnormal mastitis milk.

**Alcohol Test**

The alcohol test is used for rapid assessment of stability of milk to processing particularly for condensing and sterilization. The alcohol test is used as an indicato of disturbed salt balance in milk but not as an index for developed acidity. This tes helps in detecting abnormal milk such as colostrums, late lactation milk, and mastitis milk.

**Alizarin-alcohol Test**

This test is similar to the alcohol test and the addition of alizarin helps in detecting the acidity by judging of colour as well . The interpretation of the results is based on the size of the clot and the colour developed which in turn helps in grading of the milk.

## DAIRY ANIMALS

World milk production is almost entirely derived from cattle, buffaloes, goats, sheep and camels. Other less common milk animals are yaks, horses, reindeers and donkeys. The presence and importance of each species varies significantly among regions and countries. The key elements that determine the dairy species kept are feed, water and climate. Other factors that may influence the presence of a dairy species are market demand, dietary traditions and the socio-economic characteristics of individual households (e.g., poorer families tend to rely more on small ruminants).

Although cattle are kept in a wide range of environments, other dairy species make dairying possible in adverse environments that often cannot support any other type of agricultural production. Sheep allow milk production in semi-arid regions around the Mediterranean, goats in regions with poor soils in Africa, horses in the steppes of Central Asia, camels in arid lands, buffaloes in wet tropical regions, and yaks in high mountainous areas such as the Tibetan Plateau.

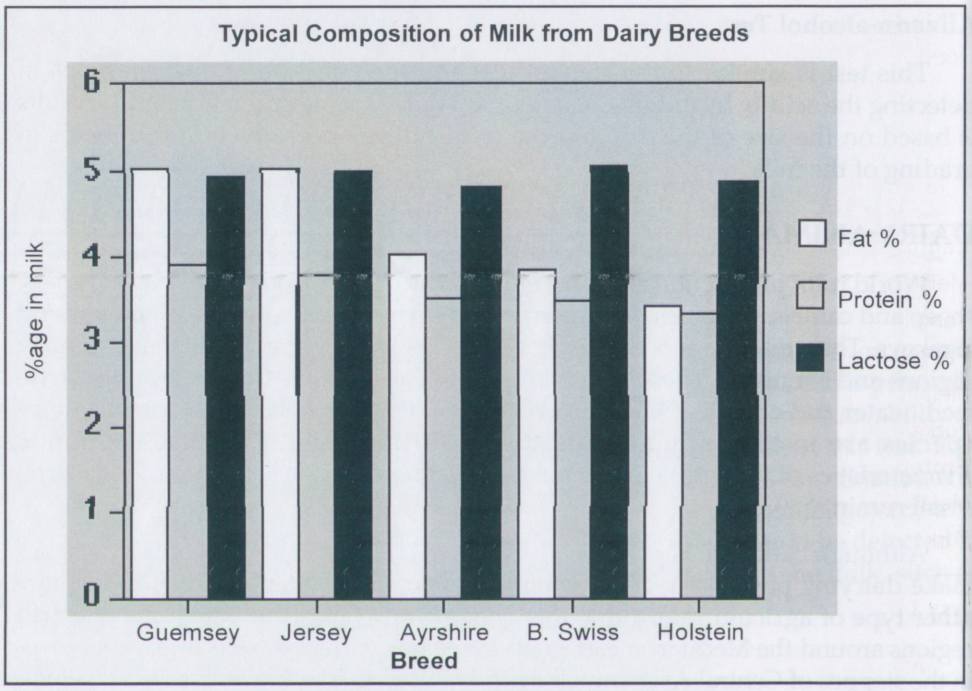
In developing countries, milk producing animals are often raised in subsistence and smallholder systems. These animals are usually multi-purpose and grow and produce under difficult conditions, such as low inputs, minimum management and harsh environments. They are well adapted to local conditions, but have low genetic potential for milk production.

## VARIABILITY

Milk fat is the most variable component of milk. It ranges from a little over 1% to greater than 50%. Aquatic mammals typically have high milk fat percentage.

Lactose ranges from only a trace to less than 7%. Some species have very little lactose in milk, such as the bear and kangaroo. These must have some other osmole in order to maintain milk in osmotic balance with blood plasma as milk is being synthesized. These species often have another sugar (for example the kangaroo has a trisaccharide) as the major osmole.

Milk protein concentration varies considerably, but not as much as milk fat varies among species. Ranges from 1% to about 14%. Generally milk protein percentage is positively correlated with milk fat percentage. If one is high, the other is usually high.

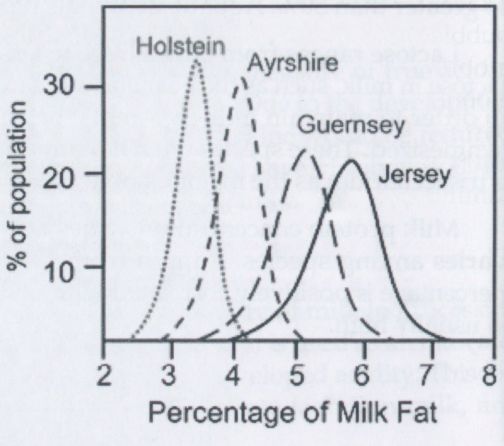



**Typical Composition of Milk from Dairy Breeds**

### Breed or Strain Variability

The composition of milk also varies within a species. The dairy cow is a good example of this (see the images below). Breed differences in milk composition, especially milk fat and protein, have been the basis for part of the premium payments that dairy producers have received for the quality of the milk they produce. Note that lactose content is fairly constant among breeds, milk fat varies extensively (Jersey and Guernsey are highest and Holstein is lowest), and protein varies somewhat among breeds.

Milk composition also varies substantially among individuals of the same breed. For example, from the image at right you can see that milk fat in Jerseys, while averaging about 5.5%, can range from less than 4% to greater than 7% for individual cows.




Differences in milk composition also occur among strains or breeds of other mammals. As an example, milk composition of a typical American breed of pig (Yorkshire) and the

Chinese Meishan breed of pigs is given below. These animals were fed the same feed and raised under the same environment.

| | Day of Lactation | | | |
|---|---|---|---|---|
| | 0 | 1 | 7 | 21 |
| **Lactose** (mg/ml) | | | | |
| Meishan | 19.9 | 31.7 | 43.5 | 51.4 |
| Yorkshire | 30.4 | 38.1 | 47.1 | 49.6 |
| **Fat** (%) | | | | |
| Meishan | 7.4 | 10.9 | 10 | 7.78 |
| Yorkshire | 5.5 | 7.8 | 7.8 | 6.58 |
| **Protein** (mg/ml) | | | | |
| Meishan | 154.4 | 28.4 | 28.4 | 28.9 |
| Yorkshire | 139.7 | 58.8 | 35.7 | 36.5 |

## CHANGES

Milk is not a system in equilibrium. It changes even while in the udder. This is partly because different components are formed at various sites in the mammary secretory cell and come into contact with one another after their formation. Furthermore, several changes can occur due to the milking, the subsequent lowering of the temperature, and so on. Changes may be classified as follows:

### Physical Changes

Physical changes occurring, for instance, when air is incorporated during milking: Because of this, additional dissolution of oxygen and nitrogen occurs in milk. Moreover, a new structural element is formed: air bubbles. Milk contains many surface-active substances, predominantly proteins, which can become attached to the air–water interface formed. Furthermore, by contact with the air bubbles, fat globules may become damaged, i.e., lose part of their membrane. Fat globules may cream. Creaming is most rapid at low temperature because the globules aggregate to large flocs during the so-called cold agglutination. On cooling, part of the milk fat starts to crystallize, the more so at a lower temperature. But even at 0°C part of the fat remains liquid. The presence of fat crystals can strongly diminish the stability of fat globules against clumping.

### Chemical Changes

Chemical changes may be caused by the presence of oxygen: Several substances may be oxidized. In particular, light may induce reactions, often leading to off-flavors. Composition of salts can vary, for example, with temperature.

### Biochemical Changes

Biochemical changes can occur because milk contains active enzymes: Examples are lipase, which causes lipolysis; proteinases, which cause proteolysis; and phosphatases, which cause hydrolysis of phosphoric acid esters.

### Microbial Changes

Microbial changes are often the most conspicuous: The best-known effect is production of lactic acid from lactose, causing an obvious decrease in pH. Numerous other changes, such as lipolysis and proteolysis, may result from microbial growth.

#### *Cooling of the Milk*

Cooling of the milk to about 4°C is generally applied to inhibit many of the changes mentioned, especially growth of microorganisms and enzyme action. In many regions, the milk is already cooled at the farm, directly after milking, in a so-called bulk tank. The milk should be kept cold during transport to the dairy and subsequent storage.

#### *Processing*

At the dairy milk is always processed. Of course, this causes changes in composition and properties of the milk, as it is intended to do. These changes can be drastic, as the following examples will show, and it can be questioned whether the resulting product can still be called milk; however, it is standard practice to do so. The most common processes applied are briefly described in the following paragraphs.

#### *Heat Treatment*

Heat treatment is virtually always applied, primarily to kill harmful bacteria. It also causes numerous chemical and other changes, the extent of which depends on temperature and duration of heating. Low pasteurization (e.g., 15 s at 74°C) is a fairly mild treatment that kills most microorganisms and inactivates some enzymes but does not cause too many other changes. High pasteurization (e.g., 1 s at 90°C, but varying widely) is more intense; all vegetative microorganisms are killed, most enzymes are inactivated, and part of the serum proteins become insoluble. Sterilization (e.g., 20 min at 118°C) is meant to kill all microorganisms including spores; all enzymes are inactivated; numerous chemical changes, such as browning reactions, occur; and formic acid is formed. UHT (ultrahigh-temperature) heating (e.g., at 145°C for a few seconds) is meant to sterilize milk while minimizing chemical changes; even some enzymes are not inactivated fully.

#### *Separation*

Separation, usually by means of a flow-through centrifuge called a cream separator, yields skim milk and cream. The skim milk has a very low fat content

0.05 to 0.08%. Milk skimmed after gravity creaming has a much higher fat content. Unless stated otherwise, the term skim milk will refer to centrifugally separated milk. By mixing skim milk and cream, milk may be standardized to a desired fat content.

### *Homogenization*

Homogenization (i.e., treatment in a high-pressure homogenizer) of milk leads to a considerable reduction in fat globule size. Such milk creams very slowly but is also altered in other respects. All types of sterilized milk or, more generally, all long-life liquid milk products are homogenized in practice.

### *Evaporation*

Evaporation removes water, producing milk that is more concentrated. Many properties are altered; the pH decreases, for example.

### *Membrane Processes*

Membrane processes may be applied to remove water; this is called reverse osmosis. Ultrafiltration separates milk into a concentrate and a permeate that is rather similar to milk serum. Electrodialysis removes some inorganic salts.

### *Fermentation or Culturing of Milk*

Fermentation or culturing of milk, usually by lactic acid bacteria, causes considerable alteration. Part of the lactose is converted to lactic acid, causing a decrease in pH to such an extent that the casein becomes insoluble. This makes the milk much more viscous. The bacteria also produce other metabolites, the kind and concentrations of which depend on the bacterial species.

### *Cheese Making*

As mentioned, milk can be clotted by adding rennet, which contains a specific proteolytic enzyme. The enzyme transforms the casein micelles in such a way that they start to coagulate. The resulting gel can be broken into pieces; stirring the material then results in the formation of curd particles and whey. The curd contains the micellar casein and most of the fat, the liquid whey contains most of the water-soluble components of the milk and some protein split off casein by the rennet. The curd is further processed to form cheese.

# 3

# Characteristics of Milk

**Topics**

*• Milk Fraction Terms • Composition of Milk • Basic Properties of Milk • Changes in Milk Composition • Milk Fat • Milk Fat - Composition • Changes in milk fat characteristics • Changes in milk during storage • Milk Proteins • Whey • Alpha-lactalbumin • Beta-lactalbumin • Milk Proteins - Reactions • Lactose • Minerals • Minor components and micronutrients • Vitamins in milk • Functions of Vitamins in Milk • Physical and chemical properties • Bacteria • Somatic Cells*

## MILK FRACTION TERMS

"**Plasma**" and "skim milk" are inter-changeable terms, but "skim milk" is more commonly used.

The serum (whey) proteins do not contain phosphorus, and these proteins remain in solution in milk at pH 4.6. The principle of coagulation, or curd formation, at reduced pH is the basis for cheese curd formation. In cow's milk, approximately 82% of milk protein is casein and the remaining 18% is serum, or whey protein.

**Milk Solids-not-fat:** The serum solids or milk solids-not-fat (MSNF) contain the lactose, caseins, whey proteins, and minerals (ash content) of the product from which they were derived. They are an important ingredient for the following beneficial reasons:

- improve the texture of ice cream, due to the protein functionality
- help to give body and chew resistance to the finished product
- are capable of allowing a higher overrun without the characteristic snowy or flaky textures associated with high overrun, due also to the protein functionality

- may be a cheap source of total solids, especially whey powder

## COMPOSITION OF MILK

| Constituent | Concentration (g/L) | Proportion solids (%) |
|---|---|---|
| Ash | 7.0 | 5.5 |
| Fat | 37.0 | 28.9 |
| Protein: casein | 27.6 | 21.6 |
| Lactose | 48.0 | 37.5 |
| Total solids | 127.9 | 100.0 |
| Non-protein nitrogen | 1.9 | 1.5 |
| Protein: whey | 6.4 | 5.0 |

The biological function of milk is to supply nutrition and immunological protection to the young mammal. In some species, milk is the only food consumed for weeks or months. Therefore, it must furnish all nutritive requirements for maintenance and growth, such as energy, amino acids, minerals, and vitamins.

This table above shows the composition of milk, detailing the major components as proportions of solids.

## BASIC PROPERTIES OF MILK

### Emulsion

An emulsion is a suspension of droplets of one liquid into another liquid. Milk is an emulsion of fat in water. Butter is an emulsion of water in fat. The solute is known as the dispersed phase and the solvent is known as the continuous phase. Other examples of emulsions include margarine, mayonaise, cream, and salad dressing.

### Colloidal Solution

A colloidal solution is when matter exists in a state of division in between a true solution, which is sugar in water, and a suspension, which is chalk in water. The characteristics of a colloid are small particle size, electrical charge, and affinity of the particles for water molecules. In milk, the whey proteins are in colloidal solution. The casein proteins are in colloidal suspension.

## CHANGES IN MILK COMPOSITION

### Changes in Composition

The composition of milk may change due to differences in relative rates of synthesis and secretion of milk components by the mammary gland. Variations are

due to differences among species, between individuals within a strain, and between conditions affecting an individual. Conditions affecting the cows may include the weather or seasons and the stage of lactation.

### Breed

The world mostly uses milk from cows of the larger breeds, such as Holstein and Brown Swiss' because of the lower fat content and greater milk production. Breeds such as the Guernseys and the Jerseys have higher fat contents in their milks. Both the Guernseys and the Jerseys have a fat content of 5.2%, where as the Holsteins and the Brown Swiss' have fat contents of 3.5%.

### Diet

The composition of the cows' diet and the form in which they are fed affect the composition of milk and especially milk fat. High fat and/or low roughage diets can reduce the fat content of milk. Diet has small effects on protein content and none on lactose content. The seasonal effect is due to the changes in the diet throughout the year.

### Stage of Lactation

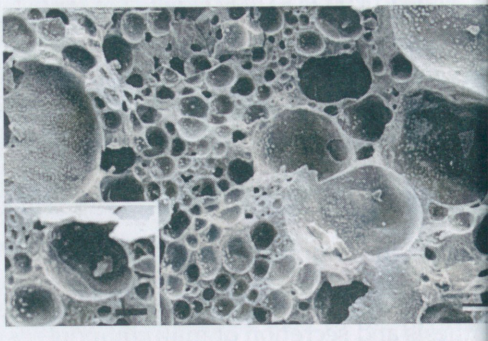
When mammals give birth, their first secreted milk is called colostrum, and it differs greatly in composition from regular milk. Colostrum contains more mineral salts and protein and less lactose than normal milk. Also, fat content, calcium, sodium, magnesium, phosphorus, and chloride are higher in colostrum than in normal milk. Whey content is about 11% in colostrum as opposed to 0.65% in normal milk.

Colostrum contains an extremely high imunoglobulin (Ig) content. Ig accumulate in the mammary gland before parturition and transfer immunity to the baby cow. These immunoglobulins protect the baby cow until it can establish its own Immunity.

## MILK FAT

Milk fats consist of a mixture of compounds including triacylglycerols, diacylglycerols, monoacylglycerols, phospholipids, cerebrosides, gangliosides, sterols and sterol esters and derivatives, carotenoids, tocopherol, vitamins A, D, E, C, $B_1$, and $B_2$ and free fatty acids. They are not soluble in water, or aqueous liquids.

Milk fat is secreted from the mammary epithelial cells as fat globules.



**Low magnification milk fat droplets**

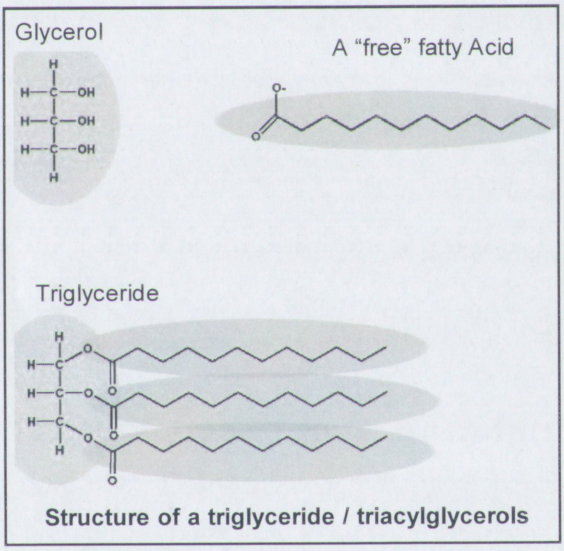
The average size of a milk fat globule is about 3um in diameter, but they can range from 1 to 18 µm.

The milk fat globule is encapsulated by a lipid bilayer membrane made up of proteins, phospholipids, lipoproteins, cerebrosides, nucleic acids, enzymes, trace elements, and bound water. The FGM (fat globule membrane) provides stabilization for the fat globule in the aqueous environment of the milk serum.

When the FGM membrane is ruptured, the fat globules join together into a solid mass of fat. (This is what happens during the production of butter).

Milk fat globules are the largest particles in milk but they are also the lightest. They have a lower density than milk serum, so the globules rise to the surface, which is how cream is formed. The density difference is used to separate milk fat from whole milk in high speed, continuous flow, centrifugal separators.

All fats belong to a group of chemical substances called esters, which are compounds of alcohols and acids. Milk fat is a mixture of different fatty acid esters called triglycerides.



**Structure of a triglyceride / triacylglycerols**

Triglycerides (triacylglycerols) account for 97-98% of the total fat in cows' milk. The remaining lipid classes are di- and mono-acylglycerols, phospholipids, free fatty acids, and cholesterol and its esters.

A triglyceride contains a glycerol backbone. This glycerol backbone is in red on the picture on this slide. Free fatty acids are attached to the glycerol backbone by an ester bond. Each glycerol molecule can bond three fatty acid molecules. About 437 fatty acids have been identified in the milk fat of cows.

These fatty acids all vary in chain length, number, position, and geometric isomerization of double bonds (cis or trans).

A fatty acid molecule is composed of a hydrocarbon chain and a carboxyl group. In saturated fatty acids, the carbon atoms are linked together in a chain by single bonds. In unsaturated fatty acids, there are one or more double bonds in the hydrocarbon chain.

Fatty acids in milk come from three main sources: from the feed, from the mobilization of reserve tissue, and from de novo (from the beginning) synthesis within the cow.

## MILK FAT - COMPOSITION

### Saturated and Unsaturated Fatty Acids

The main source of lipid is from the cow's food. Little synthesis of fatty acids occurs in the mammary gland. When the cow consumes food, the lipid is hydrolyzed to free fatty acids within the rumen of the cow. This means that unsaturated fatty acids are usually hydrogenated into saturated fatty acids. Hydrogenation is the addition of hydrogen on unsaturated bonds between carbon atoms.

Although it seems that milk fat would be mostly saturated in nature, over 30% of milk fat is still unsaturated. The unsaturated milk fat is predominately oleic acid (C18:1). Stearic acid (a saturated fatty acid) enters the mammary gland, but within the gland there is a specific C18:0 desaturase which converts stearic acid to the unsaturated oleic acid. As a result, there is a significant amount of unsaturated fat in milk. Just know that a desaturase is an enzyme which removes two hydrogen atoms from the fatty acid, creating a double bond, but you can learn more about these enzymes in upper level biochemistry classes.

From the rumen, the fatty acids have two places they can go: 1. the bloodstream to be deposited as reserve fat or to be metabolized to produce energy, or 2. the mammary gland.

### Distribution of Fatty Acids

Milk fat of cows can be separated into long-chain and short-chain fatty acids fractions by distillation or chromatography. The short chain fraction contains mostly butyrate.

## CHANGES IN MILK FAT CHARACTERISTICS

The characteristics of milk fat are highly dependent on the nature of the preformed fatty acids available in feedstuff. Therefore, it is possible to manipulate the composition of milk fat by dietary means.

The physical properties of butterfat can be controlled by dietary means because the melting properties of the triglyceride are related to fatty acid composition. For example, by dietary manipulation, butter which can be spread directly from the refrigerator can be produced. On the other hand, butter that is physically stable at high temperatures can also be made.



During colder winter months, there are more pre-formed fatty acids in the cows diet than during the summer months. During the summer, cows graze on grass and drink more water than in the winter months.

**Fatty acid composition of human and bovine milk fat[1]**

| Fatty Acid[2] | Human | | Cow | |
|---|---|---|---|---|
| | Fat-free diet | Corn oil diet | Summer | Winter |
| 4:0 | n/a | n/a | 3.6 | 3.5 |
| 6:0 | n/a | n/a | 1.3 | 1.4 |
| 8:0 | n/a | n/a | 0.9 | 1.1 |
| 10:0 | n/a | n/a | 2.4 | 2.7 |
| 12:0 | 7.9 | 3 | 2.7 | 3.9 |
| 14:0 | 9 | 2.3 | 9.8 | 12.7 |
| 15:0 | n/a | n/a | 1.1 | 1 |
| 16:0 | 23.5 | 13.1 | 25.4 | 34.4 |
| 16:1 | 6.8 | 1.4 | 0.9 | 1.3 |
| 17:0 | n/a | n/a | 0.7 | 0.7 |
| 18:0 | 3.2 | 3 | 15.8 | 11.6 |
| 18:1 | 36.9 | 31.3 | n/a | n/a |
| 18:1 cis | n/a | n/a | 24.3 | 19.9 |
| 18:1 trans | n/a | n/a | 6.4 | 2.5 |
| 18:2+3 | 7.3 | 43 | 1.9 | 1.5 |

1 Data are weight percent taken from studies of human and bovine milks.
2 Number of carbons in the chain colon number of double bonds. The cis and trans forms of 18:1 were only measured in the cow samples. The cis double bond was in the 9-position and the trans double bond in the 11-position.

These data show the differences between the milk fat of humans and cows.

The milk fat from the cow contains a set of unique short carbon chain fatty acids (4:0 – 10:0) and very little polyunsaturated fatty acids (18:2+3).

These differences are a result of the digestive system of the cow. The cow has a rumen, which you all may already know about. It is basically a large fermentation tank in which food is broken down and modified by microorganisms before it goes into the intestines. The abundant polyunsaturated lipids in the grasses, legumes, silages, and grains consumed by the cow are hydrogenated (saturated) in the rumen, which is why cows milk has saturated fats.

Acetic and beta-hydroxybutyric are products of fermentation in the rumen and they are absorbed directly from the rumen into circulation and are carried to the mammary gland where they are used to make short chain fatty acids.

When the human mother was fed corn oil, which is rich in linoleic acid (18:2), she produced a level of 43% of linoleic acid in her milk fat.

## CHANGES IN MILK DURING STORAGE

### Oxidation of Fat

Oxidation occurs at the double bonds of the unsaturated fatty acids. Th presence of iron and copper salts accelerates the onset of auto-oxidation and th development of a metallic flavor. Also, the presence of dissolved oxygen an exposure to light promotes oxidation. Oxidation of milk fat may limit the shelf li of whole milk powders, but is not usually a serious problem. Oxidation is catalyze by light, so if milk is exposed to light for too long a period of time, off-flavors wi occur. Fat oxidation can be counteracted by microorganisms in milk such as lact acid bacteria because they consume oxygen thereby reducing oxidation. Als pasteurization helps to reduce oxidation because reducing compounds such sulfhydryl groups are formed when milk Is heated. The metallic oxidation off-flav is more common in the winter than in the summer because of differences in cows diet. Summer feeds are higher in Vitamins A and C. These vitamins increa the amount of reducing substances in milk.

### Oxidation of Protein

When exposed to light, the amino acid methionine is degraded to methion by riboflavin and vitamin C. Methional causes the "sunlight flavor" in mil However, methionine is present in milk as part of the milk proteins. fragmentation of the proteins must occur to release the free amino acid methionir Factors that relate to sunlight flavor development are: Intensity of the ligh especially light from flouresecent tubes, Duration of exposure, Homogenized mi is more sensitive that non-homogenized milk, Nature of the package - opaq packages such as plastic and paper give good protection.

### Hydrolysis

Hydrolysis is the liberation of free fatty acids from the glycerol backbone. Th reaction requires the presence of a lipase, which is an enzyme that catalyzes t hydrolysis of ester bonds of lipids. A large amount lipoprotein lipase is prese naturally in milk, but fortunately fat globules with an intact milk fat globu membrane are not susceptible to hydrolysis by the enzyme. Also, lipoprotein lipa is inactivated at temperatures required for pasteurization.

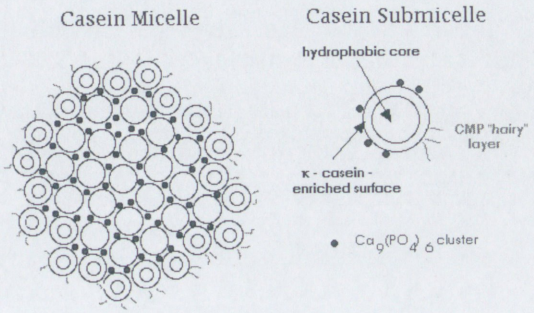
Spoilage bacteria provide a heat stable lipase, but the spoilage bacteria mu exceed normal levels, and routine bacterial control should prevent such occurrence.

### Lipolysis

The breakdown of fat into glycerol and free fatty acids is called lipolysis.

Fat that has been lipolysed tastes rancid and smells rancid. This effect is d to the presence of low molecular free fatty acids such as butyric and caproic aci

Lipolysis is enhanced by lipases and high storage temperatures. Lipases can only act if the fat globules have been damaged so that the fat is exposed. The fat glubules can be damaged by pumping, stirring, or splashing the milk. Therefore, unnecessary agitation of unpasteurized milk should be avoided to prevent the damage of the fat globules.

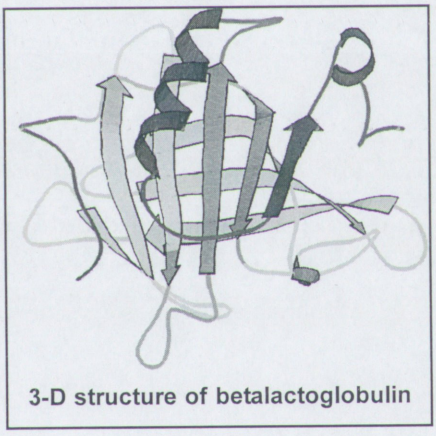



**Casein Micelle and Submicelle**

High temperature pasteurization inactivates the lipases.

## MILK PROTEINS

There are two distinct types of proteins in milk, casein and whey. Caseins make up over 80% of the total protein content and they can be further divided into five groups – the alpha$_{s1}$, alpha$_{s2}$, beta, gamma, and kappa caseins. Caseins do not have an organized structure, thus they cannot be denatured by heating.



**3-D structure of betalactoglobulin**

The casein molecules form polymers containing several identical and different molecules. Some molecules have hydrophillic regions and some have hydrophobic regions. The polymers are made up of many individual molecules and form into casein micelles. They are also formed into a colloidal solution, which gives skim milk its whitish-blue color.

The alpha and beta caseins account for the calcium sensitivity of milk protein. Casein coagulates whencalcium is added to casein solutions. This reaction is the key to cheese making and to the formation of acidic gels such as yogurt. Casein also coagulates when rennet is added. Rennet is a proteolytic enzyme that is found in the stomachs of calves.

The amino acids in casein have hydrophobic and hydrophilic regions, allowing the caseins to act as highly effective surface active agents. In other words, caseins act as stabilizers of foams and emulsions.

If an acid is added to milk or if acid producing bacteria are allowed to grow in milk, the casein micelle will change in two ways.

First, colloidal calcium hydroxyphosphate, present in the casein micelle, will dissolve and form ionized calcium. This ionized calcium will penetrate the micelle structure and create strong internal calcium bonds.

Second, the pH of the solution will approach the isoelectric points of the individual casein molecules.

So you're probably thinking great - what does this mean? What happens is this - the casein micelles get bigger because they aggregate with one another.

## WHEY

Whey is the milk serum proteins. When casein is precipitated out of skim milk, whey is left over. Casein are the curds and the liquid left over is the whey.

Whey proteins are globular proteins which may be denatured when heated above temperature above 65°C. The major components of whey are beta lactoglobulin and alpha lactalbumin. Whey is the liquid remaining after milk has been curdled and strained during the manufacture of cheese. It is used to produce ricotta and brown cheeses and is an additive in many processed foods, such as breads, crackers, pastries, and animal feed.

Many of the important nutrients that are comprise milk are partitioned into whey. It contains many important nutrients, such as lactose, water soluble vitamins, and most of the minerals, with the exceptions of considerable calcium and phosphorus which go with the casein into the cheese curd. The amino acid composition of whey is very close to that which is considered as the optimum amino acid value.

## ALPHA-LACTALBUMIN

Alpha-lactalbumin is considered the typical whey protein. It is present in milk from all mammals and it plays an important role in the synthesis of lactose in the udder.

## BETA-LACTALBUMIN

This protein is found only in ungulates and is the major whey protein component of milk from cows.

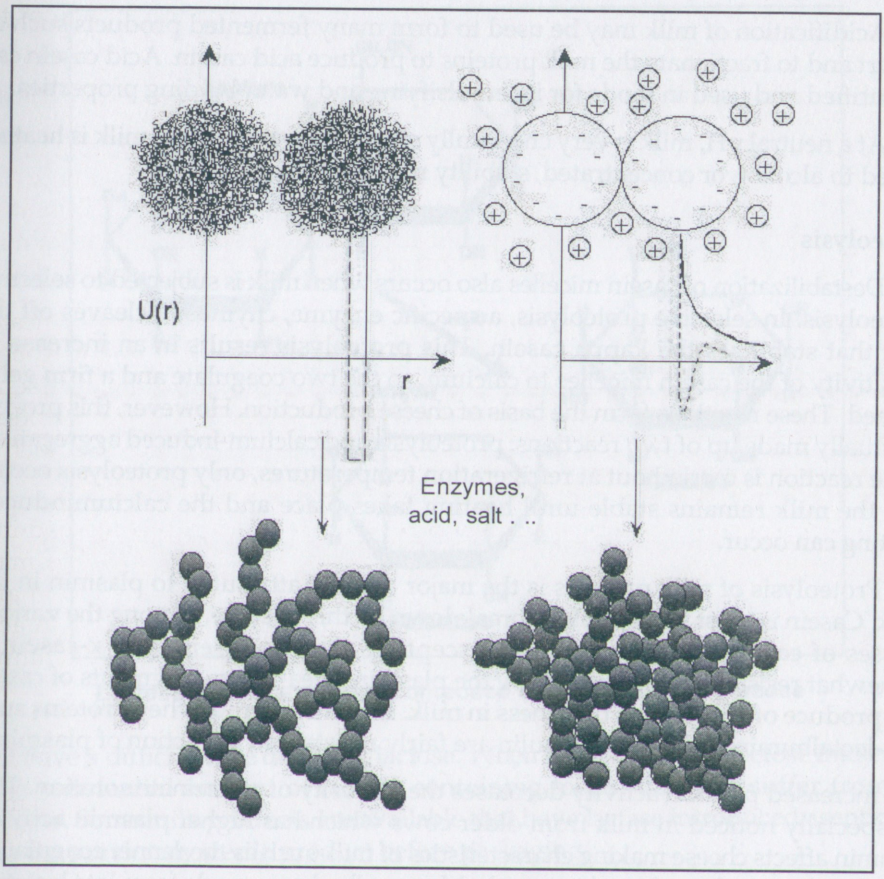
## MILK PROTEINS - REACTIONS

- Destabilization of the casein micelles
- Change in pH
- Proteolysis

### Destabilization of the casein micelles

The most important reactions of the milk proteins are those which involve destabilization of the casein micelles.

Destabilization of casein micelles in reconstituted skim milk was studied experimentally in both nonmixed and mixed conditions. The process of micelle




**Casein destabilization**

destabilization was described with a threestep kinetic model accounting for hydrolysis of casein in both stable and partially destabilized micelles. This model was used for the estimation of kinetic parameters of κ-casein hydrolysis proceeding on the surface of casein micelles and kinetic parameters of micelle destabilization. The influence of various process variables, such as mixing and enzyme concentration, on the hydrolysis of κ-casein and destabilization of casein micelles was evaluated. The presented model provides a good description of casein micelle destabilization within a specified range of process variables.

### Change in pH

As we previously mentioned, reactions can occur within casein micelles as the pH is reduced. As the pH if milk is reduced, the native micellar structure disintegrates. At the isoelectric point of casein, (the pH at which casein carries no net electrical charge - a pH of 4.6), the casein precipitates out of solution, along with any heat-denatured whey. This reaction may be reversed as the pH is increased, but native form of the micelle is not recovered.

Acidification of milk may be used to form many fermented products such as yogurt and to fractionate the milk proteins to produce acid casein. Acid casein can be purified and used in foods for its emulsifying and water binding properties.

At a neutral pH, milk is very chemically stable. However, when milk is heated, added to alcohol, or concentrated, stability may be reduced.

### Proteolysis

Destabilization of casein micelles also occurs when milk is subjected to selective proteolysis. In selective proteolysis, a specific enzyme, chymosin, cleaves off the part that stabilizes the kappa casein. This proteolysis results in an increase in sensitivity of the casein micelles to calcium, so the two coagulate and a firm gel is formed. These reactions form the basis of cheese production. However, this process is actually made up of two reactions: proteolysis and calcium-induced aggregation. If the reaction is carried out at refrigeration temperatures, only proteolysis occurs and the milk remains stable until heating takes place and the calciuminduced clotting can occur.

Proteolysis of milk proteins is the major activity attributed to plasmin in the milk. Casein is most susceptible to breakdown by this enzyme. Among the various classes of casein, β-casein is more susceptible than as-casein, and κ-casein is somewhat resistant to breakdown by the plasmin. Breakdown fragments of casein can produce off-flavor and bitterness in milk. In contrast, milk whey proteins such as a-lactalbumin and β-lactoglobulin are fairly resistant to the action of plasmin.

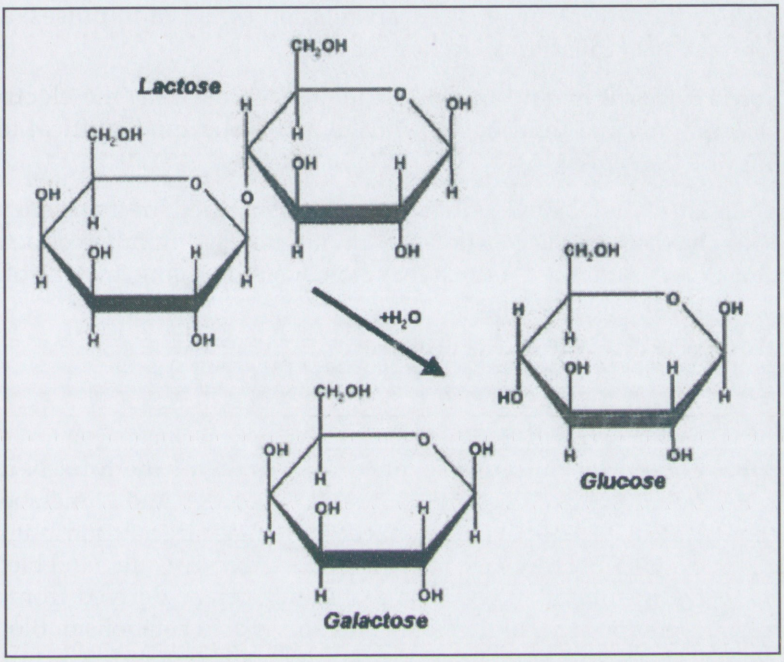
Increased plasmin activity decreases the viscosity of a caseinate solution. This is especially noticed in milk from older cows which has higher plasmin activity. Plasmin affects cheese making characteristics of milk such as the rennet coagulation time, curd strength, and curd syneresis. Mozzarella cheese made from late lactation milk, which contains higher amounts of plasmin, has inferior stretchability and melting ability. Proteolysis of casein by plasmin also increases the moisture content in cheddar cheese. Swiss cheese contains higher plasmin activity than cheddar cheese because the higher cooking temperature required for Swiss cheese destroys the inhibitors of plasmin and plasminogen activators. As a result, more breakdown of casein occurs during ripening of Swiss cheese as compared to cheddar cheese.

Thickening, gelation, and coagulation of milk occurs during storage. This is also attributed to the proteolytic activity either from milk proteases such as plasmin or proteases of bacterial origin. Proteolytic activity caused by plasmin has a particular effect on the taste of milk (appearance of bitter peptides). Bitter peptides produced by the action of proteases influence the flavor of UHT milk, UHT cream, and cheese.

## LACTOSE

Lactose is the most abundant of the milk solids.

Lactose is a useful source of energy and it is thought by some to promote the absorption of calcium from the diet. However, some people lack the enzyme lactase




**Lactose is a disaccharide composed of galactose and glucose**

and have a difficult time digesting lactose. When people who are lactose intolerant eat a substantial amount of lactose containing foods, they can suffer from GI distress. Some milk products are available that have lactase introduced aseptically into the previously sterile product before packaging.

The relative sweetness of lactose is small (only 20%) when compared to sucrose (100%).

## MINERALS

- **Calcium**: Calcium functions in the mineralization of bones and teeth, muscle contraction and relaxation, nerve functioning, and blood clotting.
- **Iron**: Iron carries oxygen as a part of hemoglobin in blood or myoglobin in muscles, and it is required for cellular energy metabolism.
- **Magnesium**: Magnesium is a factor involved in bone mineralization, the building of protein, enzyme action, normal muscular function, transmission of nerve impulses, proper immune function and maintenance of teeth.
- **Phosphorus**: Phosphorus is involved with mineralization of bones and teeth, it is important in genetic material, in cell membranes as phospholipids, in energy transfer, and in buffering systems.
- **Potassium**: Potassium facilitates reactions, including the making of protein. It is involved in the maintenance of fluid and electrolyte balance, the

support of cell integrity, the transmission of nerve impulses, and the contraction of muscles, including the heart.

- **Sodium**: Sodium, sodium chloride, and potassium (the electrolytes) maintain normal fluid balance in the body. Sodium is critical to nerve impulse transmission.
- **Zinc**: Zinc is associated with hormones, it is needed for many enzymes, it is involved in making genetic material and proteins, immune cell activation, transport of vitamin A, taste perception, wound healing, making of sperm, and normal fetal development.

## MINOR COMPONENTS AND MICRONUTRIENTS

### Non-Protein Nitrogen (NPN)

The NPN content of milk represents only 5 to 6% of the total N in milk. However, the significance of this milk N fraction to energy and N metabolism in the dairy cow has not been well characterized. The single largest contributor to the NPN fraction of milk NPN is urea. Urea equilibrates in body water, and blood urea is the primary source of milk urea. The urea in milk can be derived from at least two sources: the end product of digestion and amino acid catabolism. Blood urea N was positively associated with intakes of ruminally degradable and undegradable protein and negatively associated with intake of net energy. Consequently, it might be possible to develop a system to evaluate the dietary protein and energy status of the lactating dairy cow employing milk urea in conjunction with milk true protein.

### Nucleotides

Acid-soluble nucleotides in cow's and goat's milk at different stages of lactation were examined by ion-exchange column chromatography. In goat milk CMP5', DPN, AMP5', AMP-X, GMP5', UMP5', orotic acid, GDP-mannose, UDP-acetylglucosamine, UDP-acetylgalactosamine, UDP-glucose, UDP-galactose, GDP, UDP, UDP-uronic acid, and other unidentified nucleotides were found throughout the lactation period. Total content of acid-soluble nucleotides attained maximum at the colostral stage (330 μm per 100 ml) and decreased with the advance of lactation. The percentage of UDP-acetylhexosamine and UDP-hexose in acid-soluble nucleotides are not only highest in colostrum (68%) but also in the mid-stage of lactation (48%). Active P32 incorporation into these nucleotides was observed following the injection of P32. Milk of a goat with lactation induced by the administration of hormones showed the same chromatographic pattern as that of the milk at normal lactation. Considerable species difference of the acid-soluble nucleotides of milk was observed between cow's and goat's milk. With the exception of the colostral stage, orotic acid constituted a major part of acid-soluble nucleotides of cow milk. Cow's milk also contained CMP5', uric acid, AMP5', and an unidentified AMP derivative. There was no difference in the patterns of chromatograms between Holstein and Jersey milk.

## Milk Urea

The digestion of protein in the rumen releases ammonia. If there is excess ammonia it is absorbed through the rumen wall into the blood stream and converted to urea in the liver. Most of the urea is excreted in the cow's urine although some passes into the milk.

If the diet is deficient in nitrogen, then the urea is not excreted, but recycled back into the rumen and converted back to ammonia.

### *Interpreting MU Values*

Recommended dietary crude protein (CP) requirements and the approximate MU levels at different stages of lactation are shown in the table below.

| | Early lactation | Mid lactation | Late lactation |
|---|---|---|---|
| Minimum CP% in diet | 18 | 16 | 14 |
| Approximate MU (mg/dl) | 25-40 | 25-30 | 20-25 |

In New Zealand pasture-based systems, MU levels are higher than in systems where cows are fed a total mixed ration (i.e. USA, Western Europe). This is due to the high amount of crude protein (about 20%) in good quality pasture.

When pasture makes up more than 60% of the diet (low-input systems) MU levels can often be greater than 30 mg/dl. Research suggests that these high MU values are not detrimental to the health or reproductive performance of the cow.

When pasture is less than 60% of the diet (higher-input systems) MU may be used as a tool to help guide ration changes (e.g. help to decide when to put in or to pull out expensive protein supplements).

### *Factors affecting Milk Urea*

The main factor affecting MU in pasture-based systems is the amount of protein in the diet.

Other factors include water intake, cow condition, stage of lactation, season, genetics, milking frequency, rumen health, and liver function among others.

## Milk Enzymes

This page briefly describes the general properties of enzymes present in milk. Enzymes are proteins that have biological functions. Milk enzymes come from several sources: the native milk, airborne bacterial contamination, bacteria that are added intentionally for fermentation, or in somatic cells present in milk.

Each enzyme has a specific site of action on its target molecule, and optimal conditions (pH and temperature). There are a large number of enzymes in milk and the functions of many are not well-defined. It should be noted that the enzymes in

milk do not make a major contribution to the digestion of milk in humans, whic is accomplished by enzymes in the human stomach and small intestine.

Lipases are enzymes that degrade fats. The major lipase in milk is lipoprotei lipase. It is associated with the casein micelle. Agitation during processing ma bring the lipase into contact with the milk fat resulting in fat degradation and of flavors. Pasteurization will inactivate the lipase in milk and increase shelf life.

Proteases are enzymes that degrade proteins. The major protease in milk i plasmin. Some proteases are inactivated by heat and some are not. Protei degradation can be undesirable and result in bitter off-flavors, or it may provide desirable texture to cheese during ripening. Proteases are important in chees manufacture, and a considerable amount of information is available in the chees literature.

Alkaline phosphatase is a heat sensitive enzyme in milk that is used as indica of pasteurization. If milk is properly pasteurized, alkaline phosphatase i inactivated.

Lactoperoxidase is one of the most heat-stable enzymes found in mil Lactoperoxidase, when combined with hydrogen peroxide and thiocyanate, ha antibacterial properties. It is suggested that the presence of lactoperoxidase in ra milk inhibits the disease causing microorganisms (pathogens) present in mil However, since there is no hydrogen peroxide or thiocyanate present in fresh mil these compounds would have to be added to milk in order to achieve th antibacterial benefits. Lysozyme is another enzyme that has some antibacteri activities, although the amount of lysozyme present in milk is very small.

## VITAMINS IN MILK

### Fat Soluble Vitamins

#### *Vitamin A*

Whole milk contains some vitamin A, however levels in semi skimmed an skimmed milks are much lower. This is because vitamin A is dissolved within t milk fat fraction which is removed to varying levels when producing lower fat mi products.

Vitamin A is required for good vision, immune health and for normal grow and development of body tissues.

Whole milk contains approximately 62 µg of vitamin A per glass (200ml) whi provides about 9% of an adult's daily vitamin A requirement.

#### *Vitamin D*

Vitamin D plays an important role in the absorption of calcium and phosphor and is essential for healthy bones and teeth.

There are two sources of vitamin D, it is made in the skin by exposure to sunlight and there are a few dietary sources, such as oily fish, eggs, and fortified foods including margarine (which is required by law to contain vitamin D), some yogurts and breakfast cereals. There is very little vitamin D in milk and in fact only trace amounts and in the UK milk is not fortified with vitamin D.

Currently there is no dietary reference value (DRV) for vitamin D for those aged four to sixty-five years. This is because, until now, there has been a belief that exposure of the skin to UVB-rays contained in sunlight is sufficient for optimum vitamin D production. This belief was not based on solid scientific evidence.

The Scientific Advisory Committee on Nutrition's (SACN) report on vitamin D reinforced the need for more research before the DRVs can be reconsidered. The Food Standards Agency has funded projects, commissioned to provide evidence for the relative contributions of the two sources, dietary vitamin D and skin exposure to sunlight, to vitamin D status and to inform any future risk assessment undertaken by SACN. These projects showed low vitamin D status is a problem for some of the population in the UK and in particular for those of South Asian origin. Dietary intake was shown to make an important contribution to vitamin D status. However the research suggests that the typical daily intake of vitamin D from food contributes less than UVB rays from sunlight to average year-round vitamin status in Caucasian and Asian women.

Further appraisal of the DRVS for vitamin D will require a full risk assessment by SACN and results from the projects will need to be considered alongside other emerging research.

### *Vitamin E*

Vitamin E plays an important role in preventing damage to structures such as cell membranes. Substances which prevent damage in this way are called anti-oxidants and have been linked with reducing the risk of diseases such as cancer.

Vitamin E is naturally found in low levels within milk, a 200ml serving of semi-skimmed milk typically provides 0.04mg of vitamin E.

### *Vitamin K*

Vitamin K is essential for correct blood clotting. There is little or no vitamin K naturally found in milk although small amounts may be found in cheese.

## Water Soluble Vitamins

### *B vitamins*

Milk contains appreciable amounts of many of the B vitamins, and is particularly rich in vitamin $B_{12}$.

Vitamin $B_{12}$ is required for maintenance of healthy nerves and red blood cells, energy production and normal cell division.

It is predominantly found in foods of animal origin and therefore milk an dairy products are excellent sources.

One glass/200ml of semi skimmed milk will provide an adult (19-50 years with the full daily requirement for vitamin $B_{12}$.

Exclusion of milk and dairy products from the diet can therefore significantl reduce vitamin $B_{12}$ intakes. This is of particular concern when all animal product are excluded from the diet - as with the vegan diet - which can lead to vitamin B deficiency if suitable alternatives or supplements are not taken.

Thiamin (vitamin $B_1$) and riboflavin (vitamin $B_2$) are also present in milk.

Thiamin (vitamin $B_1$) is necessary for carbohydrate metabolism, neurologica and cardiac function. Unlike fat soluble vitamins, water soluble vitamins are no stored well in the body other than in small amounts, and therefore must come fro the diet.

One glass/200ml of semi skimmed milk will provide an adult (19-50 years with 15% of their daily requirement for thiamin.

Riboflavin (vitamin $B_2$) is necessary for the release of energy from foods an healthy membranes and skin, again riboflavin is a water-soluble vitamin and doe not store in the body, and any excess amounts are excreted unchanged in the urine

A 200ml glass of semi-skimmed milk provides 45% of an adult's (19-50 years daily requirement for riboflavin.

Niacin is involved in energy metabolism, the recommended amount fo children and adults is 6.6mg/1000kcal and milk provides an individual with up t 3% of the daily requirement for niacin.

Folate is an important vitamin essential for cell division and correc development of tissues.

A 200ml glass of semi-skimmed milk provides a 6 year old child with 18.6% o his or her daily folate requirement and an adult with 9.3%.

It is important that women planning pregnancy consume 400 micrograms o folic acid every day before conception and during early pregnancy to preven neural tube defects such as spina bifida.

Pyridoxine (Vitamin $B_6$) is an essential vitamin involved in protein metabolism and is required for the formation of red blood cells and for maintaining a healthy immune and nervous system.

This vitamin is only present in small amounts in milk.

*Vitamin C*

Vitamin C is required for the correct structure and maintenance of blood vessels, cartilage, muscle and bone.

Unlike other animals, humans cannot make vitamin C within the body and it must therefore come from the diet.

Good sources in the diet include many fruits and vegetables and milk also makes a contribution.

A glass/200ml of semi-skimmed milk will provide a child with 14% of the daily requirement for vitamin C and an adult up to 10.3%.

## FUNCTIONS OF VITAMINS IN MILK

- **Vitamin A**: Vitamin A prevents eye problems, promotes a healthy immune system, is essential for the growth and development of cells, mucous membranes, skin, bone and tooth health, reproduction, and immunity.
- **Vitamin D**: Vitamin D strengthens bones because it helps the body absorb bone-building calcium.
- **Vitamin E**: Vitamin E is an antioxidant and helps protect cells from damage. It functions in stabilization of cell membranes, support of immune function, protection of polyunsaturated fatty acids, and normal nerve development.
- **Vitamin C** (also called ascorbic acid): Vitamin C is needed to form collagen, a tissue that helps to hold cells together. It is an antioxidant, is restores vitamin E to its active form, it helps to synthesize hormones, it supports immune cell function, and helps in absorption of iron.
- **Thiamin** (also called vitamin B1): Thiamin is part of a coenzyme needed in energy metabolism. It also supports a normal appetite and nervous system function.
- **Riboflavin** (also called vitamin B2): Riboflavin is part of a coenzyme needed in energy metabolism. It also supports normal vision and skin health.
- **Vitamin $B_6$** (also called pyridoxine): Vitamin B6 is part of a coenzyme needed in amino acid and fatty acid metabolism. It helps to convert tryptophan to niacin and to serotonin, and it helps make red blood cells.
- **Vitamin $B_{12}$**: Vitamin $B_{12}$ is part of coenzymes needed to make red blood cells, and it is important for nerve cell function.
- **Folate** (also known as vitamin $B_9$, folic acid, or folacin): Folate is part of a coenzyme needed for synthesis of red blood cells. It is also needed to make DNA.
- **Niacin** (also called vitamin $B_3$): Part of coenzymes needed in energy metabolism. It helps maintain healthy skin and is important for nerve function.
- **Pantothenic Acid**: Part of a coenzyme needed in energy metabolism.
- **Biotin**: A cofactor for several enzymes needed in energy metabolism, fat synthesis, amino acid metabolism, and glycogen synthesis.

## PHYSICAL AND CHEMICAL PROPERTIES

### Density

Density is defined as an object's mass divided by it's volume. It depends on the temperature of the object, composition of the material, and whether or not the object contains air. The density of milk products can be used to convert volume into mass and mass into volume, to estimate the amount of solids present in milk, and to calculate other physical properties. The density of cows milk usually varies between 1.028 and 1.038 g/cm$^3$.

### Appearance

The opacity of milk is due to its content of suspended particles of fat, proteins and minerals. The color varies from white to yellow depending on the carotene content of the fat. Skim milk is more transparent and has a slightly bluish color.

### Freezing Point

The freezing point of milk is lower than the freezing point of water because of the dissolved components in milk. Measuring the freezing point is used as a legal standard to determine if milk has been diluted with water. The freezing point of milk is -0.552°C or 31°F.

### pH

The pH of milk is higher, or more alkaline, outside of the cow than inside the cow due to loss of carbon dioxide to the air. The pH of milk is never determined immediately after milking because the processing milk goes through removes dissolved gasses. The pH is determined after processing the milk to assure that lactic acid is being produced at the desired rate by added microorganisms during the preparation of cheeses and fermented milk. The casein in milk forms into a curd or a gel at a pH of 4.6.

### Titratable acidity

Titratable acidity is the amount of alkali required to bring the pH to neutrality. This property of milk is used to determine bacterial growth during fermentations such as cheese and yogurt making, as well as compliance with cleanliness standards. Naturally, there is no lactic acid in fresh bovine milk, however, lactic acid can be produced by bacterial contamination, but this is uncommon. The titratable acidity is due to the casein and phosphates.

## BACTERIA

Milk producers are required to exclude bacteria from milk, but some may still gain entry. The bacterial count in Grade A raw milk may not exceed 300,000/ml. When the cow has mastitis, microorganisms associated with infections are found including *Staphylococcus aureus*, *Streptococcus uberis*, and *Streptococcus agalactiae*. Mil

from cows with mastitis can not be used for human consumption. Pasteurization destroys most of the microorganisms in milk and all of the pathogens. In the US, the upper limit of bacteria in pasteurized milk is 20,000/ml.

Milk has a pH of 6.6, which is ideal for the growth of many organisms. Milk is sterile at secretion in the udder, but it becomes contaminated by bacteria before it leaves the udder. At this point, the bacteria are few and harmless, unless the cow has mastitis. Further infection of the milk by microorganisms happens during milking, storage, handling, and other activities.

### Pathogenic Bacteria

The following pathogenic bacteria are a concern in raw milk: *Mycobacterium tuberculosis, Listeria monocytogenes, Salmonella* spp., *Bacillus cereus, Yersinia enterocolitica, Escherichia coli* 0157:H7, *Campylobacter jejuni*

These pathogenic bacteria can cause foodborne illnesses by ingesting raw milk that contains these organisms or milk that was not pasteurized properly or was contaminated after processing.

### Spoilage Microorganisms

Spoilage microorganisms cause degradation of proteins, fats, and carbohydrates and the milk's texture, color, taste, and smell is deteriorated. Psychrotrophs (bacteria that grow at refrigeration temperatures) are usually involved in spoilage reactions. Most are destroyed by pasteurization, but can still produce heat stable lipolytic and proteolytic enzymes that can cause spoilage.

However, some strains and species of *Clostridium, Bacillus, Arthrobacter,* Cornebacterium, Microbacterium, Micrococcus, Streptococcus, and *Lactobacillus* can survive pasteurization and grow at refrigeration temperatures and cause spoilage.

### Coliforms

Coliforms are closely associated with the presence of pathogens, but they are not always pathogenic themselves, thus they are called indicator organisms. They are killed by proper pasteurization treatment. Their presence indicates improper or inadequate pasteurization or it indicates contamination after pasteurization. *Escherichia coli* is an example of a coliform. They are facultative anaerobes and grow best at 37°C or 98.6°F. Coliforms are able to degrade milk proteins and ferment lactose, causing milk to be spoiled quickly.

### Lactic Acid Bacteria

Lactic acid bacteria can produce chemical changes that are desirable in the production of fermented dairy products because they are able to ferment lactose to lactic acid. They are naturally present in the milk and are used as starter cultures in the production of cultured dairy products, such as yogurt and cottage cheese. Lactic acid bacteria include lactococci, lactobacilli, and *Leuconostoc.*

## SOMATIC CELLS

Somatic cells are cells from the cow (predominantly white blood cells, otherwise known as leukocytes) that are normally present in milk. During most mastitis infections, the number of somatic cells present in the udder increases to help the cow fight the infection. There are several types of somatic cells that have different functions in fighting infection. Somatic cells can contain lipolytic and proteolytic enzymes, which degrade fats and proteins, respectively. An increase in somatic cells count during a mastitis infection increases the amount of destructive enzymes present in the milk, which increases the rate of deterioration of the milk fat and protein.

### *Lactobacilli*

Lactobacilli were found in many dairy products by using enrichment techniques. The best sources were ungraded raw milk, Grade A raw milk, and sour cream. Some of the cultures, in populations of 10 ~ per milliliter, survived 61.8 ° C for 30 minutes.

The occurrence of *lactobacilli* in milk has been reported by many investigators. Nevertheless, there is relatively little information available as to the species present in various grades of milk and cream. After a culture has been isolated from milk or other sources, identification has proved to be difficult largely because of the variability of many of the characteristics which serve as taxonomic criteria. However, some reseraches have shown that these variations may be reduced by the use of nutritionally adequate media. Researchers classified *lactobacilli* with media they believed to be nutritionally adequate, and their results are quite similar.

# 4

# Milk Components

**Topics**

*Components • Trace Elements in Cow Milk • Minerals • Isolation of Casein, Lactose, and Albumin from Milk • Fats • Proteins • Carbohydrares • Polarimetry • Isolation of the Sugar, Lactose, and Albumin Proteins from Milk • Lactose • Lipids • Milk Proteins • Milk Lipids - Functional Properties • Caseins • Enzyme Coagulation • Acid Coagulation • Nutritional Components in Milk • Contaminants in Milk • Variation in Milk Vitamin Contents*

Milk provides essential nutrients and is an important source of dietary energy, high-quality proteins and fats. Milk can make a significant contribution to the required nutrient intakes for calcium, magnesium, selenium, riboflavin, vitamin $B_{12}$ and pantothenic acid. Milk and milk products are nutrient-dense foods and their consumption can add diversity to plant-based diets. Animal milk can play an important role in the diets of children in populations with very low fat intakes and limited access to other animal source foods.

The species of dairy animal, its breed, age and diet, along with the stage of lactation, parity (number of parturitions), farming system, physical environment and season influence the colour, flavour and composition of milk and allow the production of a variety of milk products:

- **Cow milk:** Fat constitutes approximately 3 to 4 percent of the solid content of cow milk, protein about 3.5 percent and lactose 5 percent, but the gross chemical composition of cow milk varies depending on the breed. For example, the fat content is usually higher in *Bos indicus* than *B. taurus* cattle. The fat content of milk from *B. indicus* cattle can be as much as 5.5 per cent.
- **Buffalo milk** has a very high fat content, which is on average twice as high as that of cow milk. The fat-to-protein ratio in buffalo milk is about 2:1. Compared with cattle milk, buffalo milk also has a higher casein-to-protein ratio. The high calcium content of casein facilitates cheese making.

- **Camel milk** has a similar composition to cow milk but is slightly saltie Camel milk can be three times as rich in vitamin C as cow milk an represents a vital source of this vitamin for people living in arid and sem arid areas, who often cannot obtain vitamin C from fruits and vegetable Camel milk is also rich in unsaturated fatty acids and B vitamins. Milk fro Bactrian camels has a higher percentage of fat than milk from dromedarie but levels of proteins and lactose are similar. Generally, camel milk consumed raw or fermented.
- **Sheep milk** has higher fat and protein contents than goat and cow mil only buffalo and yak milk contain more fat. Sheep milk also generally h a higher lactose content than milk from cows, buffaloes and goats. The hig protein and overall solid contents of sheep milk make it particular appropriate for cheese and yoghurt making. Milk from sheep is importa in the Mediterranean region, where most of it is processed into cheeses su as pecorino, caciocavallo and feta.
- **Goat milk** has a similar composition to cow milk. In Mediterranea countries and in Latin America, goat milk is generally transformed in cheese; in Africa and South Asia, it is usually consumed raw or acidifie
- **Yak milk** tastes sweet and has a fragrant, sweetish smell. Yak milk h between 15 and 18 percent solid content, 5.5 to 9 percent fat and 4 5.9 percent protein. It therefore has higher solid, fat and protein conte than cow and goat milk, and resembles buffalo milk. Raw milk is us mainly by herders and their families in milky tea. Yak milk can be process into a variety of milk products including butter, cheese and fermented m products.
- **Equine milk:** Horse and donkey milk have very similar compositio Equine milk, like human milk, is relatively low in proteins (particula caseins) and ashes and rich in lactose. Compared with that of other da species, equine milk contains low levels of fat and protein. Most equine m is consumed fermented and it is not suitable for cheese making.

## COMPONENTS

### Lactose

- is the major **carbohydrate** in milk.
- is present in milk of most mammals.
- is a readily digestible source of glucose (energy) for the neonate.
- is digested by **lactase** enzyme in the neonate.
- is unique to the mammary gland.
- is the **major osmole** of milk (the primary component that draws water i the gland).

Lactose is the **major carbohydrate** in the milk of most species. Lactose is a **disaccharide** composed of the monosaccharides D-glucose and D-galactose, joined in a β-1,4-glycosidic linkage. The chemical name for lactose is 4-0-β-D-galactopyranosyl-D-glucopyranose. It is essentially unique to milk, although it has been identified in the fruit of certain plants. Of the mammalian species where information is available, only some marsupials have an alternative sugar other than lactose, and those sugars are generally trisaccharides of glucose and galactose. Lactose plays a major role in milk synthesis. It is the major **osmole** in milk and the process of synthesis of lactose is responsible for drawing water into the milk as it is being formed in the mammary epithelial cells. Because of the close relationship between lactose synthesis and the amount of water drawn into milk, lactose content is the least variable component of milk.

Lactose is not as sweet as other disaccharides such as sucrose (a glucose-glucose sugar), or the monosaccharides fructose or glucose. Lactose is cleaved to glucose and galactose in the intestine of the neonate by an enzyme activity called **lactase** (or β-galactosidase). The galactose is then converted to another glucose by a different enzyme. Lactose is a major, readily digestible source of glucose which provides energy for the neonate. Lactose intolerance can occur in adult animals or animals who do not have lactase activity in their intestines. Aspects of lactose in milk will be discussed further in the Lactose Synthesis Lesson.

Other carbohydrates are found in milk, but at low concentrations. Low concentrations of free glucose (about 0.1 mM) and free galactose (about 0.2 mM) are found in cow milk and milk of other species. Other carbohydrates found free in milk include amino sugars, sugar phosphates, neutral and acid oligosaccharides, and nucleotide sugars. Some of the complex oligosaccharides are thought to be important in helping establish the microflora of the neonate intestine (such as the bifidis factor identified in human milk; see Human Lactation Lesson). Some milk proteins are glycosylated and some milk lipids contain carbohydrate moieties.

## MINERALS

Calcium and phosphorous are the major minerals found in milk. These minerals are required in large quantities by the rapidly growing neonate for bone growth and development of soft tissues. Calcium and phosphorous mostly are associated with the casein micelle structure. Milk also contains most other minerals found in the body.

Some minerals, such as Zn, Mg, Fe, Cu, Mn, and Mo, are required by enzymes as cofactors.

**Fe** is low in milk of many species relative to the needs of the neonate. Fe is essential for the neonate as part of hemoglobin. In many species the neonate is born with some liver stores of Fe; however, the piglet does not and needs an Fe supplement (injection) soon after birth. Fe in milk is bound to lactoferrin, transferrin, xanthine oxidase, and some to caseins.

**Zn** in cow's milk is mostly bound to casein, but some is bound to lactoferrin.

**Cu** is bound to the caseins, to ß-lactoglobulin, to lactoferrin, and some to th milk fat membranes.

**Mo** is bound to xanthine oxidase, an enzyme associated with the cell membran and on the inner surface of the milk fat globule membranes.

**Mn** is associated with the milk fat membranes.

**Co** is an essential part of vitamin $B_{12}$.

Minerals contribute to the buffering capacity of milk, the maintenance of mil pH, the ionic strength of milk, and milk's osmotic pressure.

Trace elements may enter the milk during milk synthesis or by contaminatio after the milk is removed from the cow; for example from metal containers, etc Cows raised near industrial plants can have higher milk Cd and Pb. Cows raise near highways can have higher milk Pb.

**Table 4.1: Mineral Secretion in Milk**

| Mineral | % Total | % in Soluble Form |
|---|---|---|
| Ca | 0.12 | 25 (rest of Ca is in the casein micelle form) |
| P | 0.1 | 44 (rest of P is in the casein micelle form) |
| Mg | 0.01 | 20 |
| K | 0.15 | 100 |
| Na | 0.05 | 100 |
| Cl | 0.11 | 100 |

The Ca and P are either in the casein micelles and in citrate and phosphat complexes.

**Table 4.2: Average values for milk salt constituents in whole milk**

| Constituent | mg/100ml mil |
|---|---|
| Calcium (Ca) | 123 |
| Phosphorous (P) | 95 |
| Magnesium (Mg) | 12 |
| Potassium (K) | 141 |
| Sodium (Na) | 58 |
| Chlorine (Cl) | 119 |
| Sulfur (S) | 30 |
| Citric acid | 160 |

## TRACE ELEMENTS IN COW MILK

The milk concentration of some elements can be increased by increasing the amount in the diet. These particularly include I, B, Br, Co, Mn, Mo, Se, and Zn. The table below shows examples of the concentrations of various elements in milk when fed a normal diet vs. a diet where that particular element was supplemented.

**Table 4.3: Trace elements in cow milk (micrograms per liter)**

| Cow Receiving Element | Normal Ration | Supplement of Element |
|---|---|---|
| Aluminum | 460 | 810 |
| Arsenic | 50 | 450 |
| Boron | 270 | 660 |
| Bromine | 600 | Increases |
| Bromine (costal areas) | 2800 | - |
| Cadmium | 26 | No increase |
| Chromium | 15 | - |
| Cobalt | 0.6 | 2.4 |
| Copper | 130 | No increase |
| Fluorine | 150 | Increases |
| Iodine | 43 | Up to 2700 |
| Iron | 450 | No increase |
| Lead | 40 | Increases |
| Manganese | 22 | 64 |
| Molybdenum | 73 | 371 |
| Nickel | 27 | No increase |
| Selenium (low Se area) | 40 | Increases |
| Selenium (high Se area) | Up to 1270 | - |
| Silicon | 1430 | No increase |
| Silver | 47 | - |
| Strontium | 171 | - |
| Vanadium | 0.092 | - |
| Zinc | 3900 | 5100 |

## ISOLATION OF CASEIN, LACTOSE, AND ALBUMIN FROM MILK

### Introduction

Milk is a food of exceptional interest. Not only is milk an excellent food for the very young, but humans have also adapted milk, specifically cow's milk, as a food substance for persons of all ages. Many specialized milk products like cheese, yogurt, butter, and ice cream are staples of our diet.

Milk is probably the most nutritionally-complete food that can be found in nature. This property is important for milk, since it is the only food young mammals consume in the nutritionally significant weeks following birth. Whole milk contains vitamins (principally thiamine, riboflavin, pantothenic acid, and vitamins A, D, and K), minerals (calcium, potassium, sodium, phosphorus, and trace metals), proteins (which include all the essential amino acids), carbohydrates (chiefly lactose), and lipids (fats).

The only important elements in which milk is seriously deficient are iron and Vitamin C. Infants are usually born with a storage supply of iron large enough to meet their needs for several weeks. Vitamin C is easily secured through an orange juice supplement. The average composition of the milk of each of several mammals is summarized in the accompanying table.

**Tabl 4.4: Average Percentage Composition of Milk from Various Mammals**

| | Cow | Human | Goat | Sheep | Horse |
|---|---|---|---|---|---|
| Water | 87.1 | 87.4 | 87.0 | 82.6 | 90.6 |
| Protein | 3.4 | 1.4 | 3.3 | 5.5 | 2.0 |
| Fats | 3.9 | 4.0 | 4.2 | 6.5 | 1.1 |
| Carbohydrates | 4.9 | 7.0 | 4.8 | 4.5 | 5.9 |
| Minerals | 0.7 | 0.2 | 0.7 | 0.9 | 0.4 |

## FATS

Whole milk is an oil-water type of emulsion, containing about 4% fat dispersed as very small (5-10 microns in diameter) globules. The globules are so small that a drop of milk contains about a million of them. Because the fat in milk is so finely dispersed, it is digested more easily than fat from any other source. The fat emulsion is stabilized to some extent by complex phospholipids and proteins that are adsorbed on the surfaces of the globules. The fat globules, which are lighter than water, coalesce on standing and eventually rise to the surface of the milk, forming a layer of cream.

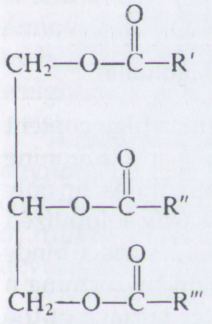
Since vitamins A and D are fat-soluble vitamins, they are carried to the surface with the cream. Commercially, the cream is often removed by centrifugation and skimming and is either diluted to form coffee cream ("half and half"), sold as **whipping cream,** converted to **butter,** or converted to ice cream. The milk that remains is called skimmed **milk.**

Skimmed milk, except for lacking the fats and vitamins A and D, has approximately the same composition as whole milk. If milk is **homogenized,** its fatty content will not separate. Milk is homogenized by forcing it through a small hole. This breaks up the fat globules and reduces their size to about 1 to 2 microns in diameter.

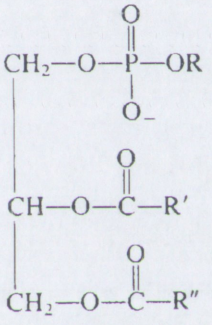
The fats in milk are primarily triglycerides; about two thirds of all the fatty acids in milk are saturated, and about one third are unsaturated. Milk is unusual in that about 12% of the fatty acids are shortchain fatty acids ($C_2$-$C_{10}$) like butyric, caproic, and caprylic acids.

Additional lipids (fats and oils) in milk include small amounts of cholesterol, phospholipids, and lecithins (phospholipids conjugated with choline). The structures of phospholipids and lecithins are shown.

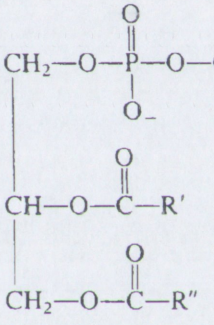
The phospholipids help to stabilize the whole milk emulsion; the phosphate groups help to achieve partial water solubility for the fat globules. All the fat can be removed from milk by extraction with petroleum ether or a similar organic solvent.



**A triglyceride**



**A phospholipid**



**A lecithin**

## PROTEINS

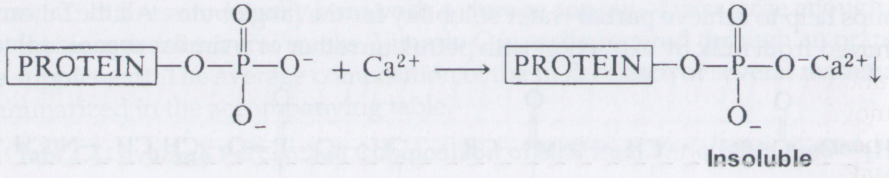
Proteins may be classified broadly in two general categories: fibrous and globular. Globular proteins are those that tend to fold back on themselves into compact units that approach nearly spheroidal shapes. These types of proteins do not form intermolecular interactions between protein units (H bonds and so on) as fibrous proteins do, and they are more easily solubilized as colloidal suspensions. There are three kinds of proteins in milk: caseins, lactalbumins, and lactoglobulins. All are globular.

**Casein** is a phosphoprotein, which has phosphate groups are attached to some of the amino acid sidechains. These are attached mainly to the hydroxyl groups of the serine and threonine moieties. Actually, casein is a mixture of at least three similar proteins, which differ primarily in molecular weight and amount of phosphorus they contain (number of phosphate groups).

**Table 4.5**

| Casein | MW | Phosphate Group/Molecule |
|---|---|---|
| α | 27,300 | ~9 |
| β | 24,100 | ~4-5 |
| κ | ~8, 000 | ~1.5 |

Casein exists in milk as the calcium salt, calcium caseinate. This salt has a complex structure. It is composed of α, β, and κ caseins which form a **micelle,** or a solubilized unit. Neither the α nor the β casein is soluble in milk, singly or in combination. If κ casein is added to either one, or to a combination of the two, however, the result is a casein complex that is soluble owing to the formation of the micelle. A structure proposed for the casein micelle is shown on the following page. The κ casein is thought to stabilize the micelle. Since both α and β casein are phosphoproteins, they are precipitated by calcium ions.




The κ casein protein, however, has fewer phosphate groups and a high content of carbohydrate bound to it. It is also thought to have all its serine and threonine residues (which have hydroxyl groups), as well as its bound carbohydrates, on only one side of its outer surfaces. This portion of its outer surface is easily solubilized in water since these polar groups are present. The other portion of its surface binds well to the water-insoluble α and β caseins and solubilizes them by forming a protective colloid or micelle around them. Since the entire outer surface of the micelle can be solubilized in water, the unit is solubilized as a whole, thus bringing the α and β caseins, as well as κ casein, into solution.

Calcium caseinate has its isoelectric (neutrality) point at pH 4.6. Therefore, it is insoluble in solutions of pH less than 4.6. The pH of milk is about 6.6; therefore casein has a negative charge at this pH and is solubilized as a salt. If acid is added to milk, the negative charges on the outer surface of the micelle are neutralized (the phosphate groups are protonated) and the neutral protein precipitates:

$$Ca^{2+}\text{Caseinate} + 2HCl \rightarrow \text{Casein}\downarrow + CaCl_2$$

The calcium ions remain in solution. When milk sours, lactic acid is produced by bacterial action, and the consequent lowering of the pH causes the same **clotting** reaction. The isolation of casein from milk will be carried out in this experiment.

The casein in milk can also be clotted by the action of an enzyme called **rennin.** Rennin is found in the fourth stomach of young calves. However, both the nature of the clot and the mechanism of clotting differ when rennin is used. The clot formed using **rennin, calcium paracaseinate,** contains calcium.

$$Ca^{2+}\text{Caseinate} + \text{rennin} \rightarrow Ca_2 + \text{Paracaseinate}\downarrow + \text{a small peptide}$$

Rennin is a hydrolytic enzyme (peptidase) and acts specifically to cleave peptide bonds between phenylalanine and methionine residues. It attacks the κ casein, breaking the peptide chain so as to release a small segment of it. This destroys the water-solubilizing surface of the κ casein, which protects the inner α and β caseins and causes the entire micelle to precipitate as calcium paracaseinate.

Milk can be decalcified by treatment with oxalate ion, which forms an insoluble calcium salt. If the calcium ions are removed from milk, a clot will not be formed when the milk is treated with rennin.

The clot, or curd, formed by the action of rennin is sold commercially as cottage cheese. The liquid remaining is called the whey. The curd can also be used in producing various types of cheese. It is washed, pressed to remove any excess whey, and chopped. After this treatment, it is melted, hardened and ground. The ground curd is then salted, pressed into molds, and set aside to age.

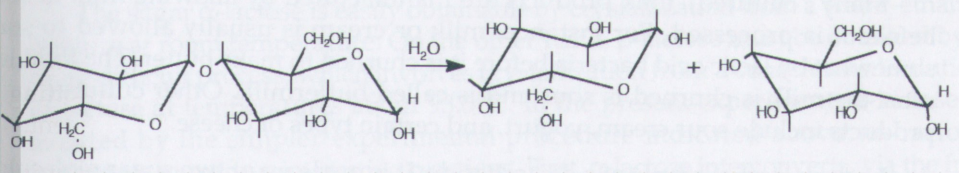
**Albumins** are globular proteins that are soluble in water and in dilute salt solutions. They are, however, denatured and coagulated by heat. The second most abundant protein types in milk are the lactalbumins. Once the caseins have been removed, and the solution has been made acidic, the lactalbumins can be isolated by heating the mixture to precipitate them. The typical albumin has a molecular weight of about 41,000.

A third type of protein in milk is the lactoglobulins. They are present in smaller amounts than the albumins and generally denature and precipitate under the same conditions as the albumins. The lactoglobulins carry the immunological properties of milk. They protect the young mammal until its own immune system has developed.

## CARBOHYDRARES

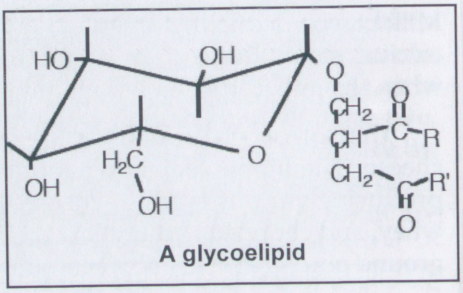
When the fats and the proteins have been removed from milk, the carbohydrates remain, as they are soluble in aqueous solution. The main carbohydrate in milk is **lactose**.

Lactose, a disaccharide, is the only carbohydrate that mammals synthesize. It is synthesized in the mammary glands. Hydrolyzed, it yields one molecule of D-glucose and one of D-galactose.




α-Lactose: D-Galactose + D-Glucose D-Galactose D-Glucose

In this process, one molecule of glucose is converted to galactose and joined to another of glucose. The galactose is apparently needed by the developing infant to build brain and nervous tissue. Brain cells contain glycolipids as a part of their structure. A glycolipid is a triglyceride in which one of the fatty acid groups has been replaced by a sugar, in this case galactose. Galactose is more stable (to metabolic oxidation) than glucose and affords a better material for forming structural units in cells.



A glycoelipid

Although almost all human infants can digest lactose, some adults lose this ability on reaching maturity, since milk is no longer an important part of their diet. An enzyme called lactase is necessary to digest lactose. Lactase is secreted by the cells of the small intestine, and it cleaves lactose into its two component sugars, which are easily digested. Persons lacking the enzyme lactase do not digest lactose properly. As it is poorly absorbed by the small intestine, it remains in the digestive tract, where its osmotic potential causes an influx of water. This results in cramps and diarrhea for the affected individual. Persons with a lactase deficiency cannot tolerate more than one glass of milk a day. The deficiency is most common among African-Americans and older Caucasians.

When milk is allowed to stand at room temperature, it sours. Many bacteria are present in milk, particularly lactobacilli. These bacteria act on the lactose in milk to produce the sour lactic acid. These microorganisms actually hydrolyze lactose and produce lactic acid only from the galactose portion of the lactose. Since the production of the lactic acid also lowers the pH of the milk, the milk clots when it sours:

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

lactose — galactose glucose

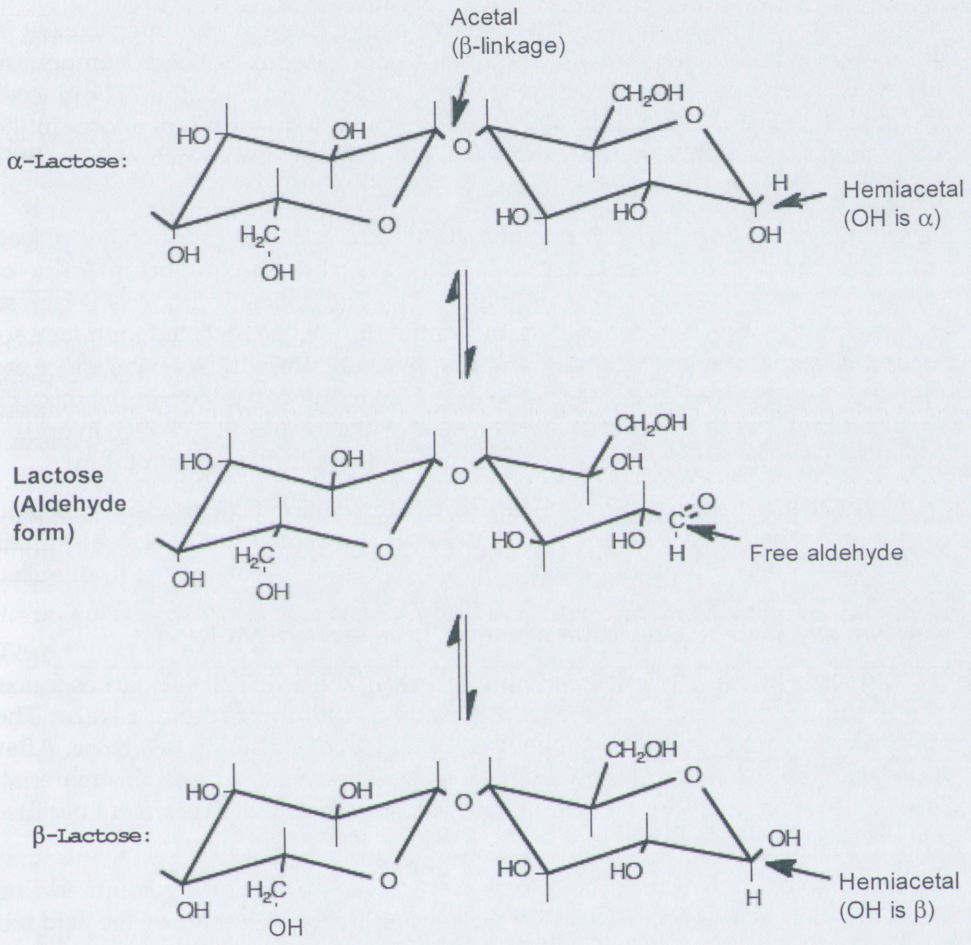
$$C_6H_{12}O_6 \xrightarrow{\text{lactobacilli}} \text{CH(OH)(CH}_3\text{)CO}_2\text{H}$$

galactose — lactic acid

Many "cultured" milk products are manufactured by allowing milk to sour before it is processed. For instance, milk or cream is usually allowed to sour somewhat by lactic acid bacteria before it is churned to make butter. The fluid left after the milk is churned is sour and is called buttermilk. Other cultured milk products include sour cream, yogurt, and certain types of cheese.

Lactose is an example of a disaccharide. It is made up of two sugar molecules: galactose and glucose. In the following structures, the galactose portion is on the left and glucose is on the right. Galactose is bonded through an acetal linkage to glucose.

Notice that the glucose portion can exist in one of two isomeric hemiacetal structures: α-lactose and β-lactose. Glucose can also exist in a free aldehyde form. This aldehyde form (open form) is an intermediate in the equilibration (interconversion) of α- and β-lactose. Very little of this free aldehyde form exists in the equilibrium mixture. The isomeric α- and β-lactose are diastereomers because they differ in the configuration at one carbon atom, called the anomeric carbon atom.




The sugar α-lactose is easily obtainable by crystallization from a water-ethanol mixture at room temperature. On the other hand, β-lactose must be obtained by a more difficult process, which involves crystallization from a concentrated solution of lactose at temperatures about 93.5°C. In the present experiment, α-lactose is isolated by the simpler experimental procedure indicated above. α-Lactose undergoes numerous interesting reactions. First, α-lactose interconverts, via the free aldehyde form, to a large extent, to the β-isomer in aqueous solution. This causes a change in the rotation of polarized light from +92.60° to +52.30° with increasing time. The process that causes the change in optical rotation with time is called **mutarotation.**

## POLARIMETRY

In this experiment, you will study the mutarotation of lactose by polarimetry. The disaccharide α- lactose, made up of galactose and glucose, can be isolated from

milk. As you can see in the structures shown above, the glucose unit can exist in one of two isomeric hemiacetal structures, α- and β-lactose. These isomers are diastereomers because they differ in configuration at one carbon atom. The glucose part can also exist in a free aldehyde form. This aldehyde form (open form in the equation above) is an intermediate in the equilibration of α- and β-lactose. Very little of this free aldehyde form exists in the equilibrium mixture.

α-Lactose has a specific rotation at 20°C of +92.60. However, when it is placed in water, the optical rotation decreases until it reaches an equilibrium value of +52.30. β-Lactose has a specific rotation of +34°. The optical rotation of β-lactose *increases* in water until it reaches the same equilibrium value obtained for α-lactose. At the equilibrium point, both the a and β isomers are present. However, since the equilibrium rotation is closer in value to the initial rotation of β-lactose, the mixture must contain more of this isomer. The process, which results in a change in optical rotation over time to approach an equilibrium value, is called **mutarotation**.

Lactose can be removed from whey by adding ethanol. Lactose is insoluble in ethanol, and when the ethanol is mixed with the aqueous solution, the lactose is forced to crystallize. This is the process used in this experiment to isolate lactose from milk.

### Isolation of a Protein, Casein, and a Sugar, Lactose, from Milk

In this experiment, you will separate several of the chemical substances found in milk. First, you will isolate a phosphorus-containing protein, **casein**. The remaining milk mixture will then be used as a source of a sugar, **α-lactose**. After you isolate the milk sugar, you will make several chemical tests on this material. Fats, which are present in whole milk, are not isolated in this experiment because powdered nonfat milk is used.

First, the casein is precipitated by warming the powdered milk and adding dilute acetic acid. It is important that the heating not be excessive or the acid too strong, because these conditions also hydrolyze lactose into its components, glucose and galactose. After the casein has been removed, the excess acetic acid is neutralized with calcium carbonate, and the solution is heated to its boiling point to precipitate the initially soluble protein, albumin. The liquid containing the lactose is poured away from the albumin. Alcohol is added to the solution, and any remaining protein is removed by centrifugation. α-lactose crystallizes on cooling.

### Cautions

The isolation of lactose requires the use of a bench-top centrifuge. Be sure to balance the centrifuge with two tubes of equal weight when operating, or damage can occur to the centrifuge and the centrifuge tube may shatter.

### Isolation and Purification of Casein

Make a dilute solution (approx. 10%) of acetic acid by adding 1 mL glacial (100%) acetic acid to 10 ml distilled water in 10-mL-Erlenmeyer flask. Mix thoroughly and set aside.

Place 4.0 g of powdered nonfat milk and 10 mL of water into a 50- or 100-mL beaker. Heat on a sand bath to about 40°C ( top of sand bath at about 50°C). Monitor the temperature of the milk solution with a thermometer.

When the mixture has reached 40°C, add the dilute acetic acid dropwise to the warm milk. *Do not add all of the dilute acetic acid at one time!* Maintain the solution at about 40°C and **after every 5 drops**, stir the solution gently using a small spatula. Using the spatula, push the precipitated casein onto the side of the beaker so that most of the liquid drains from the solid (you will have to hold it on the side of the beaker to drain the liquid).

Then transfer the congealed casein to a 20-mL vial in small portions. The casein will stick together and be hard to transfer if you use large pieces. If any liquid separated from the casein in the vial, use a Pasteur pipet to transfer the liquid back into the beaker. Slowly continue the dropwise addition of the 1 mL of dilute acetic acid solution to the beaker to complete the casein precipitation. Remove as much casein as possible from the beaker and transfer it to the vial. Avoid adding an excess of acetic acid to the milk solution, as this will cause the lactose in the milk to hydrolyze to glucose and galactose.

When most of the casein has been removed from the milk solution, add 0.2 g of calcium carbonate to the milk in the beaker. Stir this mixture for a few minutes and save it for use in the isolation of lactose below. (The mixture should be used as soon as possible during the current lab period.)

Transfer the casein from the 20 mL vial to a Hirsch suction filter funnel. Draw a vacuum on the casein for about 5 min to remove as much liquid as possible, pressing the casein with a spatula during this time. (The liquid contains the albumins and lactose—so a great loss of liquid at this point will result in decreased yields of these other two components.)

Transfer the casein to a 7- to 10-cm piece of filter paper, fold this over onto the casein, and press gently to absorb any remaining liquid. Place the solid on a ***tared*** watch glass, let air dry until the next lab period, and weigh. Casein is used to make white glue, so it is important that you don't leave it on the filter paper or it will become glued to it! Calculate the weight percent of casein isolated from the powdered milk.

## ISOLATION OF THE SUGAR, LACTOSE, AND ALBUMIN PROTEINS FROM MILK

After the isolation of casein, the milk mixture contains the sugar (lactose) and the protein (albumin).

Heat the milk mixture to about 75°C for about 5 min. on a sand bath. Heating results in a nearly complete denaturization and precipitation of the albumins from the solution.

Decant the liquid in the beaker away from the solid into a clean plastic 10-mL centrifuge tube (borrowed from the rack next to the Beckman centrifuge located at

the end of lab 215). You may need to hold the solid with a spatula when transferring the liquid. Press the solid albumins with a spatula to remove as much liquid as possible and pour the liquid into the centrifuge tube. Save the albumins in the original beaker. You should now have about 7 mL of liquid. If you have less than 2 mL, you need to add an additional 1 mL to the albumin residue, heat to about 75°C, and decant, combining the two liquid portions. This procedure will minimize the loss of lactose that may have crystallized along with the albumin precipitate.

When the liquid has cooled to about room temperature, place it in the centrifuge. Make sure the centrifuge is balanced by placing another plastic centrifuge tube filled with water to the same level as your lactose tube, at a position 180° opposite the lactose tube. Centrifuge for 5 min according to the instructions posted on the wall above the centrifuge.

Following centrifugation, decant the liquid away from the solid into a 50- or 100-mL beaker. Add 15 mL of 95% ethanol to the beaker. Solids will precipitate. Heat this solution on a sand bath to about 60°C to dissolve some of the solid. Pour equal amounts of the hot solution into two 10-mL plastic centrifuge tubes and centrifuge this solution as soon as possible before it cools appreciably. Centrifuge for 2 min. It is important to centrifuge the solution while it is still warm to prevent premature crystallization of the lactose. A considerable quantity of solid material is deposited on the bottom of the centrifuge tubes.

Remove the warm, supernatant liquid from the tube using a Pasteur pipet, and transfer the liquid to a 25- or 50-mL Erlenmeyer flask. (You can discard the solid remaining in the centrifuge tube.) Stopper the flask and allow the lactose to crystallize for at least two days. Granular crystals will form during this time.

Collect the lactose by vacuum filtration on a Hirsch funnel. Use about 3 mL of 95% ethanol to aid the transfer and to wash the product. α-Lactose crystallizes with one molecule of water of hydration per molecule of lactose and therefore its formula is $C_{12}H_{22}O_{11}.H_2O$. Weigh the product after it is thoroughly dry. Calculate the weight percent of the α-Lactose isolated from the powdered milk. Measure the optical activity of α -lactose by weighing and dissolving all of your sample in 2 mL of distilled water and analyzing the solution by polarimetry. Try to obtain a reading as soon as the polarimeter readings stabilize to the nearest tenth of a degree (+0.1°C). Over a period of time, one can observe a gradual reduction in the specific rotation.

**Important**: If the polarimeter continues to scan in the positive direction, reverses, and scans to the negative values, and back again, then your sample is probably too cloudy. (Sometimes particles of albumin will precipitate while the lactose crystals are growing.) If this is the case, you can remove the sample solution from the cell and rinse the cell with methanol. Centrifuge the solution and return it to the cell. If centrifuging does not remove the suspended particles (that is, the suspended particles are really fine), fold a piece of filter paper in a cone shape and gravity filter the solution, and return it to the cell.

Allow the albumins to dry for 2-3 days in the original beaker in which they were precipitated. Break up the solid and weigh it. Calculate the weight percent of albumins isolated from the powdered milk.

### Cleaning Up

Clean the plastic centrifuge tubes with soap and water, rinse with distilled water, NOT ACETONE, and place upside down in the rack by the Beckman centrifuge. All the filtrates from the above isolations are easily biodegraded and can therefore be flushed down the drain.

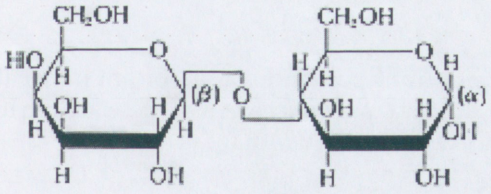
### Final Report

Use the data for cow's milk given in the table titled *Average Percentage Composition of Milk from Various Mammals* to discuss your percent yields from dry fat-free milk and how these compare to what is found in whole milk or might be calculated for whole milk using your experimental data in conjunction with the table data.

## LACTOSE

- is the major carbohydrate in milk.
- is present in milk of most mammals.
- is a readily digestible source of glucose (energy) for the neonate.
- is digested by lactase enzyme in the neonate.
- is unique to the mammary gland.
- is the major osmole of milk (the primary component that draws water into the gland).

Lactose is a disaccharide composed of D-galactose and D-glucose.




Lactose is the major carbohydrate in the milk of most species. Lactose is a disaccharide composed of the monosaccharides D-glucose and D-galactose, joined in a β-1,4-glycosidic linkage. The chemical name for lactose is 4-0-β-D-galactopyranosyl-D-glucopyranose. It is essentially unique to milk, although it has been identified in the fruit of certain plants. Of the mammalian species where information is available, only some marsupials have an alternative sugar other than lactose, and those sugars are generally trisaccharides of glucose and galactose.

Lactose plays a major role in milk synthesis. It is the major osmole in milk and the process of synthesis of lactose is responsible for drawing water into the milk as it is being formed in the mammary epithelial cells. Because of the close relationship between lactose synthesis and the amount of water drawn into milk, lactose content is the least variable component of milk.

Lactose is not as sweet as other disaccharides such as sucrose (a glucose-glucose sugar), or the monosaccharides fructose or glucose. Lactose is cleaved to glucose and galactose in the intestine of the neonate by an enzyme activity called lactase (or β-galactosidase). The galactose is then converted to another glucose by a different enzyme. Lactose is a major, readily digestible source of glucose which provides energy for the neonate. Lactose intolerance can occur in adult animals or animals who do not have lactase activity in their intestines.

## Nutritional Benefits of Lactose

### *Promotes Intestinal Health*

Lactose has been shown to promote the growth of beneficial intestinal bacteria, such as Bifidobacterium bifidum and Lactobacilli, both lactic acid bacteria. At the same time, lactose inhibits the growth of some types of pathogenic bacteria and endotoxins. The intake of lactose by infants and children may increase their resistance to intestinal infections and contribute to the maintenance of a healthy intestinal flora.

### *Provides a Source of Energy*

Lactose is natural, milk derived carbohydrate, a nutrient required by the body for energy. It has only about 25% of the sweetness of sucrose and is often used as an energy source in foods for infants and children where too much sweetness is undesirable. Lactose has a low glycemic index and causes a slow, modest rise in blood sugar. Diets containing low glycemic foods are known to have health benefits during aging.

### *Aids Mineral Absorption*

Lactose helps stimulate the absorption and retention of important minerals in infants, such as calcium, magnesium, zinc and manganese. All of these are required for normal growth and development and for good health.

## Chemical and Biochemical Properties

Lactose is very stable and inert from a chemical point of view. It has, apart from some special cases, no significant tendency to react with the drug or other components of a formulation.Some remarks on the chemical properties of lactose are useful however.The low hygroscopicity of crystalline lactose supports its virtual chemical inertness. Most chemical reactions of lactose occur noticeably only in aqueous environment. Because lactose has a very low tendency to attract moisture, water in dry lactose preparations is normally not present in amounts sufficient for

chemical reactions to proceed at a noticeable speed. The water of crystallisation is bound so tightly in the crystal lattice of the lactose that it is chemically inert.

### *Dehydration and Pyrolysis*

At temperatures above 100°C, α-lactose monohydrate gradually loses its water of crystallisation. At 140°C, the loss of crystal water is completed. The loss of water of crystallisation is accompanied by a change of the crystalline structure of the lactose. The lactose becomes anhydrous. Further heating of lactose to higher temperatures causes the lactose to decompose. This process is called pyrolysis. The primary reaction products of pyrolysis tend to polymerize, resulting in brown and black coloured macromolecules. Eventually the lactose becomes black on heating.

### *Maillard Reaction*

In aqueous solution, lactose, as all reducing sugars (e.g. glucose, galactose, maltose, maltodextrines), tends to react with compounds possessing primary- and secondary amino-groups, such as proteins and peptides. This reaction is called the Maillard reaction. The chemical pathways of the Maillard reaction are very complicated and will not be dealt with further. High temperature and alkaline pH promote the reaction. In an advanced stage, yellow and brown polymers are formed, which ultimately turn the reaction mixture black. Purified lactose, even of pharmaceutical grade, still contains traces of proteinaceous matter. These are the reason why the Maillard reaction cannot be completely excuded, even from some dry lactose preparations. However, the Maillard reaction requires some free water, although very little may suffice. Here again the low hygroscopicity of lactose proves favourable. α-Lactose monohydrate and roller dried lactose have a very low tendency to adsorb moisture. Furthermore, they have a moisture content which is sufficiently low to prevent the Maillard reaction to occur noticeably. Even over periods of years significant changes in colour are normally not observed. Spray dried lactose, being somewhat hygroscopic, may have some tendency to become slightly coloured at long storage times when moisture adsorption occurs. In this respect dry storage in an adequate packing is important. Formulating lactose with drugs of a protein or peptide character, in general, should pose no problems of drug stability, provided that free moisture content of the formulation can be kept very low. Examples on the market are Levothyroxin and Alendronic acid.

### *Fermentation*

Lactose is a carbohydrate and as such an easy substrate for microorgamisms, like bacteria, yeasts and moulds. Controlled use of this is made in several industrial fermentation processes where lactose is used as the carbon source.Uncontrolled fermentation of lactose, i.e. microbial spoilage of the lactose product, is of course highly undesirable.Here again the low hygroscopicity of lactose adds to its microbial stability as well. Microorganisms require a certain water activity ($A_w$) to grow. Storage of lactose under dry and cool conditions will prevent microbial growth to occur.

Lactose is more soluble in molar solutions of calcium chloride or of calcium nitrate than in pure water. Increase in the concentration of salt brings further increase in the solubility of lactose. This phenomenon is believed to be related to the anomalous rotations of lactose in salt solutions.

The increased solubility of lactose in calcium chloride solutions is due to the formation of a molecular compound of alpha lactose and calcium chloride in solution. A study of the solubility of lactose in calcium chloride solutions indicated the existence of only one compound at 25°C. This compound was isolated. It has the composition alpha lactose $\cdot CaC_{12} \cdot 7H_2O$.

Hydrated lactose is exceedingly soluble in concentrated solutions of calcium chloride, (or calcium bromide), but these solutions are supersaturated with respect to the compound just mentioned. Such solutions are often exceedingly stable, and may resist all attempts to induce crystallization.

Faith in the reliability of polariscopic data as evidence of the formation of lactose compounds in salt solutions has been strengthened by the fact that lactose, in the solutions tested, exhibited an increased solubility, and furthermore, by the fact that one such compound has been isolated. Such data indicate that many salts may combine with lactose. The existence of such molecular compounds is possibly a factor contributing to the stability of such supersaturated solutions of lactose as are found in ice cream and in milk powders.

## LIPIDS

### Chemical Properties

The fat content of milk is of economic importance because milk is sold on the basis of fat. Milk fatty acids originate either from microbial activity in the rumen, and transported to the secretory cells via the blood and lymph, or from synthesis in the secretory cells. The main milk lipids are a class called triglycerides which are comprised of a glycerol backbone binding up to three different fatty acids. The fatty acids are composed of a hydrocarbon chain and a carboxyl group. The major fatty acids found in milk are:

*Long chain*

- C14 - myristic 11%
- C16 - palmitic 26%
- C18 - stearic 10%
- C18:1 - oleic 20%

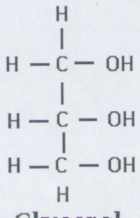
*Short chain (11%)*

- C4 - butyric*
- C6 - caproic

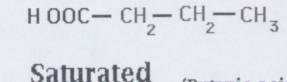
- C8 - caprylic
- C10 - capric

* butyric fatty acid is specific for milk fat of ruminant animals and is resposible for the rancid flavour when it is cleaved from glycerol by lipase action.

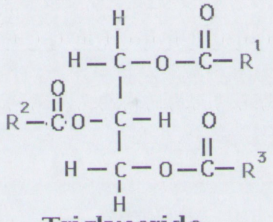
Saturated fatty acids (no double bonds), such as myristic, palmitic, and stearic make up two thirds of milk fatty acids. Oleic acid is the most abundant unsaturated fatty acid in milk with one double bond. While the cis form of geometric isomer is the most common found in nature, approximately 5% of all unsaturated bonds are in the trans position as a result of rumen hydrogenation.



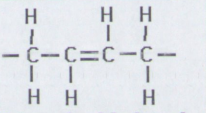
**Glycerol**



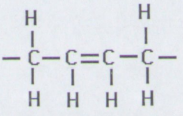
**Saturated Fatty Acid** (Butyric acid)



**Triglyceride**



Trans double bond



Cis double bond

**Unsaturated**

Triglycerides account for 98.3% of milkfat. The distribution of fatty acids on the triglyceride chain, while there are hundreds of different combinations, is not random. The fatty acid pattern is important when determining the physical properties of the lipids. In general, the SN1 position binds mostly longer carbon length fatty acids, and the SN3 position binds mostly shorter carbon length and unsaturated fatty acids. For example:

- C4 - 97% in $SN_3$
- C6 - 84% in $SN_3$
- C18 - 58% in $SN_1$

The small amounts of mono- , diglycerides, and free fatty acids in fresh milk may be a product of early lipolysis or simply incomplete synthesis. Other classes of lipids include phospholipids (0.8%) which are mainly associated with the fat globule membrane, and cholesterol (0.3%) which is mostly located in the fat globule core.

**Physical Properties**

- The physical properties of milkfat can be summerized as follows:
- density at 20° C is 915 kg m(-3)*
- refractive index (589 nm) is 1.462 which decreases with increasing temperature

- solubility of water in fat is 0.14% (w/w) at 20° C and increases with increasing temperature
- thermal conductivity is about 0.17 J m(-1) s(-1) K(-1) at 20° C
- specific heat at 40° C is about 2.1kJ kg(-1) K(-1)
- electrical conductivity is <10(-12) ohm(-1) cm(-1)
- dielectric constant is about 3.1

*the brackets around numbers denote superscript

At room temperature, the lipids are solid, therefore, are correctly referred to as "fat" as opposed to "oil" which is liquid at room temperature. The melting points of individual triglycerides ranges from -75° C for tributyric glycerol to 72° C for tristearin. However, the final melting point of milkfat is at 37° C because higher melting triglycerides dissolve in the liquid fat. This temperature is significant because 37° C is the body temperature of the cow and the milk would need to be liquid at this temperature. The melting curves of milkfat are complicated by the diverse lipid composition:

trans unsaturation increases melting points

odd-numbered and branched chains decrease melting points

Crystallization of milkfat largely determines the physical stability of the fat globule and the consistency of high-fat dairy products, but crystal behaviour is also complicated by the wide range of different triglycerides. There are four forms that milkfat crystals can occur in; alpha, β , β'1, and β'2, however, the alpha form is the least stable and is rarely observed in slowly cooled fat.

## MILK PROTEINS

The primary structure of proteins consists of a polypeptide chain of amino acids residues joined together by peptide linkages, which may also be cross-linked by disulphide bridges. Amino acids contain both a weakly basic amino group, and a weakly acid carboxyl group both connected to a hydrocarbon chain, which is unique to different amino acids. The three-dimensional organization of proteins, orconformation, also involves secondary, tertiary, and quaternary structures. The secondary structure refers to the spatial arrangement of amino acid residues that are near one another in the linear sequence. The alpha-helix and β-pleated sheat are examples of secondary structures arising from regular and periodic steric relationships. The tertiary structure refers to the spatial arrangement of amino acid residues that are far apart in the linear sequence, giving rise to further coiling and folding. If the protein is tightly coiled and folded into a somewhat spherical shape, it is called a globular protein. If the protein consists of long polypeptide chains that are intermolecularly linked, they are called fibrous proteins. Quaternary structure occurs when proteins with two or more polypeptide chain subunits are associated.

### Milk Protein Fractionation

The nitrogen content of milk is distributed among caseins (75%), whey proteins (18%), miscellaneous proteins (2%) and non-protein nitrogen (5%). This nitrogen distribution can be determined by the Rowland fractionation method:

1. Precipitation at pH 4.6 - separates caseins from whey nitrogen
2. Precipitation with sodium acetate and acetic acid (pH 5.0) - separates total proteins from whey NPN

Ninety-five percent of the nitrogen is associated with protein. The average concentration of proteins in milk is as follows (Table 4.6) (although there can be considerable natural variation):

**Table 4.6**

| | grams/ litre | % of total protein |
|---|---|---|
| Total Protein | 33.0 | 100 |
| Total Caseins | 26.0 | 78.8 |
| $\alpha_{s1}$-casein | 10.7 | 32.4 |
| $\alpha_{s2}$-casein | 2.8 | 8.5 |
| beta-casein | 8.6 | 26.1 |
| kappa-casein | 3.1 | 9.4 |
| gamma-casein | 0.8 | 2.4 |
| Total Whey Proteins | 6.4 | 19.4 |
| alpha lactalbumin | 1.2 | 3.6 |
| beta lactoglobulin | 3.2 | 9.8 |
| BSA | 0.4 | 1.2 |
| Immunoglobulins | 0.8 | 2.4 |
| Proteose peptone | 0.8 | 2.4 |
| Miscellaneous | 0.6 | 1.8 |

Caseins, as well as their structural form - casein micelles, whey proteins, and milk enzymes will now be examined in further detail.

## MILK LIPIDS - FUNCTIONAL PROPERTIES

Like all fats, milkfat provides lubrication. They impart a creamy mouth feel as opposed to a dry texture. Butter flavour is unique and is derived from low levels of short chain fatty acids. If too many short chain fatty acids are hydrolyzed (separated) from the triglycerides, however, the product will taste rancid. Butter fat also acts as a reservoir for other flavours, especially in aged cheese. Fat globules produce a 'shortening' effect in cheese by keeping the protein matrix extended to give a soft texture. Fat substitutes are designed to mimic the globular property of milk fat. The spreadable range of butter fat is 16-24° C. Unfortunately butter is not

spreadable at refrigeration temperatures. Milk fat provides energy (1g = 9 cal.), and nutrients (essential fatty acids, fat soluble vitamins).

## CASEINS

The casein content of milk represents about 80% of milk proteins. The principal casein fractions are alpha(s1) and alpha(s2)-caseins, ß -casein, and kappa-casein. The distinguishing property of all caseins is their low solubility at pH 4.6. The common compositional factor is that caseins are conjugated proteins, most with phosphate group(s) esterified to serine residues. These phosphate groups are important to the structure of the casein micelle. Calcium binding by the individual caseins is proportional to the phosphate content.

The conformation of caseins is much like that of denatured globular proteins. The high number of proline residues in caseins causes particular bending of the protein chain and inhibits the formation of close-packed, ordered secondary structures. Caseins contain no disulfide bonds. As well, the lack of tertiary structure accounts for the stability of caseins against heat denaturation because there is very little structure to unfold. Without a tertiary structure there is considerable exposure of hydrophobic residues. This results in strong association reactions of the caseins and renders them insoluble in water.

Within the group of caseins, there are several distinguishing features based on their charge distribution and sensitivity to calcium precipitation:

**alpha(s1)-casein: (molecular weight 23,000; 199 residues, 17 proline residues)**

Two hydrophobic regions, containing all the proline residues, separated by a polar region, which contains all but one of eight phosphate groups. It can be precipitated at very low levels of calcium.

**alpha(s2)-casein: (molecular weight 25,000; 207 residues, 10 prolines)**

Concentrated negative charges near N-terminus and positive charges near C-terminus. It can also be precipitated at very low levels of calcium.

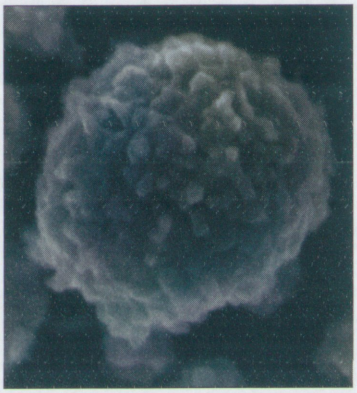
**ß -casein: (molecular weight 24,000; 209 residues, 35 prolines)**

Highly charged N-terminal region and a hydrophobic C-terminal region. Very amphiphilic protein acts like a detergent molecule. Self association is temperature dependant; will form a large polymer at 20° C but not at 4° C. Less sensitive to calcium precipitation.

**kappa-casein: (molecular weight 19,000; 169 residues, 20 prolines)**

Very resistant to calcium precipitation, stabilizing other caseins. Rennet cleavage at the Phe105-Met106 bond eliminates the stabilizing ability, leaving a hydrophobic portion, para-kappa-casein, and a hydrophilic portion called kappa-casein glycomacropeptide (GMP), or more accurately, caseinomacropeptide (CMP).

Most, but not all, of the casein proteins exist in a colloidal particle known as the casein micelle. Its biological function is to carry large amounts of highly insoluble CaP to mammalian young in liquid form and to form a clot in the stomach for more efficient nutrition. Besides casein protein, calcium and phosphate, the micelle also contains citrate, minor ions, lipase and plasmin enzymes, and entrapped milk serum. These micelles are rather porous structures, occupying about 4 mL/g and 6-12% of the total volume fraction of milk. Size ranges from 50-250 nm in diameter.



There have been many models developed over the years to explain the structure of the casein micelle, based on all of the information available about its composition and reactivity. The casein sub-micelle model was prominent for many years, but there is sufficient evidence now to conclude that there is not a defined sub-micellar structure to the micelle at all. More recent models suggest a more open structure comprised of aggregates of protein around calcium phosphate nanoclusters. Each of the casein proteins has unique abilitites to either bind with CaP or with other caseins, which gie rise to the aggregates. The nanoclusters provide regions of more or less density. The structure is sufficiently porous to hold a considrable amount of water, and for the surface, and even part of the interior, to be reactive to other substances. All models agree that the k-casein is mostly present as a stabilizng layer around the exterior of the micelle. Please see any of the following references for great detail about micelle structures and models.

**Casein Micelle Stability**

Colloidal calcium phosphate (CCP) acts as a cement between the hundreds or even thousands of submicelles that form the casein micelle. Binding may be covalent or electrostatic. Submicelles rich in kappa-casein occupy a surface position, whereas those with less are buried in the interior. The resulting hairy layer, at least 7 nm thick, acts to prohibit further aggregation of submicelles by steric repulsion. The casein micelles are not static; there are three dynamic equilibria between the micelle and its surroundings:

- the free casein molecules and submicelles
- the free submicelles and micelles
- the dissoved colloidal calcium and phosphate

The following factors must be considered when assessing the stability of the casein micelle:

*Role of $Ca^{++}$*

More than 90% of the calcium content of skim milk is associated in some way or another with the casein micelle. The removal of $Ca^{++}$ leads to reversible dissociation of β-casein without micellular disintegration. The addition of $Ca^{++}$ leads to aggregation.

*H Bonding*

Some occurs between the individual caseins in the micelle but not much because there is no secondary structure in casein proteins.

*Disulphide Bonds*

alpha(s1) and β-caseins do not have any cysteine residues. If any S-S bonds occur within the micelle, they are not the driving force for stabilization.

*Hydrophobic Interactions*

Caseins are among the most hydrophobic proteins and there is some evidence to suggest they play a role in the stability of the micelle. It must be remembered that hydrophobic interactions are very temperature sensitive.

*Electrostatic Interactions*

Some of the subunit interactions may be the result of ionic bonding, but the overall micellar structure is very loose and open.

*van der Waals Forces*

No sucess in relating these forces to micellular stability.

*Steric stabilization*

As already noted, the hairy layer interferes with interparticle approach.

There are several factors that will affect the stability of the casein micelle system:

*Salt content*

Affects the calcium activity in the serum and calcium phosphate content of the micelles.

*pH*

Lowering the pH leads to dissolution of calcium phosphate until, at the isoelectric point (pH 4.6), all phosphate is dissolved and the caseins precipitate.

*Temperature*

At 4° C, beta-casein begins to dissociate from the micelle, at 0° C, there is no micellar aggregation; freezing produces a precipitate called cryo-casein.

*Heat Treatment*

Whey proteins become adsorbed, altering the behaviour of the micelle.

*Dehydration*

By ethanol, for example, leads to aggregation of the micelles.

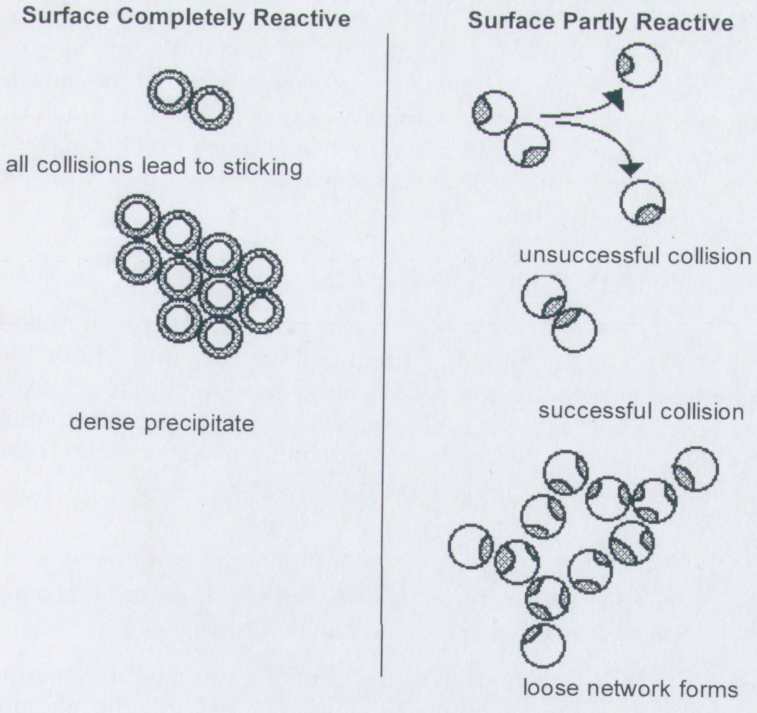
When two or more of these factors are applied together, the effect can also be additive.

**Casein micelle aggregation**

Caseins are able to aggregate if the surface of the micelle is reactive. The Schmidt model further illustrates this.

Although the casein micelle is fairly stable, there are four major ways in which aggregation can be induced:

- chymosin - rennet or other proteolytic enzymes as in Cheese manufacturing
- acid
- heat
- age gelation.




**The Schmidt Model**

## ENZYME COAGULATION

Chymosin, or rennet, is most often used for enzyme coagulation. During the primary stage, rennet cleaves the Phe(105)-Met(106) linkage of kappa-casein resulting in the formation of the soluble CMP which diffuses away from the micelle and para-kappa-casein, a distinctly hydrophobic peptide that remains on the micelle. The patch or reactive site, as illustrated in the above image, that is left on the micelles after enzymatic cleavage is necessary before aggregation of the paracasein micelles can begin.

During the secondary stage, the micelles aggregate. This is due to the loss of steric repulsion of the kappa-casein as well as the loss of electrostatic repulsion due to the decrease in pH. As the pH approaches its isoelectric point (pH 4.6), the caseins aggregate. The casein micelles also have a strong tendency to aggregate because of hydrophobic interactions. Calcium assists coagulation by creating isoelctric conditions and by acting as a bridge between micelles. The temperature at the time of coagulation is very important to both the primary and secondary stages. With an increase in temperature up to 40° C, the rate of the rennet reaction increases. During the secondary stage, increased temperatures increase the hydrophobic reaction. The tertiary stage of coagulation involves the rearrangement of micelles after a gel has formed. There is a loss of paracasein identity as the milk curd firms and syneresis begins.

## ACID COAGULATION

Acidification causes the casein micelles to destabilize or aggregate by decreasing their electric charge to that of the isoelectric point. At the same time, the acidity of the medium increases the solubility of minerals so that organic calcium and phosphorus contained in the micelle gradually become soluble in the aqueous phase. Casein micelles disintegrate and casein precipitates. Aggregation occurs as a result of entropically driven hydrophobic interactions.

## NUTRITIONAL COMPONENTS IN MILK

This page describes the function of nutritional components in milk: Energy, Water, Carbohydrate, Fat, Protein, Vitamins, Minerals, and Minor Biological Proteins & Enzymes. Links are provided to move the reader to pages that present the content of specific nutrients in milk, important background information on the chemistry of milk carbohydrate (lactose), fat, protein, and enzymes, and other topics that are covered in more depth in other sections of this website.

### Energy

The energy in milk comes from its protein, carbohydrate and fat content, with the exception of skim milk that has virtually no fat (Tables 4.7).

Food provides energy to the body in the form of calories (kcal). There are many components in food that provide nutritional benefits, but only the macronutrients

protein, carbohydrate and fat provide energy. The energy value of a food is calculated based on the calories provided by the amount of protein (4 kcal/gram), carbohydrate (4 kcal/gram), and fat (9 kcal/gram) that is present.

**Water**

Milk is approximately 87% water, so it is a good source of water in the diet (Tables 4.7).

Water does not provide a nutritional benefit in the same manner as proteins or vitamins, for example. However, water is extremely important in human metabolism. Water is a major component in the body. Water maintains blood volume, transports nutrients like glucose and oxygen to the tissues and organs, and transports waste products away from tissues and organs for elimination by the body. Water helps to lubricate joints and cushions organs during movement. Water maintains body temperature regulation through sweating. Lack of water (dehydration) results in fatigue, mental impairment, cramping, and decreased athletic performance. Severe dehydration can be life-threatening.

**Carbohydrate**

Milk is approximately 4.9% carbohydrate in the form of lactose (Tables 4.7).

Carbohydrates are the primary source of energy for activity. Glucose is the only form of energy that can be used by the brain. Excess glucose is stored in the form of glycogen in the muscles and liver for later use. Carbohydrates are important in hormonal regulation in the body. Lack of adequate levels of glucose in the blood and carbohydrate stores leads to muscle fatigue and lack of concentration.

Lactose is a disaccharide made up of glucose and galactose bonded together. Before it can be used by the body, the bond must be broken by the enzyme lactase in the small intestine. People that have decreased activity of lactase in the small intestine may have problems digesting lactose and this is referred to as lactose intolerance or malabsorption.

**Fat**

Milk is approximately 3.4% fat (Tables 4.7).

Fats are a structural component of cell membranes and hormones. Fats are a concentrated energy source and are the main energy source used by the body during low intensity activities and prolonged exercise over 90 minutes. Fat is the main storage form of excess energy in the body. Fats cushion organs during movement.

There are 2 fatty acids that are considered "essential" that cannot be made by the body and must come from the diet, and these are linoleic (18:2) and linolenic (18:3) acids. These fatty acids are used to synthesize the longer chain fatty acids arachidonic acid (AA, 20:4o-6), docopentaenoic acid (DPA, 22:4o-6), eicosapentaenoic acid (EPA, 20:5o-3) and docohexaenoic acid (DHA, 22:6o-3). These

fatty acids are essential for the synthesis of hormones such as prostaglandins, thromboxanes, and leukotrienes that are involved in muscle contraction, blood clotting, and immune response.

The fatty acids in milk fat are approximately 65% saturated, 29% monounsaturated, and 6% polyunsaturated. The polyunsaturated fatty acids in milk fat include small amounts of the essential fatty acids linoleic and linolenic, and approximately 5% trans fatty acids. An important trans fatty acid in milk fat is conjugated linoleic acid (CLA, 18:2). There are several types (isomers) of CLA in milk that have been shown to inhibit cancer and help maintain lean body mass while promoting the loss of body fat. The health benefits of CLA consumption are discussed in the Milk and Human Health section.

The health concerns associated with fats are often linked to the chemical differences in the fatty acids. Saturated and trans unsaturated fats have been associated with high blood cholesterol and heart disease. However, the relationships are not simple. The length of the fatty acid chain and source of the unsaturated bond (naturally-occurring or man-made through processing) can greatly influence the health consequences of a specific fat in the human diet. In addition, the genetics and health status of an individual greatly influences the impact of consuming different types of fats. The subject of fats and health is complex and constantly being updated in the medical literature.

Cholesterol (Tables 4.7) is an important component of cell membranes and as a starting material for the production of bile salts and steroid hormones. The body manufactures cholesterol to ensure that an adequate level of cholesterol is available for body functions. High levels of blood cholesterol are associated with increased risk for heart disease and are discussed in the Milk and Human Health section. Cholesterol is associated with fat so the content will vary depending on the fat content of the dairy product. An 8 oz serving of 2% milk contains 8% of the Daily Reference Intake (DRI) for cholesterol.

**Protein**

Milk is approximately 3.3% protein and contains all of the essential amino acids (Tables 4.7).

Proteins are the fundamental building blocks of muscles, skin, hair, and cellular components. Proteins are needed to help muscles contract and relax, and help repair damaged tissues. They play a critical role in many body functions as enzymes, hormones, and antibodies. Proteins may also be used as an energy source by the body.

Nine amino acids must be obtained from the diet and are referred to as the "essential" amino acids: leucine, isoleucine, valine, phenylalanine, tryptophan, histidine, threonine, methionine, and lysine. Proteins that contain all 9 essential amino acids are often called "complete" proteins. Proteins of animal origin and soy are complete proteins, whereas proteins from grains and legumes are missing 1 or

more of the essential amino acids, which means that consumers must eat complementary foods in order to get all of the essential amino acids.

Milk protein consists of approximately 82% casein and 18% whey (serum) proteins. Both casein and whey proteins are present in milk, yogurt, and ice cream. In most cheeses the casein is coagulated to form the curd, and the whey is drained leaving only a small amount of whey proteins in the cheese. During cheese making, the 6-casein is cleaved between specific amino acids and results in a unique protein fragment that is drained with the whey. This fragment, called milk glycomacropeptide, does not have any phenylalanine and can be used as a source of protein for people with phenylketonuria, the inability to digest proteins that contain phenylalanine. Whey proteins have become popular ingredients in foods as an additional source of protein or for functional benefits. Whey proteins are used as a protein source in high protein beverages and energy bars targeted to athletes. Some examples include the use of whey proteins to bind water in meat and sausage products, provide a brown crust in bakery products, and provide whipping properties that replace a portion of egg whites.

Whey proteins contain immunoglobulins which are important in the immune responses of the body. Whey proteins contain branched chain amino acids (leucine, isoleucine, and valine) and have been proposed to have some benefits to athletes for muscle recovery and for preventing mental fatigue.

**Vitamins**

Vitamins have many roles in the body including metabolism co-factors, oxygen transport and antioxidants. They help the body use carbohydrates, protein, and fat. The functions of vitamins are described below in alphabetical order.

The content of vitamin A in milk is shown in the Nutrient Content Table 4.7. Vitamin A is a fat soluble vitamin involved in vision, gene expression, reproduction, and immune response. The compounds with vitamin A activity are called retinoids and are found in foods in different forms – typically animal foods provide retinol and retinyl esters, and plant foods provide β-carotene, a starting molecule (precursor) for vitamin A synthesis. Milk contains retinol, retinyl esters, and ß-carotene. Dairy products are a good source of vitamin A, although the vitamin A content will vary with the fat content of the product. An 8 oz serving of 2% milk contains approximately 15% of the daily reference intake (DRI) for vitamin A.

The content of thiamin (vitamin $B_1$) in milk is shown in the Nutrient Content Tables. Thiamin is a water soluble vitamin that is an enzyme cofactor involved in the metabolism of carbohydrates and branched chain amino acids. An 8 oz serving of 2% milk contains approximately 8% of the DRI for thiamin.

The content of riboflavin (vitamin $B_2$) in milk is shown in the Nutrient Content Table 4.7. Riboflavin is a water soluble vitamin that is an enzyme cofactor involved in electron transport reactions. Milk is a recommended source of riboflavin and an 8 oz serving of 2% milk provides approximately 35% of the DRI for riboflavin.

Niacin is a water soluble vitamin that is an enzyme cofactor involved in electron transport reactions required for energy metabolism. There is a small amount of niacin in milk, an 8 oz serving of 2% milk contains less than 2% of the DRI for niacin.

Pantothenic acid is a water soluble vitamin that is an enzyme cofactor in fatty acid metabolism. Milk is a good source of pantothenic acid and an 8 oz serving of 2% milk contains approximately 17% of the DRI for pantothenic acid.

The content of vitamin $B_6$ (pyridoxine) in milk is shown in the Nutrient Content Tables. Vitamin $B_6$ is a water soluble vitamin involved in the metabolism of proteins and glycogen (energy stored in the liver and muscles), and in the metabolism of sphingolipids in the nervous system. An 8 oz serving of 2% milk contains approximately 7% of the DRI for vitamin $B_6$.

Vitamin $B_{12}$ is a water soluble vitamin involved in protein metabolism and blood functions. Milk is a recommended source of vitamin $B_{12}$. An 8 oz serving of 2% milk contains approximately 47% of the DRI for vitamin $B_{12}$.

Vitamin C is a water soluble vitamin that is an important antioxidant. It has a role in collagen formation in connective tissue and helps in iron absorption and healing of wounds and injuries. There is a negligible amount of vitamin C in milk, and a serving of milk contains less than 1% of the DRI for Vitamin C.

Vitamin D is a fat soluble vitamin that is important in maintaining blood calcium and phosphorus balance and assists calcium metabolism. Milk is typically fortified with vitamin D. Fortified milk is a good source of vitamin D, and an 8 oz serving of 2% milk contains over 50% of the DRI for vitamin D.

Vitamin E is a fat soluble vitamin that has antioxidant activity. The compounds with vitamin E activity are the tocopherols and tocotrienols. Milk contains a small amount of vitamin E, which increases with increasing fat content of dairy products. An 8 oz serving of whole milk contains 1% vitamin E, and an 8 oz serving of 2% milk contains only 0.5% of the DRI for vitamin E.

Folate is one of the water soluble B vitamins. Folate is an enzyme cofactor important in the metabolism of proteins and nucleic acids and blood functions. There is a small amount of folate in milk. An 8 oz serving of 2% milk contains 3% of the DRI for folate.

Vitamin K is a fat soluble vitamin involved in blood clotting, bone metabolism, and protein synthesis. Milk contains a small amount of vitamin K, which increases with the fat content in dairy products. An 8 oz serving of milk contains less than 1% of the DRI for vitamin K.

**Minerals**

Minerals have many roles in the body including enzyme functions, bone formation, water balance maintenance, and oxygen transport. They help the body use carbohydrates, protein, and fat. The functions of minerals are described below in alphabetical order.

Calcium plays an essential role in bone formation and metabolism, muscle contraction, nerve transmission and blood clotting. Dairy products are a significant source of calcium in the diet. Milk is a recommended source of calcium, and an 8 oz serving contains almost 30% of the DRI for calcium.

Copper is a component of enzymes used in iron metabolism. Milk contains a small amount of copper. An 8 oz serving of 2% milk contains approximately 3% of the DRI for copper.

Iron is a component of blood and many enzymes. It is involved in blood metabolism and oxygen transport. Milk contains a small amount of iron, and an 8 oz serving of milk contains less than 1% of the DRI for iron.

Magnesium is an enzyme cofactor and is important in bone metabolism. Milk is a recommended source of magnesium, and an 8 oz serving of 2% milk contains approximately 7% of the DRI for magnesium.

Manganese is involved in bone formation, and in enzymes involved in amino acid, cholesterol, and carbohydrate metabolism. There is a small amount of manganese in milk. An 8 oz serving contains less than 1% of the DRI.

Phosphorus is involved in maintaining body pH, in storage and transfer of energy, and in nucleotide synthesis. Milk is a recommended source of phosphorus, and an 8 oz serving of milk contains over 30% of the DRI for phosphorus.

Potassium is an electrolyte that is important in the maintenance of water balance, blood volume and blood pressure. Dairy products are a recommended source of potassium, and an 8 oz serving of milk contains approximately 8% of the DRI for potassium.

Selenium is important in oxidative stress response, electron transport, and regulation of thyroid hormone. Milk is a good source of selenium, and an 8 oz serving of 2% milk contains approximately 11% of the DRI for selenium.

Sodium is an electrolyte that is important in the maintenance of water balance and blood volume. An 8 oz serving of milk contains approximately 7% of the DRI for sodium.

Zinc is a component of many enzymes and proteins, and is involved in gene regulation. Milk is a good source of zinc, and an 8 oz serving contains approximately 10% of the DRI for zinc.

**Minor Biological Proteins & Enzymes**

Other minor proteins and enzymes in milk that are of nutritional interest include lactoferrin and lactoperoxidase. There are many other enzymes in milk but these do not have a role in human nutrition.

Lactoferrin is an iron binding protein that plays a role in iron absorption and immune response. Many other functions of lactoferrin have been proposed, but their confirmation is still under study, including protection against bacterial and

## NUTRITION FACTS

**Table 4.7. Nutrient content of milk varieties by serving; 1 serving = 1 cup (8 oz; 244 g)**

| Component | Unit | Cow | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Whole (3.25% fat) | Reduced Fat (2% fat)[1] | Low fat (1% fat)[1] | Skim[1] | Goat | Sheep | Water Buffalo |
| | | -- Amount per 8 oz serving (1 cup; 244 g) --- | | | | | | |
| **Overall Composition** | | | | | | | | |
| Water | g | 215.50 | 217.97 | 219.4 | 222.56 | 212.35 | 197.72 | 203.47 |
| Energy | kcal | 146 | 122 | 102 | 83 | 168 | 265 | 237 |
| Carbohydrate2 | g | 11.03 | 11.42 | 12.18 | 12.15 | 10.86 | 13.13 | 12.64 |
| Fat | g | 7.93 | 4.81 | 2.37 | 0.20 | 10.10 | 17.15 | 16.81 |
| Protein | g | 7.86 | 8.05 | 8.22 | 8.26 | 8.69 | 14.65 | 9.15 |
| Minerals (Ash) | g | 1.68 | 1.73 | 1.83 | 1.84 | 2.00 | 2.35 | 1.93 |
| **Vitamins** | | | | | | | | |
| Vitamin A | µg | 68 | 134 | 142 | 149 | 139 | 108 | 129 |
| Thiamin (Vitamin $B_1$) | mg | 0.107 | 0.095 | 0.049 | 0.110 | 0.117 | 0.159 | 0.127 |
| Riboflavin (Vitamin $B_2$) | mg | 0.447 | 0.451 | 0.451 | 0.446 | 0.337 | 0.870 | 0.329 |
| Niacin (Vitamin $B_3$) | mg | 0.261 | 0.224 | 0.227 | 0.230 | 0.676 | 1.022 | 0.222 |
| Pantothenic Acid (Vitamin $B_5$) | mg | 0.883 | 0.869 | 0.881 | 0.875 | 0.756 | 0.997 | 0.468 |
| Vitamin $B_6$ (Pyridoxine) | mg | 0.088 | 0.093 | 0.090 | 0.094 | 0.112 | 0.147 | 0.056 |
| Vitamin $B_{12}$ (Cobalamin) | µg | 1.07 | 1.12 | 1.07 | 1.30 | 0.17 | 1.74 | 0.88 |
| Vitamin C | mg | 0.0 | 0.5 | 0.0 | 0.0 | 3.2 | 10.3 | 5.6 |
| Vitamin D | IU | 98 | 105 | 127 | 100 | 29 | ND3 | ND |
| Vitamin E | mg | 0.15 | 0.07 | 0.02 | 0.02 | 0.17 | ND | ND |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Folate | µg | 12 | 12 | 12 | 12 | 2 | 17 | 15 |
| Vitamin K | µg | 0.5 | 0.5 | 0.2 | 0.0 | 0.7 | ND | ND |
| **Minerals (Ash)** | | | | | | | | |
| Calcium | mg | 276 | 285 | 290 | 306 | 327 | 473 | 412 |
| Copper | mg | 0.027 | 0.029 | 0.024 | 0.032 | 0.112 | 0.113 | 0.112 |
| Iron | mg | 0.07 | 0.07 | 0.07 | 0.07 | 0.12 | 0.25 | 0.29 |
| Magnesium | mg | 24 | 27 | 27 | 27 | 34 | 44 | 76 |
| Manganese | mg | 0.007 | 0.007 | 0.007 | 0.007 | 0.044 | 0.044 | 0.044 |
| Phosphorus | mg | 222 | 229 | 232 | 247 | 271 | 387 | 285 |
| Potassium | mg | 349 | 366 | 366 | 382 | 498 | 336 | 434 |
| Selenium | µg | 9.0 | 6.1 | 8.1 | 7.6 | 3.4 | 4.2 | ND |
| Sodium | mg | 98 | 100 | 107 | 103 | 122 | 108 | 127 |
| Zinc | mg | 0.98 | 1.05 | 10.2 | 1.03 | 0.73 | 1.32 | 0.54 |
| **Carbohydrate Detail** | | | | | | | | |
| Lactose4 | g | 12.83 | 12.22 | 12.69 | 12.47 | ND | ND | ND |
| **Fat Detail** | | | | | | | | |
| Cholesterol | mg | 24 | 20 | 12 | 5 | 27 | 66 | 46 |
| Fatty acids, total saturated | g | 4.551 | 3.067 | 1.545 | 0.287 | 6.507 | 11.277 | 11.217 |
| 4:0 | g | 0.183 | 0.188 | 0.059 | 0.010 | 0.312 | 0.500 | 0.673 |
| 6:0 | g | 0.183 | 0.098 | 0.044 | 0.000 | 0.229 | 0.355 | 0.373 |
| 8:0 | g | 0.183 | 0.049 | 0.032 | 0.002 | 0.234 | 0.338 | 0.173 |
| 10:0 | g | 0.183 | 0.120 | 0.066 | 0.005 | 0.634 | 0.980 | 0.344 |
| 12:0 | g | 0.188 | 0.134 | 0.071 | 0.002 | 0.303 | 0.586 | 0.407 |
| 13:0 | g | 0.000 | 0.005 | ND | ND | ND | ND | ND |
| 14:0 | g | 0.725 | 0.427 | 0.222 | 0.020 | 0.793 | 1.617 | 1.715 |

(*contd.*)

| Component | Unit | Cow | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Whole (3.25% fat) | Reduced Fat (2% fat)[1] | Low fat (1% fat)[1] | Skim[1] | Goat | Sheep | Water Buffalo |
| | | -- Amount per 8 oz serving (1 cup; 244 g) --- | | | | | | |
| 15:0 | g | 0.000 | 0.049 | 0.024 | ND | ND | ND | ND |
| 16:0 | g | 2.023 | 1.362 | 0.700 | 0.061 | 2.223 | 3.974 | 4.878 |
| 17:0 | g | 0.000 | 0.027 | 0.015 | ND | ND | ND | ND |
| 18:0 | g | 0.891 | 0.593 | 0.307 | 0.022 | 1.076 | 2.203 | 1.664 |
| 20:0 | g | 0.000 | 0.010 | 0.005 | ND | ND | ND | ND |
| Fatty acids, total monounsaturated | g | 1.981 | 1.366 | 0.676 | 0.115 | 2.706 | 4.224 | 4.360 |
| 14:1 | g | 0.000 | 0.034 | 0.017 | ND | ND | ND | ND |
| 15:1 | g | 0.000 | 0.010 | 0.005 | ND | ND | ND | ND |
| 16:1 unspecified | g | 0.000 | 0.066 | 0.041 | 0.007 | 0.200 | 0.314 | 0.346 |
| 16:1 cis | g | ND | 0.066 | 0.041 | ND | ND | ND | ND |
| 17:1 | g | 0.000 | 0.012 | 0.005 | ND | ND | ND | ND |
| 18:1 unspecified | g | 1.981 | 1.237 | 0.610 | 0.044 | 2.384 | 3.817 | 3.821 |
| 18:1 cis | g | ND | 1.049 | 0.520 | ND | ND | ND | ND |
| 18:1 trans | g | ND | 0.190 | 0.090 | ND | ND | ND | ND |
| Fatty acids, total polyunsaturated | g | 0.476 | 0.178 | 0.085 | 0.017 | 0.364 | 0.755 | 0.356 |
| 18:2 unspecified | g | 0.293 | 0.151 | 0.073 | 0.005 | 0.266 | 0.443 | 0.171 |
| 18:2o-6,cis,cis | g | ND | 0.134 | 0.066 | ND | ND | ND | ND |
| 18:2 i | g | ND | 0.017 | 0.007 | ND | ND | ND | ND |
| 18:3 unspecified | g | 0.183 | 0.020 | 0.010 | 0.002 | 0.098 | 0.311 | 0.185 |
| 18:3o-3,cis,cis,cis | g | ND | 0.020 | 0.010 | ND | ND | ND | ND |

| **Protein Detail** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Alanine | g | 0.251 | 0.271 | 0.259 | 0.245 | 0.288 | 0.659 | 0.322 |
| Arginine | g | 0.183 | 0.261 | 0.234 | 0.176 | 0.290 | 0.485 | 0.278 |
| Aspartic Acid | g | 0.578 | 0.730 | 0.759 | 0.595 | 0.512 | 0.804 | 0.754 |
| Cystine | g | 0.041 | 0.261 | 0.283 | 0.310 | 0.112 | 0.086 | 0.117 |
| Glutamic Acid | g | 1.581 | 1.901 | 1.908 | 1.649 | 1.527 | 2.497 | 1.164 |
| Glycine | g | 0.183 | 0.149 | 0.154 | 0.123 | 0.122 | 0.100 | 0.195 |
| Histidine | g | 0.183 | 0.178 | 0.205 | 0.184 | 0.217 | 0.409 | 0.190 |
| Isoleucine | g | 0.403 | 0.447 | 0.456 | 0.367 | 0.505 | 0.828 | 0.495 |
| Leucine | g | 0.647 | 0.808 | 0.915 | 0.801 | 0.766 | 1.438 | 0.893 |
| Lysine | g | 0.342 | 0.569 | 0.700 | 0.617 | 0.708 | 1.257 | 0.683 |
| Methionine | g | 0.183 | 0.203 | 0.203 | 0.152 | 0.195 | 0.380 | 0.237 |
| Phenylalanine | g | 0.359 | 0.395 | 0.407 | 0.355 | 0.378 | 0.696 | 0.395 |
| Proline | g | 0.834 | 0.898 | 0.876 | 0.840 | 0.898 | 1.421 | 0.888 |
| Serine | g | 0.261 | ND | 0.508 | 0.412 | 0.442 | 1.205 | 0.554 |
| Threonine | g | 0.349 | 0.251 | 0.217 | 0.201 | 0.398 | 0.657 | 0.444 |
| Tyrosine | g | 0.371 | 0.373 | 0.346 | 0.363 | 0.437 | 0.688 | 0.447 |
| Tryptophan | g | 0.183 | 0.098 | 0.098 | 0.098 | 0.107 | 0.206 | 0.129 |
| Valine | g | 0.468 | 0.532 | 0.529 | 0.441 | 0.586 | 1.098 | 0.534 |

[1]Vitamin A added.

[2]Carbohydrate determined by difference.

[3]ND = not determined.

[4]Lactose determined by analysis.

**Table 4.8. Nutrient content of milk varieties by 100 g reference amount**

| Component | Unit | Cow | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Whole (3.25% fat) | Reduced Fat (2% fat)[1] | Lowfat (1% fat)[1] | Skim[1] | Goat | Sheep | Water Buffalo |
| | | --- Amount per 100 g --- | | | | | | |
| **Overall Composition** | | | | | | | | |
| Water | g | 88.32 | 89.33 | 89.92 | 90.84 | 87.03 | 80.70 | 83.39 |
| Energy | kcal | 60 | 50 | 42 | 34 | 69 | 108 | 97 |
| Carbohydrate[2] | g | 4.52 | 4.68 | 4.99 | 4.96 | 4.45 | 5.36 | 5.18 |
| Fat | g | 3.25 | 1.97 | 0.97 | 0.08 | 4.14 | 7.00 | 6.89 |
| Protein | g | 3.22 | 3.30 | 3.37 | 3.37 | 3.56 | 5.98 | 3.75 |
| Minerals (Ash) | g | 0.69 | 0.71 | 0.75 | 0.75 | 0.82 | 0.96 | 0.79 |
| **Vitamins** | | | | | | | | |
| Vitamin A | µg | 28 | 55 | 58 | 61 | 57 | 44 | 53 |
| Thiamin (Vitamin $B_1$) | mg | 0.044 | 0.039 | 0.02 | 0.045 | 0.048 | 0.065 | 0.052 |
| Riboflavin (Vitamin $B_2$) | mg | 0.183 | 0.185 | 0.185 | 0.182 | 0.138 | 0.355 | 0.135 |
| Niacin (Vitamin $B_3$) | mg | 0.107 | 0.092 | 0.093 | 0.094 | 0.277 | 0.417 | 0.091 |
| Pantothenic Acid (Vitamin $B_5$) | mg | 0.362 | 0.356 | 0.361 | 0.357 | 0.310 | 0.407 | 0.192 |
| Vitamin B6 (Pyridoxine) | mg | 0.036 | 0.038 | 0.037 | 0.037 | 0.046 | 0.060 | 0.023 |
| Vitamin B12 (Cobalamin) | µg | 0.44 | 0.46 | 0.44 | 0.53 | 0.07 | 0.71 | 0.36 |
| Vitamin C | mg | 0.0 | 0.2 | 0.0 | 0.0 | 1.3 | 4.2 | 2.3 |
| Vitamin D | IU | 40 | 43 | 52 | 41 | 12 | ND3 | ND |
| Vitamin E | mg | 0.06 | 0.03 | 0.01 | 0.01 | 0.07 | ND | ND |
| Folate | µg | 5 | 5 | 5 | 5 | 1 | 7 | 6 |
| Vitamin K | µg | 0.2 | 0.2 | 0.1 | 0.0 | 0.3 | ND | ND |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Minerals (Ash)** | | | | | | | | |
| Calcium | mg | 113 | 117 | 119 | 125 | 134 | 193 | 169 |
| Copper | mg | 0.011 | 0.012 | 0.010 | 0.013 | 0.046 | 0.046 | 0.046 |
| Iron | mg | 0.03 | 0.03 | 0.03 | 0.03 | 0.05 | 0.10 | 0.12 |
| Magnesium | mg | 10 | 11 | 11 | 11 | 14 | 18 | 31 |
| Manganese | | mg | 0.003 | 0.003 | 0.003 | 0.003 | 0.018 | 0.018 |
| 0.018 | | | | | | | | |
| Phosphorus | mg | 91 | 94 | 95 | 101 | 111 | 158 | 117 |
| Potassium | mg | 143 | 150 | 150 | 156 | 204 | 137 | 178 |
| Selenium | µg | 3.7 | 2.5 | 3.3 | 3.1 | 1.4 | 1.7 | ND |
| Sodium | mg | 40 | 41 | 44 | 42 | 50 | 44 | 52 |
| Zinc | mg | 0.40 | 0.43 | 0.42 | 0.42 | 0.30 | 0.54 | 0.22 |
| **Carbohydrate Detail** | | | | | | | | |
| Lactose4 | g | 5.26 | 5.01 | 5.20 | 5.09 | ND | ND | ND |
| **Fat Detail** | | | | | | | | |
| Cholesterol | mg | 10 | 8 | 5 | 2 | 11 | 27 | 19 |
| Fatty acids, total saturated | g | 1.865 | 1.257 | 0.633 | 0.117 | 2.677 | 4.603 | 4.597 |
| 4:0 | g | 0.075 | 0.077 | 0.024 | 0.004 | 0.128 | 0.204 | 0.276 |
| 6:0 | g | 0.075 | 0.040 | 0.018 | 0.000 | 0.094 | 0.145 | 0.153 |
| 8:0 | g | 0.075 | 0.020 | 0.013 | 0.001 | 0.096 | 0.138 | 0.071 |
| 10:0 | g | 0.075 | 0.049 | 0.027 | 0.002 | 0.260 | 0.400 | 0.141 |
| 12:0 | g | 0.077 | 0.055 | 0.029 | 0.001 | 0.124 | 0.239 | 0.167 |
| 13:0 | g | 0.000 | 0.002 | ND | ND | ND | ND | ND |
| 14:0 | g | 0.297 | 0.175 | 0.091 | 0.008 | 0.325 | 0.660 | 0.703 |
| 15:0 | g | 0.000 | 0.020 | 0.010 | ND | ND | ND | ND |

(contd.)

| Component | Unit | Cow | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Whole (3.25% fat) | Reduced Fat (2% fat)[1] | Lowfat (1% fat)[1] | Skim[1] | Goat | Sheep | Water Buffalo |
| | | --- Amount per 100 g --- | | | | | | |
| 16:0 | g | 0.829 | 0.580 | 0.287 | 0.025 | 0.911 | 1.622 | 1.999 |
| 17:0 | g | 0.000 | 0.011 | 0.006 | ND | ND | ND | ND |
| 18:0 | g | 0.365 | 0.243 | 0.126 | 0.009 | 0.441 | 0.899 | 0.682 |
| 20:0 | g | 0.000 | 0.004 | 0.002 | ND | ND | ND | ND |
| Fatty acids, total monounsaturated | g | 0.812 | 0.560 | 0.277 | 0.047 | 1.109 | 1.724 | 1.787 |
| 14:1 | g | 0.000 | 0.014 | 0.007 | ND | ND | ND | ND |
| 15:1 | g | ND | 0.004 | 0.002 | ND | ND | ND | ND |
| 16:1 unspecified | g | 0.000 | 0.027 | 0.017 | 0.003 | 0.082 | 0.128 | 0.142 |
| 16:1 cis | g | ND | 0.027 | 0.017 | ND | ND | ND | ND |
| 17:1 | g | ND | 0.005 | 0.002 | ND | ND | ND | ND |
| 18:1 unspecified | g | 0.812 | 0.507 | 0.250 | 0.018 | 0.977 | 1.558 | 1.566 |
| 18:1 cis | g | ND | 0.430 | 0.213 | ND | ND | ND | ND |
| 18:1 trans | g | ND | 0.078 | 0.037 | ND | ND | ND | ND |
| Fatty acids, total polyunsaturated | g | 0.195 | 0.073 | 0.035 | 0.007 | 0.149 | 0.308 | 0.146 |
| 18:2 unspecified | g | 0.120 | 0.062 | 0.030 | 0.002 | 0.109 | 0.181 | 0.070 |
| 18:2o-6,cis,cis | g | ND | 0.055 | 0.027 | ND | ND | ND | ND |
| 18:2 i | g | ND | 0.007 | 0.003 | ND | ND | ND | ND |
| 18:3 unspecified | g | 0.075 | 0.008 | 0.004 | 0.001 | 0.040 | 0.127 | 0.076 |
| 18:3o-3,cis,cis,cis | g | ND | 0.008 | 0.004 | ND | ND | ND | ND |

| **Protein Detail** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Alanine | g | 0.103 | 0.111 | 0.106 | 0.100 | 0.188 | 0.269 | 0.132 |
| Arginine | g | 0.075 | 0.107 | 0.096 | 0.072 | 0.119 | 0.198 | 0.114 |
| Aspartic Acid | g | 0.237 | 0.299 | 0.311 | 0.243 | 0.210 | 0.328 | 0.309 |
| Cystine | g | 0.017 | 0.107 | 0.116 | 0.123 | 0.046 | 0.035 | 0.048 |
| Glutamic Acid | g | 0.648 | 0.779 | 0.782 | 0.673 | 0.626 | 1.019 | 0.477 |
| Glycine | g | 0.075 | 0.061 | 0.063 | 0.050 | 0.050 | 0.041 | 0.080 |
| Histidine | g | 0.075 | 0.073 | 0.084 | 0.075 | 0.089 | 0.167 | 0.078 |
| Isoleucine | g | 0.165 | 0.183 | 0.187 | 0.150 | 0.207 | 0.338 | 0.203 |
| Leucine | g | 0.265 | 0.331 | 0.375 | 0.327 | 0.314 | 0.587 | 0.366 |
| Lysine | g | 0.140 | 0.233 | 0.287 | 0.252 | 0.290 | 0.513 | 0.280 |
| Methionine | g | 0.075 | 0.083 | 0.083 | 0.062 | 0.080 | 0.155 | 0.097 |
| Phenylalanine | g | 0.147 | 0.162 | 0.167 | 0.145 | 0.155 | 0.284 | 0.162 |
| Proline | g | 0.342 | 0.368 | 0.359 | 0.343 | 0.368 | 0.580 | 0.364 |
| Serine | g | 0.107 | ND | 0.208 | 0.168 | 0.181 | 0.492 | 0.227 |
| Threonine | g | 0.143 | 0.103 | 0.089 | 0.082 | 0.163 | 0.268 | 0.182 |
| Tyrosine | g | 0.152 | 0.153 | 0.142 | 0.148 | 0.179 | 0.281 | 0.183 |
| Tryptophan | g | 0.075 | 0.040 | 0.040 | 0.040 | 0.044 | 0.084 | 0.053 |
| Valine | g | 0.192 | 0.218 | 0.217 | 0.180 | 0.240 | 0.448 | 0.219 |

[1]Vitamin A added.
[2]Carbohydrate determined by difference.
[3]ND = not determined.
[4]Lactose determined by analysis.

viral infections, and it's role in inflammatory response and enzyme activity. The use of lactoferrin as an antimicrobial agent is discussed in the section on Antibacterial Properties of Milk in this website.

Lactoperoxidase is an enzyme that, in the presence of hydrogen peroxide and thiocyanate, has antibacterial properties. The use of lactoperoxidase as an antimicrobial agent is discussed in the section on Antibacterial Properties of Milk in this website. Lactoperoxidase does not provide antimicrobial protection to fresh milk because hydrogen peroxide is not normally present in milk – it must be added to activate this system.

Lipases, a group of enzymes that break down fats, are present in milk but are inactivated by pasteurization, which increases the shelf life of milk. A popular belief among raw milk consumers is that the native lipase in milk plays an important role in the digestion of fat. Fat digestion begins in the stomach with gastric lipase, and the majority of fat digestion occurs in the small intestine, using enzymes secreted by the pancreas. The relative importance of the native milk lipase in digestion compared to the pancreatic lipases is not clear.

Lactase (β-galactosidase) is the enzyme responsible for the breakdown of lactose into glucose and galactose for digestion. There is no lactase present in fresh milk. Any lactase present in milk products comes from lactic acid bacteria that are either added to milk on purpose, as in the case of yogurt and cheese, or that enter milk from airborne or other contamination. A popular belief is that people with lactose intolerance are able to drink raw milk but not pasteurized milk because the lactase present in raw milk is inactivated during pasteurization. Because there is no lactase present in fresh milk, this concept is a myth. People with lactose intolerance have, themselves, lower levels of lactase which creates problems when it comes to digesting large amounts of lactose in a timely manner. Naturally occurring lactase used to digest milk is normally secreted by the small intestine. Lactase found in any lactic acid bacteria present will minimally help to digest lactose when it is released as the milk is digested in the small intestine.

## CONTAMINANTS IN MILK

### Introduction

Milk production has inextricably a linked to the environment. The latter depends largely on human activities. Uncontrolled processes interfering with the food delivering ecological system or with its natural compounds lead to residues in milk, which are not very easy to be controlled. Most of the foods produced for human consumption grow using pesticides. Chemical control of weeds, insects, fungi and rodents has enabled agricultural productivity and intensity to increase. However, these economic benefits are not without their risks to human and environmental health. Pesticides are not the only contaminants in food that may affect children's health adversely. In addition to pesticides, a large number of environmental contaminants can enter the cow as a residue of herbicides on feedstuffs or as drugs given to the cow orally, by injection, or as intra mammary

infusions for the treatment of mastitis. Contaminants also enter milk from equipment after milking. Many of these compounds are fat-soluble and will be stored in adipose tissue or secreted in milk fat. Residues, particularly organochlorine pesticides which tend to accumulate in body fat, or enter to milk fat. Less lipophilic compounds and their metabolites may excrete in urine. The general types of compounds which have been or are found in milk are chlorinated pesticides, organophosphates, herbicides, fungicides, anti helminthetic drugs, antibiotic and sulfonamides drugs, detergents and disinfectants, Poly Chlorinated (PCBs), Poly Brominated Biphenyles (PBBs), dioxins, mycotoxins, heavy metals and somatotropin hormone. Any of these compounds may persist at collection, preparation processes of dairy products and they considered residues. The potential to cause toxicological harm to consumers is one characteristic in common for all of chemical contaminants. Chemical contaminants in milk and dairy products may know to be contributory factors in several diseases such as cancer, heart disease, Alzheimer's disease and Parkinsonism. The idea that synthetic chemicals such as DDT are major contributors to human cancer has been inspired. The occurrence of chemical residues in the milk of lactating cows is a matter of public health concern, since dairy products are widely consumed by infants, children and many adults throughout the world. In view of this, many countries have enacted regulations that limit the level of chemical residues in milk and dairy products. Milk chemical safety is important for public health. Governments need to assessment of chemical risks in people who consume contaminated milk to achieve risk communication and risk management. Total quality management and the Hazard Analysis Critical Control Points (HACCP) approach has an important role for preventing and controlling of chemical contaminants in milk and dairy products, especially antibiotics in raw milk shipped from the farm.

### Sources of Chemical Contaminants

The sources of chemical contaminants in raw materials of animal origin, mainly milk, are to a large extent comparable to those of raw materials originating from plants. The most of chemical contaminants in milk and dairy products are veterinary drugs such as antimicrobials (antibiotics and sulfonamides), hormones, anthelminthitic drugs, pesticides and etc.

### Antibiotic Drugs

Much of the veterinary treatment of dairy cattle involves intra mammary infusion of antibiotics to control mastitis. Some drugs apply to control endoparasites, ectoparasites and several illnesses and to increase milk production. The most commonly used antimicrobials in dairy cattle can group into five major classes. These include the beta-lactams (e.g, penicillins and cephalosporins), tetracyclines (e.g., Oxytetracycline, tetracycline and chlortetracycline), amino glycosides (e.g., streptomycine, neomycin and gentamycin), macrolides (e.g., erythromycin) and sulfanomides (e.g., sulfamethazines). These drugs are administered to animals by many routes such as injection routs, orally in the food and water, topically on the skin and by intra mammary and intrauterine infusions.

Theoretically, all of these routes may lead to residues appearing in milk and dairy products. Whenever any route with an antibiotic treats a lactating cow, measurable levels of the antibiotic are usually detectable in the milk for a few days after the last.

**Steroid Hormones**

Milk can also consider as a rich source of steroid hormones. The amounts of lipophilic hormones depend on the fat content of the milk and dairy products. Not only progesterone but also estrone increases with fat content. Food processing does not seem to influence the amount and ratios of the hormones. Food processing, such as heating or churning, appears to have no effect on the hormones in milk and dairy products although cheese ripening does. In fresh cheese as well as in ripened cheese, testosterone was detected (0.1-0.5 mg $kg^{-1}$). Probably not only propionic acid bacteria but also other fermenting bacteria or clotting enzymes are responsible for the formation of testosterone during the fermentation process.

**Bovine Growth Hormone**

Bovine Growth Hormone (BGH) or Bovine Somatotropin (BST) is a genetically engineered protein hormone either identical or similar to the natural bovine pituitary product. Its primary function is to increase milk production dairy lactating cattle. Therefore, BST is a protein hormone that increases milk production in cows between 10 and 15%. An increase in milk yield typically occurs with 5 days after beginning of treatment. When BST-treated cows were consuming sufficient quantities of nutrients to meet the energy needs for additional milk synthesis, body lipid mobilization did not increase, but lipid synthesis was instead reduced. BGH increases activity and/or longevity of mammary secretary cells, probably via Insulin-like Growth Factor (IGF)-I produced by the liver and/or the mammary gland. IGF-I is a portion of the effects of BST on lactation in dairy cows. The raw milk and pasteurized milk could have been levels of IGF-I of 5.6 and 8.2 ng $mL^{-1}$, respectively. The infant formula could have been only trace amounts of IGF-I of 0.7 ng $mL^{-1}$. Therefore, IGF-I is not destroyed by the pasteurization process but the heating of milk for the preparation of infant formula denaturizes IGF-I and significantly, reduction (35-48%) levels of IGF-I compared to raw milk. BGH is probably stimulating immunological responses of animals and hence increasing the milk cell count. It can cause to increase incidence of clinical mastitis in cows and sub clinical mastitis in ewes.This prompted concern that increased use of antibiotics to treat the mastitis might lead to increased residues of such drugs in milk. Therefore, the risk to human health comes not from BGH residues but from the possibility that residues from the antibiotics used to treat the udder infections could end up in the milk supply. There are many conventions about BST treated in cows. For example, an international meeting of scientists of the United States and Europe has concluded that beef raised with hormonal supplements has no adverse effect on humans and they are safe for cows and humans. However, in news of the World Trade Organization has partially accepted European Union (EU) contentions for prohibiting the use of growth-promoting hormones in cattle.

**Parasiticide drugs**

Parasiticide drugs also use to remove internal parasites such as flukes, tapeworms (helminths) and nematodes (round worms) are important in animal production systems. The oxylosanide, closantel and rafoxanide are active against Fasciola hepatica (liver fluke). Albendazole is a widely used benzimidazole anthelmentic that has shown efficacy against the important species of round worms, tapeworms and flukes from animals and humans. Following administration to cattle and sheep, albendazole is readily absorbed from the gut and rapidly transform to various metabolites, the major metabolites being albendazole sulfoxide, albendazole sulfone and albendazole 2-amino sulfone. These metabolites can account for all residues found in milk and dairy products at any time point that are both bioavailable and of toxicological significance. Residues of benzimidazole compounds can occur in milk and dairy products and it is necessary to observe withdrawal periods for milk after therapy. Toxicological studies of animals have shown albendazole and its metabolites to be mutagenic. To protect the consumer from residues in marketed. A provisional maximum residue limit for total albendazole residues of 100 ng $ml^{-1}$ established within the European Union.

**Pesticides and Insecticides**

Contamination of feeds arises in the field or store where treatment with pesticides occurs. Chlorinated pesticides and related compounds such as DDT, Polychlorinated biphenyls (PCBs) and Dioxins can enter milk and dairy products. The chlorinated hydrocarbons are extremely durable, persistent, endocrine-disrupting activities, bio-accumulating and widely distributed toxic compounds that find their way into the food chain usually through use in controlling environmental or animal pests. As much as 20% of an ingested chlorinated hydrocarbon excretes in milk. Chlorinated hydrocarbons adhere to milk fat and butter contains a much higher proportion of these insecticides. DDT can accumulate in fatty tissues and can transfer into milk and dairy products. Organochlorine pesticides such as DDT and Hexachlorocyclohexane (HCH) have banned in China since 1983. Residues of such compounds may persist in the environment and cause contamination through the food chain. Presented organochlorine pesticide residues (owing to their use in sanitary actions) indicating a human exposure through milk and dairy products.

**Mycotoxins**

Some of the moulds produce various toxic metabolites under appropriate temperature and moisture conditions. These metabolites, which may be hazardous for human health, are called mycotoxins. Aflatoxin M1 (AFM1) may be found in the milk of animals that are fed with Aflatoxin B1 (AFB1) containing feed. It can produce by moulds of feeds that have been harvested damp, have not adequately dried, or are improperly stored. These substances through the feedstuff can infect dairy cattle. The content of AFM1 in milk is entirely dependent on the presence of the precursor AFB1 in the ration of dairy cattle and it can numerically express as

feed to milk ratio. The forming of AFM1, metabolite of AFB1, occurs in liver and it secrets into milk in the mammary gland of dairy cows. Many researchers reported that there was a linear relationship between the amount of AFM1 in milk and AFB1 in feed consumed by the animals. On the other hand, AFM1 levels in milk show a seasonal variation and the toxin amount have differences in the products, which are produced from the toxin containing milk. It reported that after uncovering of negative effects of aflatoxins on public health, various international foundations related with this subject paid attention and in 1993, International Agency for Research on Cancer (IARC) of WHO included AFB1 as primary and AFM1 as secondary groups of carcinogenic compounds. Many countries have carried out various control and inspection programs on this subject concerning about public health for many years. According to the results obtained, maximum aflatoxin levels were determined for food and feed by considering each country's conditions and finally regulations were established.

Heat treatments like pasteurization were not effective in the reduction of formation of AFM1. It has relatively increased in cheese samples because of its affinity to casein fraction in milk and due to the water solubility of this toxin lower level of AFM1 was found in cream and butter than that of bulk-tank milk. All of these findings indicated that AFM1 with different levels could be available in dairy products made from contaminated milk. Consequently, this subject is a serious problem for the public health since all the age groups including infants and children consume these products worldwide. For this reason, milk and dairy products have to be inspected continuously for AFM1 contamination at least twice a year. Beside this, it is important to have low levels of AFM1 in the feeds of dairy animals and in order to achieve this purpose, feeds of dairy cows should be kept away from contamination as much as possible.

**Heavy Metals**

Heavy metals enter the human body mainly by the routes of inhalation and ingestion. With increasing environmental pollution, a heavy metal exposure assessment study is necessary. Heavy metals produce toxic effects by replacing essential metal ions existing in the chelates present in body. The intake via ingestion depends upon food habits. It is well established that lead (Pb) and cadmium (Cd) are toxicfor human and children are more sensitive to these metals than adults. The metals, namely copper (Cu) and zinc (Zn), are essential micronutrients and have a variety of biochemical functions in all living organisms. While Cu and Zn are essential, they can be toxic when taken in excess; both toxicity and necessity vary from element-to-element. Milk is the fundamental food for infants and the daily intake of the heavy metals Pb, Cd, Cu and Zncanb determine by different age groups of infants through different milks and baby foods. Heavy metals can enter to milk and dairy products and affect the health of people who have consumed contaminate milk and dairy products.

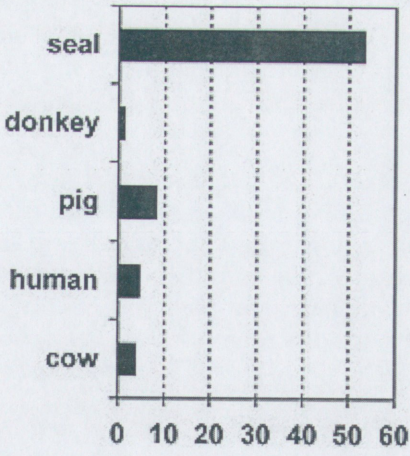
## VARIATION IN MILK VITAMIN CONTENTS

The milk fatty acid (FA) profile is far from the optimal fat composition in regards to human health. The natural sources of variation, such as feeding or genetics, could be used to increase the concentrations of unsaturated fatty acids. The impact of feeding is well described. However, genetic effects on the milk FA composition begin to be extensively studied. This paper summarizes the available information about the genetic variability of FAs. The greatest breed differences in FA composition are observed between Holstein and Jersey milk. Milk fat of the latter breed contains higher concentrations of saturated FAs, especially short-chain FAs. The variation of the delta-9 desaturase activity estimated from specific FA ratios could explain partly these breed differences. The choice of a specific breed seems to be a possibility to improve the nutritional quality of milk fat. Generally, the proportions of FAs in milk are more heritable than the proportions of these same FAs in fat. Heritability estimates range from 0.00 to 0.54. The presence of some single nucleotide polymorphisms could explain partly the observed individual genetic variability. The polymorphisms detected on SCD1 and DGAT1 genes influence the milk FA composition. The SCD1 V allele increases the unsaturation of C16 and C18. The DGAT1 A allele is related to the unsaturation of C18. So, a combination of the molecular and quantitative approaches should be used to develop tools helping farmers in the selection of their animals to improve the nutritional quality of the produced milk fat.

### Species Variability

Milk fat is the most variable component of milk. It ranges from a little over 1% to greater than 50 %. Aquatic mammals typically have high milk fat percentage.

Lactose ranges from only a trace to less than 7%. Some species have very little lactose in milk, such as the bear and kangaroo. These must have some other osmole in order to maintain milk in osmotic balance with blood plasma as milk is being synthesized. These species often have another sugar (for example the kangaroo has a trisaccharide) as the major osmole.




**Milk fat % in milks of various species**

Milk protein concentration varies considerably, but not as much as milk fat varies among species. Ranges from 1% to about 14%. Generally milk protein percentage is positively correlated with milk fat percentage. If one is high, the other is usually high.

# 5

# Colloids in Milk

**Topics**

*Properties of Colloids • Classifying Colloids • Milk Fat Size Distribution • Milk Fat Size Distribution • Milk Fat Globule Composition, Synthesis, and Size Distribution • Enzyme Coagulation • Acid Coagulation • Heat • Age Gelation • Milk Protein Gels • Acid milk gels • Protein gels • Mechanism of the acid coagulation • Gelation Properties of Skim Milk Gels*

In a colloid, one substance is dispersed in another in a finer state than an emulsion but the particle size is larger than that in a true solution. Colloidal systems are classified according to the physical state of the two phases. In a colloid, solid particles consisting of groups of molecules float freely. The particles in a colloid are much smaller than those in a suspension and a colloid is much more stable.

A colloid is one of the three primary types of mixtures, with the other two being a solution and suspension. A colloid is a solution that has particles ranging between 1 and 1000 nanometers in diameter, yet are still able to remain evenly distributed throughout the solution. These are also known as colloidal dispersions because the substances remain dispersed and do not settle to the bottom of the container. In colloids, one substance is evenly dispersed in another. The substance being dispersed is referred to as being in the dispersed phase, while the substance in which it is dispersed is in the continuous phase.

## PROPERTIES OF COLLOIDS

To be classified as a colloid, the substance in the dispersed phase must be larger than the size of a molecule but smaller than what can be seen with the naked eye. This can be more precisely quantified as one or more of the substance's dimensions must be between 1 and 1000 nanometers. If the dimensions are smaller than this the substance is considered a solution and if they are larger than the substance is a suspension.

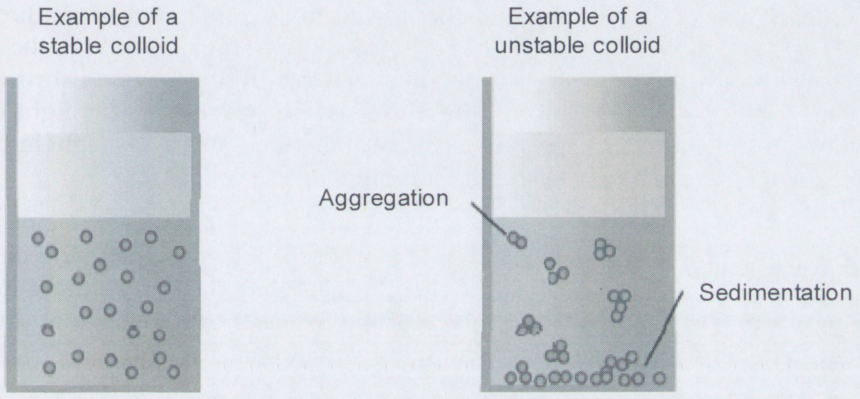



**Fig. 5.1. Examples of a stable and of an unstable colloidal dispersion.**

## CLASSIFYING COLLOIDS

A common method of classifying colloids is based on the phase of the dispersed substance and what phase it is dispersed in. The types of colloids includes sol, emulsion, foam, and aerosol.

1. **Sol** is a colloidal suspension with solid particles in a liquid.
2. **Emulsion** is between two liquids.
3. **Foam** is formed when many gas particles are trapped in a liquid or solid.
4. **Aerosol** contains small particles of liquid or solid dispersed in a gas.

When the dispersion medium is water, the collodial system is often referred to as a hydrocolloid. The particles in the dispersed phase can take place in different phases depending on how much water is available. For example, Jello powder mixed in with water creates a hydrocolloid. A common use for hydrocolloids is in the creation of medical dressings.

**Table 5.1: Examples of Colloids**

| Dispersion Medium | Dispersed Phase | Type of Colloid | Example |
|---|---|---|---|
| Solid | Solid | Solid sol | Ruby glass |
| Solid | Liquid | Solid emulsion/gel | Pearl, cheese |
| Solid | Gas | Solid foam | Lava, pumice |
| Liquid | Solid | Sol | Paints, cell fluids |
| Liquid | Liquid | Emulsion | Milk, oil in water |
| Liquid | Gas | Foam | Soap suds, whipped cream |
| Gas | Solid | Aerosol | Smoke |
| Gas | Liquid | Aerosol | Fog, mist |

An easy way of determining whether a mixture is colloidal or not is through use of the Tyndall Effect. When light is shined through a true solution, the light passes cleanly through the solution, however when light is passed through a colloidal solution, the substance in the dispersed phases scatters the light in all directions, making it readily seen. An example of this is shining a flashlight into fog. The beam of light can be easily seen because the fog is a colloid.

Another method of determining whether a mixture is a colloid is by passing it through a semipermeable membrane. The larger dispersed particles in a colloid would be unable to pass through the membrane, while the surrounding liquid molecules can. Dialysis takes advantage of the fact that colloids cannot diffuse through semipermeable membranes to filter them out of a medium.

## MILK FAT SIZE DISTRIBUTION

### Introduction

The role of milk in nature is to nourish and provide immunological protection. Milk has been a food source for humans since prehistoric times; from human, goat, buffalo, sheep, yak, and domesticated cow milk. Milk is also a very complex food with over 100,000 different molecular species found. There are many factors that can affect milk composition such as breed variations, including management and feed considerations, seasonal variations, and geographic variations.

Milk is an emulsion of fat globules within a water-based fluid. Each fat globule is surrounded by a membrane consisting of phospholipids and proteins; these emulsifiers keep the individual globules from joining together into noticeable grains of fat and also protect the globules from the fat-digesting activity of enzymes found in the fluid portion of the milk. The fat-soluble vitamins A, D, E, and K are found within the milk fat portion of the milk. Not only is the composition important in determining the properties of milk, but the physical structure must also be examined. Due to its role in nature, milk is in a liquid form. This may seem curious if one takes into consideration the fact that milk has less water than most fruits and vegetables. Milk can be described as:

- an **oil-in-water emulsion** with the fat globules dispersed in the continuous serum phase
- a **colloid suspension** of casein micelles, globular proteins and lipoprotein particles
- a **solution** of lactose, soluble proteins, minerals, vitamins other components.

The main structural components of milk, fat globules and casein micelles, are shown in Fig. 5.2.

### Milk Fat Globules - Particle Size

Properties such as flavor, emulsion stability and sensory feel (mouth feel) are related to the particle size of the fat droplets present in milk. More than 95% of the

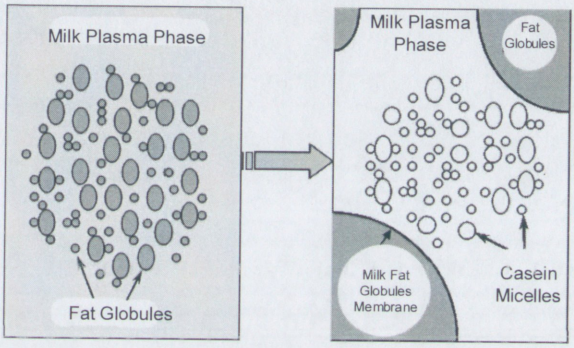



**Fig. 5.2 Main structural components of milk**

total milk lipid is in the form of globules ranging in size from 0.1 to 15 μm in diameter, with milk fat having size ranging from 1-10 μm, depending on cow breed and season. If the droplet size is too large creaming, due to poor stability, could be generated, thus, providing a product with a greasy taste. On the other hand, a size that is too small may cause may lead to flocculation.

### Particle Size Measurements

Four different types of milk, fat free, low fat (1%), reduced fat (2%), and whole milk (3.25%) obtained from a local market, were analyzed with the Beckman Coulter LS 13 320 laser diffraction particle size analyzer. For the determination of the size distribution a refractive index of 1.462 (RI for milk fat) was used. Figure 5.2 is an overlay of the results obtained.

## MILK FAT GLOBULE COMPOSITION, SYNTHESIS, AND SIZE DISTRIBUTION

The lipid composition of a MFG is traditionally considered to be solely a function of the length and degree of unsaturation of the fatty acids. Fatty acids tend to be esterified to different lipid fractions depending on their carbon chain length and degree of unsaturation. In addition, milk fat globules span a wide range of sizes with quite different size classes having different TG/PL composition. Hence, differences in the TG/PL ratios in the MFGs of different diameters also indicate different FA compositions. The composition of the membrane phospholipids and the fatty-acid profile within the phospholipid fraction certainly play important roles in the milk fat globule membrane's physical and functional properties. Nonetheless, our ability to control membrane properties and the processes of milk fat globule assembly, need to extend beyond simply fatty acid composition.

The MFGs triglycerides are synthesized at the rough endoplasmic reticulum, accumulate into triglyceride-rich domains varied in size, which are released as discrete droplets to the cytoplasm, coated with polar lipids and proteins derived from the ER. From their site of origin, lipid droplets migrate to the apical pole of the cell from which they will be secreted. Milk fat globules follow a distinct export

process in which the plasma membrane itself enrobes the emerging globule. In the secreted milk, lipid globule diameters range from 0.2 to more than 15 μm which implies that some lipid droplets grow substantially between the time of their formation and the time of their secretion. Furthermore, post-secretion, some of the larger milk fat globules may fuse with smaller ones and by that increase their diameter. However, whether this process is explicitly directed by cellular processes or whether the globules spontaneously self-assemble according to the composition of the respective surfaces, is unknown. The fact that milk lipid fraction, consist of MFG in various diameters implies that their size distribution within milk may have additional role than simply deliver fat to the infant. Moreover the diameter distribution might be altered under different physiological conditions since it has been shown to change in different lactation stages and subjected to nutrition administrations. Because MFG synthesis and secretion pathway is unique, major studies were conducted during the last two decades, pursuing ways to understand the MFG biological mechanisms enabling their synthesis and secretion. However the intracellular mechanisms remain illusive. If the basic mechanisms of the processes that dictate globule size distribution were known, and if indeed the nutritional importance is established, they could be controlled, and the size of particles, the compositions of surfaces and net composition of milk lipids (Fig. 5.3) be explicitly guided to desired goals.

The most studied lipid of the entire cream fraction of milk is the triacylglycerol core of the droplets which typically consist of more than 99% of milk lipids. Nonetheless, this overwhelming predominance of neutral lipids obscures the

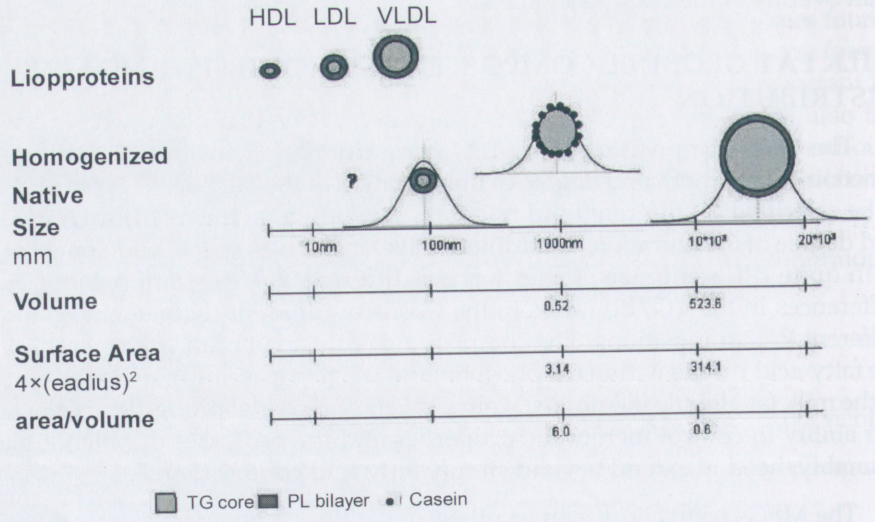



**Fig. 5.3. Milk fat globules size, volume and surface area parameters in native and homogenized milk compared with lipoproteins. Distribution plots indicated size distribution by number of the milk fat globules as was found in human breast milk and bovine homogenized milk.**

complexity of the processes, structures and functions of actual milk fat particles. Once those globules are secreted into the milk they are completely coated with the cellular bilayer membranes of the epithelial cells of the mammary gland. Therefore all the apical membrane physical, chemical and functional features are carried over to milk by the milk fat globule membrane. Although extensive proteomics studies were conducted in order to characterize the proteins in the MFGM it seems that a parallel lipidomic study has not yet been conducted. In the nutritional, as well as industrial point of view, this kind of research might be highly valuable. Because protein distribution is dictated by genes, the ability to alter their distribution within milk is somewhat restricted. However, the lipid fraction of the milk can be modified nutritionally. Therefore in order to enable control of the milk lipid macrostructure, further understanding of the regulating factors as well as the bioactivities of the macrostructure, is required.

## ENZYME COAGULATION

Chymosin, or rennet, is most often used for enzyme coagulation. During the primary stage, rennet cleaves the Phe(105)-Met(106) linkage of kappa-casein resulting in the formation of the soluble CMP which diffuses away from the micelle and para-kappa-casein, a distinctly hydrophobic peptide that remains on the micelle. The patch or reactive site, as illustrated in the above image, that is left on the micelles after enzymatic cleavage is necessary before aggregation of the paracasein micelles can begin.

During the secondary stage, the micelles aggregate. This is due to the loss of steric repulsion of the kappa-casein as well as the loss of electrostatic repulsion due to the decrease in pH. As the pH approaches its isoelectric point (pH 4.6), the caseins aggregate. The casein micelles also have a strong tendency to aggregate because of hydrophobic interactions. Calcium assists coagulation by creating isoelctric conditions and by acting as a bridge between micelles. The temperature at the time of coagulation is very important to both the primary and secondary stages. With an increase in temperature up to 40° C, the rate of the rennet reaction increases. During the secondary stage, increased temperatures increase the hydrophobic reaction. The **tertiary stage** of coagulation involves the rearrangement of micelles after a gel has formed. There is a loss of paracasein identity as the milk curd firms and syneresis begins.

## ACID COAGULATION

Acidification causes the casein micelles to destabilize or aggregate by decreasing their electric charge to that of the isoelectric point. At the same time, the acidity of the medium increases the solubility of minerals so that organic calcium and phosphorus contained in the micelle gradually become soluble in the aqueous phase. Casein micelles disintegrate and casein precipitates. Aggregation occurs as a result of entropically driven hydrophobic interactions.

## HEAT

At temperatures above the boiling point casein micelles will irreversibly aggregate. On heating, the buffer capacity of milk salts change, carbon dioxide is released, organic acids are produced, and tricalcium phophate and casein phosphate may be precipitated with the release of hydrogen ions.

## AGE GELATION

Age gelation is an aggregation phenomenon that affects shelf-stable, sterilized dairy products, such as concentrated milk and UHT milk products. After weeks to months storage of these products, there is a sudden sharp increase in viscosity accompanied by visible gelation and irreversible aggregation of the micelles into long chains forming a three-dimensional network. The actual cause and mechanism is not yet clear, however, some theories exist:

1. Proteolytic breakdown of the casein: bacterial or native plasmin enzymes that are resistant to heat treatment may lead to the formation of a gel
2. Chemical reactions: polymerization of casein and whey proteins due to Maillard type or other chemical reactions
3. Formation of kappa-casein-β-lactoglobulin complexes

## MILK PROTEIN GELS

Gelation of milk proteins is the crucial first step in both cheese and yogurt manufacture. Several types of milk gels are discussed, with an emphasis on recent developments in our understanding of how these gels are formed and some of their key physical properties. Areas discussed include the latest dual-binding model for casein micelles; some recent developments in rennet-induced gelation; review of the methods that have been used to monitor milk coagulation; and a discussion of some of the possible causes for the wheying-off defect in yogurts. Casein micelles are the primary building blocks of casein-based gels; however, controversy about its structure continues. The latest model proposed for the formation of casein micelles is the dual-binding model proposed by Horne, 1998, which suggests that casein micelles are formed as a result of two binding mechanisms, namely hydrophobic attraction and colloidal calcium phosphate (CCP) bridging. Most previous models for the casein micelle have treated milk gelation from the viewpoint of simple particle destabilization and aggregation, but they have not been able to explain several unusual rheological properties of milk gels. Although there have been many techniques used to monitor the milk gelation process over the past few decades, only a few appear attractive as possible in-vat coagulation sensors. Another important aspect of milk gels is the defect in yogurts called wheying-off, which is the appearance of whey on the gel surface. The factors responsible for its occurrence are still unclear, but they have been investigated in model acid gel systems.

## ACID MILK GELS

Acidified milk products like yoghurt are an important food product but there are relatively few reports on the mechanisms involved in gel formation and the effects of processing variables such as heat treatment and gelation temperature on the important physical properties (such as whey separation) of acid-induced gels. Most previous reviews have described the microbiology of the starter cultures and technologies used in yoghurt manufacture. Recent developments are reviewed including the use of techniques such as dynamic low amplitude oscillatory rheology to monitor the gel formation process; confocal scanning laser microscopy to examine gel microstructure; and various models for the aggregation of particle gels are discussed in terms of possible mechanisms involved in the formation of acid-induced milk gels.

### Properties of acid-coagulated milk gels

Acidified milk gels are one of the oldest and the most popular foodstuffs produced throughout the entire world. The popularity of fermented milk products such as fresh acid-coagulated cheese varieties (cream cheese, Cottage cheese, Quarg, tvorog, and frais), and yogurt, is due to various health claims and curative benefits. Irrespective of the commercial importance of these acidified milk products, there is not much information regarding formation, structures and physico-chemical properties of acid-coagulated milk products.

A wide variety of acidified milk products are produced. Some of the main products are fresh acid-coagulated cheese as stated above and yogurt. The manufacture and technologies engaged in the production of fresh acid cheese varieties have been reviewed. Additionally, there has been significant research on acid gels made with thermophilic cultures for the yogurt production. The objective of this article is mainly to give overall information of acidified milk gels and their physical, rheological and microstructural properties.

## PROTEIN GELS

Several kinds of proteins are able to form gels, and the type of gel network contributes to the texture characteristics of many food products by acting as a matrix, holding water with fat and other components. In addition, proteins are able to form different types of network structures depending on several factors such as temperature, pH, and salts. Figure 5.4 shows different types of network structures, which can be divided roughly into finestranded gels (Fig. 5.4(a) and (b)) and course aggregated gels (Fig. 5.4(c) and (d)), which commonly occurs in biopolymer gels. Fine-stranded gels are formed by an ordered association of molecules, and the networks are so small that these gel structures are transparent. The other type of networks, the aggregated gels, is formed into particulate gels that are not transparent, and this type of gels is common in milk and egg products.

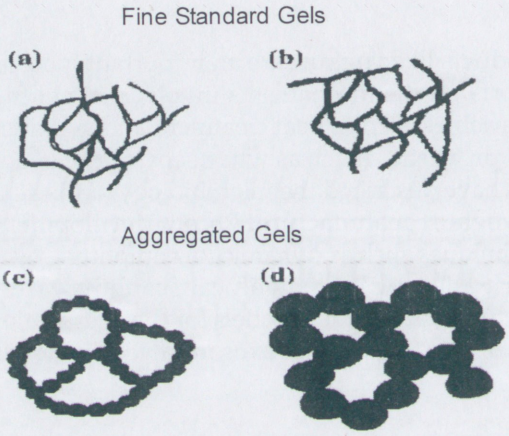



**Fig. 5.4. Diagrams of different types of gel networks: fine-stranded gels made up of (a) macromolecules and (b) globular proteins; (c) and (d) particulate gels.**

### Method of Acidification of Milk

The acidification method of milk can be done by using bacterial cultures, which ferment lactose to lactic acid, direct addition of acids, such as HCl, or the utilization of glucono-δ-lactone (GDL), which is hydrolyzed to gluconic acid, resulting in a reduction in pH. An extensive study of the formation and properties of milk gels made by cold acidification with direct addition of HCl and successive heating to the gelation temperature has been reported.

The comparison of the formation of acidified milk gels made with a bacterial culture or GDL has also been reported. From their study, the rate of acidification of milk gels using different media is found to be different. GDL is rapidly hydrolyzed to gluconic acid; on the contrary, in milk added starter culture, the pH drops little initially, but then decreases steadily with time.

Effect of acidification on the properties of casein micelles Caseins are phosphoproteins containing approximately 80% of the total content of milk proteins. Caseins are made up of many components. The main ones are $\alpha_{s1}$-casein, $\alpha_{s2}$-casein, β-casein, and κ-casein with considerable quantities of micellar or colloidal calcium phosphate (CCP) in the form of aggregates called casein micelles. Upon acidification, many of the physico-chemical properties of the casein micelles go through significant changes, especially in the pH range of 5.5 to 5.0, which includes voluminosity, salvation and dissociation of the caseins. In both heated and unheated milk, as the pH of milk is reduced to ~5.1 at a temperature above 22°C, most of the CCP is dissolved, and caseins are liberated into the serum phase. Although the internal casein micelle structure is changed caused by the loss of CCP, the average hydrodynamic diameter of casein micelles remains the same. Consequently, aggregation of casein occurs as the isoelectric point (pH ~4.6) is reached.

## MECHANISM OF THE ACID COAGULATION

### Theoretical Approaches

Gels formed from milk protein are irreversible, unlike many other food gels, and are involved in the manufacture of many dairy and non-dairy foods. Acid milk gels are types of gel that are aggregated particle networks forming continuous structures expanding throughout the entire volume. There are at least three theoretical models, which are adhesive sphere model, percolation models and fractal models, which have been used to explain the formation of acid-induced milk gels.

### Adhesive Sphere Model

It has been proposed, in this model, that casein micelles are sterically stabilized by the glycomacropeptide (GMP) part of κ-casein. The GMP acts as a polyelectrolyte brush, which collapses on the surface of the micelle as the pH of the system reaches the pKa of the charged groups on the brush, or while the charge density is reduced. Hence, this model has been used to quantitatively describe the aggregation reactions of destabilized casein micelles while lowering pH. The steric stabilization of the casein micelles declines, resulting in attractive interactions among casein micelles, while pH is being reduced. Although the concept of gelation is well explained, the adhesive sphere model could solely apply to circumstances where weak attraction between particles is present. In addition, this model fails to describe the elastic properties of the gel and the kinetics of the development in the shear modulus.

### Percolation models

It is assumed, in this theory, that percolation clusters form random bonds in a lattice between adjacent micelles, resulting in an increase in the size of clusters as number of bonds increases, which can be calculated in computer simulation. When the aggregation of percolation clusters reaches a certain threshold, it can be detected and marked as a gelation point in a rheometer where the elasticity is measurable. Analogies can be drawn between this model and gelation, which is that as particles aggregate, they establish numbers of links and bonds until a certain threshold, a cluster is created, and it spans the container. At this stage, only a fraction of bonds have been incorporated into the system-spanning cluster and this mechanism could well explain the continued increase in storage modulus of the gel after gelation. The drawback of the percolation model is that, it is relevant only close to the gelation point and it is unable to predict the amplitude of the elastic moduli. Additionally, this model cannot easily explain the mechanical properties of acid gels.

### Fractal models

The fractal theory has been used to describe the formation of many casein gels, and has been recently reviewed by the researchers. This model can be mathematically described with an assumption that hard sphere particles of radius

'a' can move by the Brownian movement and that when they come across each other, they would aggregate, resulting in aggregation of aggregated forms.

**Physico-chemical mechanisms**

At normal milk pH, most proteins have a net negative charge value causing long-range electrostatic repulsion and short-range hydration repulsion between protein molecules, which stabilizes casein micelles (Bringe and Kinsella, 1987). Three pH regions from pH 6.7 to 4.6 of interest are discussed below.

*pH 6.7 to ~ 6.0*

The net negative charge on the casein micelles is lowered because of the decrease in pH, resulting in the reduction of electrostatic repulsion. The structural size of the casein micelles is mostly the same, because only a small amount of CCP is dissolved above pH 6.0.

*pH 6.0 to ~ 5.0*

The charged κ-casein hairs on the micelle surface provide a stabilizing layer including both sterically and electrostatically. Upon acidification, the net negative charge continues to get lowered, resulting in the decrease in both electrostatic repulsion and steric stabilization, which are both largely responsible for micelle stability. Therefore, the charged hairs may shrink, and the micelles are more sensitive to aggregation. In addition, the decrease in pH provokes a reduction in the ionization of the acidic functions of the caseins (aspartic - glutamic - phosphoserine residues). Thus, the surface potential is decreased. Accordingly, the sequestering power of the $\alpha_s$ - α caseins simultaneously lessens, causing increases of the solubility of the phosphocalcic salt in water. This consequence causes a progressive transfer of the calcium and the inorganic phosphate from micelle to the aqueous phase, that is, the CCP in milk is dissolved entirely by pH around 5.0. Moreover, the dissociation of casein from the micelle largely depends on both temperature and pH. The maximum pH of casein dissociation at temperature <20°C is 5.2-5.4, owing to loosening of the molecular interactions between caseins as a result of the dissolved CCP, thereby causing increased electrostatic repulsion among the newly-exposed phosphoserine groups.

*pH < 5.0*

The acidification from pH 5.0 to 4.6 induces the disassociation of the calcium complexes by the phosphoserines, whereas the previous pH region (pH 6.6 to 5.0) induces mainly the solubilization of the calcium phosphate. This association appears to induce a disorganization of the micelles and a reorganization of the micellar sub-units. While the pH decreases and approaches the isoelectric point, the net negative charge is neutralized; thereby causing a reduction in the amphiphilic character of the â and ê caseins. Owing to calcium phosphate solubilization, electrostatic interaction is strengthened, whereas the electrostatic repulsion is weakened, causing the depolymerizations of the ás-caseins. Therefore, the

hydrophobic interaction is increased. Consequently, this causes the aggregation and formation of chains and cluster linked together as a three dimensional network.

Researchers stated that hydrophobic interactions are the main driving force for interactions between proteins for coagulation, gelation and gel syneresis. Therefore, the casein gels should be weaker with a decrease in temperature, because the hydrophobic interaction would be weaker. However, Researchers reported that hydrophobic interactions are not the major role for the strength of acidified milk gels, because the strength of the gels, as seen from viscoelastic moduli, are greater when the temperature is lowered. Where milk gels were acidified with 1.3% (w/w) GDL at two different incubation temperatures (30 and 40°C). Acidified milk gels incubated at lower temperature (30°C) had higher storage modulus (G2 ) values than those incubated at higher temperature (40°C).

Gelation in unheated milk gels, where acidification is the only coagulation method, occurs around pH 4.9; unless the acidification is performed at a very high temperature, where a higher gelation pH can be seen. Additionally, the strength of gels made from unheated milk is low. Thus they have low values for the viscoelastic moduli because of the dense clusters of aggregated casein particles from the extensive particle rearrangements during the gel formation. As a result, many particles from those clusters would not be entirely cross linked throughout the network.

Acidified milk gels made from heated milk form a gel at higher pH values because of the denaturation of whey proteins by high heat treatment. Consequently, when heated milk is acidified and become susceptible to aggregation, the denatured whey proteins, associated with the casein micelles, especially κ-caseins, have a high isoelectric pH (e.g. the main whey protein, β-lactoglobulin has an isoelectric pH of ~ 5.3). This isoelectric pH is higher than that of the caseins, resulting in the high pH of gelation of heated milk. In addition, the strength of gels made from heated milk is higher than those made from unheated milk, because denatured whey proteins associated with casein micelles interact with each other and act as bridging materials, thereby increasing the strength and number of bonds between protein particles. Gels made from milk heated at 80°C for 30 min prior to GDL addition had higher G2 values than those made from unheated milk.

### *Physical properties of acidified milk gels*

#### *Rheological properties*

Acid gels are viscoelastic materials. The rheological and textural properties can be determined from several test methods, such as small amplitude oscillatory rheology (SAOR), large amplitude oscillatory shear, penetration, and texture profile analysis. The ideal technique should be able to observe the gel formation process, since gelation is the primary step in the formation of most acid-type cheese. Hence, in order to study the gelation phase of an acidified milk gel, dynamic non-destructive techniques, such as SAOR, are needed.

*Microstructure and Textural Properties*

The textural properties of acidified milk gels are noticeably affected by the microstructure of these gels. The microstructural changes in casein micelles during the acidification of milk have been recently observed by reserachers. They studied the microstructure of acidified milk gels obtained from scanning electron microscopy (SEM). pH-induced changes in casein micelles during direct acidification and bacterial fermentation of reconstituted skim milk at 20°C were monitored. Acidified milk samples were taken by dipping an ANODISC® alumina inorganic membrane with an average pore diameter of 0.2 μm. The membranes with attached casein micelles were either directly dehydrated using an alcohol gradient and dried to $CO_2$ critical point, or dipped in pH-adjusted simulated milk ultrafiltrate (SMUF). The latter samples were then rapidly frozen in Freon 22 (cooled to -155°C with liquid nitrogen) and then freeze-dried at -80°C. Dried samples were gold-coated and examined with a JEOL JSM- 6400F field emission scanning electron microscope operated at 15 kV. Five main pH ranges of microstructural changes in casein micelles during milk acidification were proposed, which were:

1) The first stage of aggregation (pH 6.7 to 5.8), micelles started to loose their individuality, came closer and formed clusters, but the initial shape was still seen (Figure 5.5a,b; Figure 5.6a);

2) pH 5.5 to 5.3, most casein particles were significantly deformed, stretched, and extensively coalesced; thus forming a pseudo network with a very open structure (Figure 5.5c,d; Figure 5.6b,c);

3) pH 5.3 to 4.8, the network became denser and fragmented into small units with new casein particles acting like individual characteristics (Figure 5.6c, d, e);

4) pH 4.8 and 4.7, casein particles with the previous stage of fusion were followed by a stage of contraction and rearrangement, resulting in new casein particles with spherical shapes (Fig. 5.6e, f);

5) pH 4.6, the formation of acidified milk gels was completed with the casein particles aggregated into a true three-dimensional network of chains and clusters (Fig. 5.5e, f).

Confocal laser scanning microscopy (CLSM) has also been used to study on acid milk gels. CLSM is one of the most useful microscopy techniques to observe the microstructure of a wide variety of foods, especially dairy foods. A most important quality of the CLSM is its ability to "optically section" through samples, giving a 3-dimensional view of a food product with minimal sample disturbance, where other microscopy techniques would require physical disruption of the sample. Some researchers have found by using this technique that acid gels consist of a course particulate network of casein particles linked together in clusters, chains and strands, as shown in Figures 5.7 and 5.8.

A study performed at Moorepark examined the effect of whey protein and ionic environment on microstructure of acid-induced milk protein gels. Phosphocasein

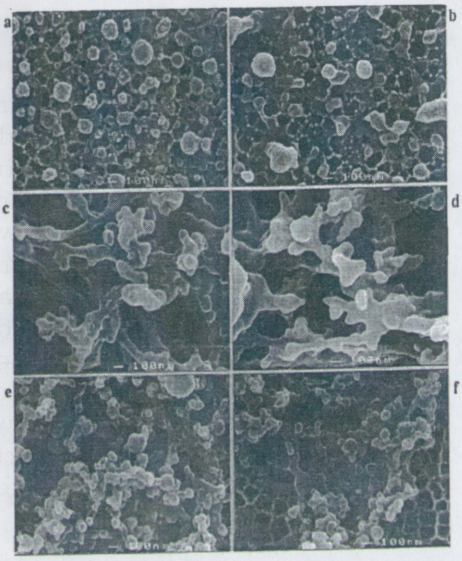


**Fig. 5.5. SEM micrographs of acidified milk samples with a comparison of the two different techniques of sample preparation for SEM. Critical point dried samples: a) pH 6.7, c) pH 5.3, e) pH 4.6; Freeze dried samples: b) pH 6.7, d) pH 5.3, f) pH 4.6. Scale bar represents 100 nm.**

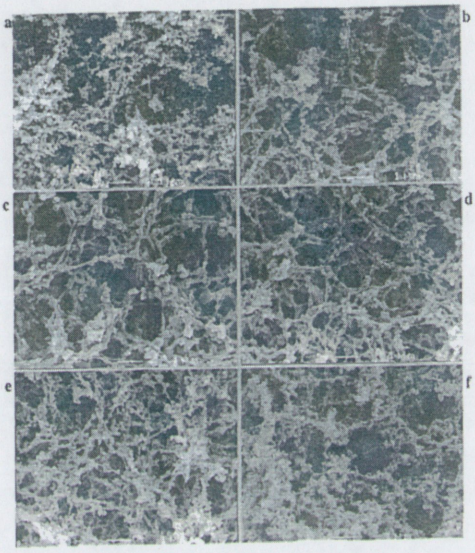


**Fig. 5.6. SEM micrographs of acidified milk critical-point dried samples; a) pH 5.8, b) pH 5.5, c) pH 5.3, d) pH 5.0, e) pH 4.8, f) pH 4.7. Scale bar represents 1 µm.**

(obtained by micro-filtration) and heat denatured whey protein (DWP) were dispersed (5% protein) in water, simulated milk ultra-filtrate (SMUF) or two-fold concentrated SMUF. Individual protein dispersions and 80:20 phosphocasein:whey protein mixtures were acidified at 40°C with glucono-δ-lactone until reaching pH 4.6 after two hours. CSLM allowed direct visualization of protein aggregation and subsequent network formation. The phosphocasein gels consisted of a coarse, open network of aggregated protein, whereas DWP gels appeared much finer networks with smaller pores. CSLM showed that increasing the SMUF concentration decelerated the onset of gelation. It was also shown that pre-denatured whey proteins interacted with casein micelles at comparatively low temperatures (25-40°C) with the presence of SMUF, causing the initiation of gelation at a higher pH (6.2). These results indicated that gel microstructure and viscoelastic properties can be controlled by the inclusion of denatured whey proteins and manipulation of ionic environment. These findings will help food technologists to create novel textures.

Researchers studied micellar casein gelation at high sucrose content by investigating the effect of sucrose addition on the formation of casein gels by acidification and/or renneting of pure micellar casein. Gelation kinetics and gel properties were followed by rheological methods, and microscopy and syneresis measurements were used to obtain a complete portrayal of the structures formed. CLSM was used to visualize the microstructures of the gels. To stain the proteins,

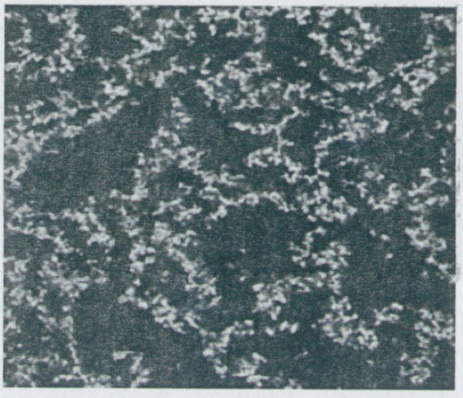


**Fig. 5.7. Confocal laser scanning microscopy image of acid casein gel. Protein network appears white/gray on dark background.**



**Fig. 5.8. A 3D projection of a stack of confocal laser scanning images of an acid casein gel. Protein network appears gray.**

Rhodamine B (0.001% wt/wt) was added to the dispersions, and then GDL or rennet was added and mixed. A small amount of the sample was placed on a microscope slide with a cavity and then covered with a cover slip. The samples were stored for 24 h at 20°C, and then placed on a temperature-controlled stage. Visualization was carried out with laser excitation of 488 nm, provided by CLSM. The microstructures of casein gels produced by acidification, renneting, or by a combination of acidification and renneting, in the presence or absence of sucrose, were compared. In the absence of sucrose, large water-containing pores are present between the casein aggregates, whereas in the presence of sucrose, the gel is more homogeneous with smaller aggregates linked together to form a fine meshed network. The network remains intact on storage, indicating that sucrose can prevent the local phase separation normally present in acid- or rennetinduced casein gels.

The textural properties of acidified milk gels are influenced by several factors, such as casein concentration, pH, temperature, the history of pH and temperature, total solid contents, and processing parameters such as homogenization pressure and incubation temperature. An extremely firm texture can be caused by a very high total solid content of the mix (both casein and fat) or an excessive amount of added stabilizers. On the contrary, the weak texture can be because of factors such as a low total solids (fat content) in the mix, deficient heat treatment of the milk, low acidity (high pH value), and too low gelation temperature.

## Conclusion

Although acidified milk gels have been made for thousands of years, the microbiological and technological features of the products have not been thoroughly studied until recently. During the past two decades, extensive research has been done on the formation and physical properties of acidified milk gels. Recent developments regarding possible mechanisms involved in the formation of acid-induced milk gels were discussed. Modern techniques were also reviewed,

containing the use of dynamic low amplitude oscillatory rheology to observe the process of gel formation, and confocal laser scanning microscopy to non-destructively monitor the gel microstructure. The author believes that future work is still needed in many areas; for instance, comparison of acidified milk gels made by different acidification techniques and development or modification of existing theoretical models for the formation of acidified milk gels.

## GELATION PROPERTIES OF SKIM MILK GELS

The effect of three milk pH values, 6.0, 6.3 and 6.7, on gelation properties was monitored by small amplitude oscillatory rheology as well as a large deformation (yield) test for gels induced by the plant coagulants, *Cynara cardunculus* L. and *Cynara humilis* L., and chymosin. Gel microstructure was studied using confocal scanning laser microscopy. For each coagulant, a decrease in pH of milk resulted in a decrease in gelation time (tg), and an increase in the rate of increase in storage modulus (G'). At pH 6.0 and 6.3, plant coagulant-induced gels reached a maximum value in G' (G'max) followed by a decrease in G' values during the rest of the experiment, reflecting higher proteolytic activity of plant coagulants towards caseins as determined by SDS-PAGE. Gels produced at pH 6.0 and 6.3, exhibited a minimum in loss tangent (tan d) followed by slight increase in tan d during gel aging, that may have been associated with faster rearrangements of the gel network structure. In gels aged for ~6 h, the values for G', tan d at low frequency (0.006 Hz) and yield stress were higher for chymosin than for plant-induced gels. For gels with the same pH value, no major differences were observed in microstructure between coagulants. Gels made at low pH values (6.3 and 6.0) appeared to have a denser or more interconnected structure than gels made at pH 6.7. Our results suggest that, at a low pH, the type of coagulant used in gelation is likely to have a considerably impact on gel/cheese structure.

# 6

# Properties of Milk

**Topics**

*Milk Composition • Constituents of Milk • Major Constituents • Physical Properties of Milk • Milk Carbohydrate (Lactose) • Milk Fat • Milk Protein • Vitamins and Minerals in Milk • Milk Enzymes*

Milk and dairy-based ingredients are used as components of many food products. Their contributions consist of unique flavor, desirable texture, excellent nutritive value, and a widely accepted "natural" image. In many instances, the success of the product in the marketplace is significantly enhanced by incorporation of traditional functional ingredients familiar to the consumer. Thus, dairy ingredients provide a consumer-friendly label on packaged foods.

Dairy ingredients contribute a number of characteristics critical to a food product. These include the emulsifying and stabilizing ability of caseinates, the gelling properties of whey protein concentrates and isolates, the water-absorption capacity of high-heat nonfat dry milk, and the browning of lactose during heat processing. Furthermore, the crystallization characteristics of lactose and the hydrolytic activity of the enzyme lactase are important in confectionery and frozen products. In addition, butter flavor carryover can be achieved with enzyme-modified butterfat and various cheese flavors imparted by enzyme-modified cheeses. Therefore, a food developer can select an appropriate dairy-based ingredient to create certain desirable attributes in foods. An understanding of the functional properties of dairy ingredients allows food technologists to utilize their potential contribution to product characteristics to meet consumer expectations.

## MILK COMPOSITION

Milk may be defined various ways. Chemically speaking, milk is a complex fluid in which more than 100 separate chemical compounds have been found. Its major components are water, fat, lactose, casein, whey proteins, and minerals (or

ash) in amounts varying with the milk of various species of animals. However, for any given species, the range of values for the constituents of milk is fairly constant.

From a physiological standpoint, milk is the secretion of the normally functioning mammary gland of the females of all mammals, which is produced for some time following parturition for the nourishment of the young of the species during the initial period of growth. In terms of physical chemistry, milk is an opaque, whitish fluid of multidisperse phases. The true solution contains lactose, vitamins, acids, enzymes, and some inorganic salts. The colloidal phase contains casein, calcium phosphate, and globular proteins. Fat exists in the form of an oil-in-water type of emulsion, with fat globules varying from 0.1 to 22 μm in diameter.

As a food ingredient or consumed by itself, milk provides an excellent nutritional profile in the human diet. Nutrition experts consider milk an exceptionally complete food because it contains significant levels of required nutrients such as protein, fat, carbohydrates, minerals, and several vitamins. Low-fat and nonfat milks are increasingly popular in fat-reduced and fat-free food formulations.

Worldwide, milk of the cow is by far of more commercial importance than milk of any other mammal. In the United States, the term "milk" legally refers to cow's milk. Milk from other species is labelled to indicate the type: sheep's milk, goat's milk, etc. Milk is the whole, clean lacteal secretion of one or more healthy cows, properly fed and kept, excluding that obtained within 15 days before calving and three to five days after. Colostrum, the milk secreted immediately after giving birth, is not considered milk from a legal standpoint. The U.S. Public Health Service's definition of Grade A milk is "the lacteal secretion practically free from colostrum, obtained by complete milking of one or more healthy cows, which contains not less than 8.25% milk solids-not-fat (MSNF) and not less than 3.25% milkfat."

## CONSTITUENTS OF MILK

Milk is composed of water, milkfat, and MSNF. The MSNF consists of protein, lactose, and minerals. These solids are also referred to as skim solids, or serum solids. The term total solids refers to the serum solids plus the milkfat.

## MAJOR CONSTITUENTS

The major constituents of milk vary more widely in individual cow's milk than in pooled market milk. Factors affecting the milk such as breed of cow, intervals of milking, stages of milking, different quarters of udder, lactation period, season, feed, nutritional level, environmental temperature, health status, age, weather, oestrum, gestation period, and exercise are known to cause variations in fat, protein, lactose, and mineral levels in milk derived from individual cows. In general, these variations tend to average out but display an interesting seasonal pattern in commercial milk used by food processors, which may have an important impact on properties of the finished products. An approximately 10% variation in fat and protein is observed in milk received in July and August (lowest level) compared to that received in October and November (highest level). Variations in protein and fat during the year affect

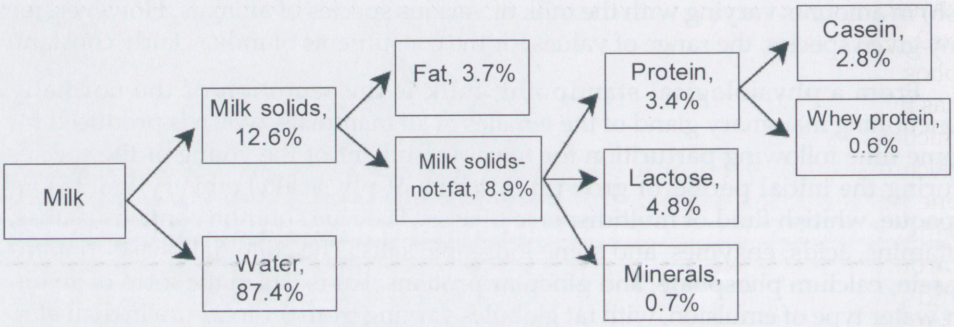



**Fig. 6.1. Gross composition of milk, showing major constituents.**

yogurt and cheese manufacture and whey protein production. For example, appropriate adjustments, such as fortification with additional nonfat milk solids, are necessary in yogurt production to ensure uniform viscosity throughout the year.

Milk proteins. Caseins make up approximately 80% of milk proteins. The remaining proteins are classified as whey proteins. Milk proteins and their concentration in milk are shown in Table 6.1.

Besides having a biological and nutritional role, caseins and caseinates are important because of their structure, charge, and physical proper-ties. Caseins become insoluble when the milk is acidified and the pH is reduced to 4.6, while the whey proteins remain in solution. Caseins exist in milk as particles called *micelles,* which are made up of calcium phosphate and casein complexes. The micelles are spherical particles varying in size from 50 to 200 nm and containing thousands of protein molecules. Caseins are further divided into $\alpha_{s1}$, $\alpha_{s1}$, β, and κ fractions. A γ-fraction is derived from the breakdown of β-casein by inherent proteolytic enzymes of milk. Each of the casein fractions exhibits distinct

**Table 6.1. Milk Proteins**

| Type | Nomenclature | Concentration (g/L of milk) |
|---|---|---|
| Casein | | 24–28 |
| | $\alpha_{s1}$-Casein | 12–15 |
| | $\alpha_{s2}$-Casein | 3–4 |
| | β-Casein | 9–11 |
| | κ-Casein | 3–4 |
| | γ-Casein | 1–2 |
| Whey protein | | 5–7 |
| | β-Lactoglobulin | 2–4 |
| | α-Lactalbumin | 1–1.5 |
| | Bovine serum albumin | 0.1–0.4 |
| | Immunoglobulins | 0.6–1.0 |
| | Proteose peptones | 0.6–1.8 |

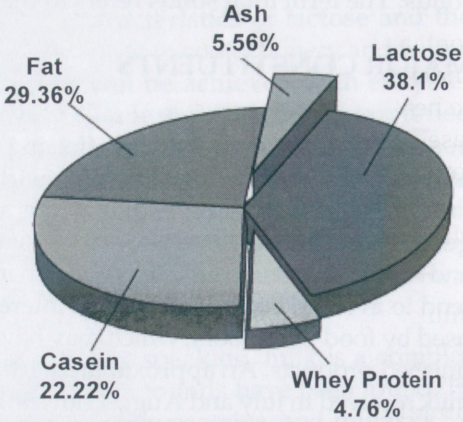



**Fig. 6.2. Composition of milk solids of whole milk.**

chemical properties. For example, αs1 and αs2 caseins have eight and 10–13 phosphoserine units, respectively. β-Casein has five phosphoserine units. All the casein fractions except κ-casein are precipitated by calcium, whereas κ- casein, which contains one phosphoserine unit, is not calcium-sensitive. Only κ-casein contains a carbohydrate moiety. Calcium (30 m*M*) and phosphate (20 m*M*) are complexed with αs1, αs2, and β- caseins, whereas κ-casein stabilizes the colloidal particles (micelles) by surface binding. During cheesemaking, the stabilizing κ-casein is cleaved by the enzyme *rennin*, resulting in destabilization of the micelle and subsequent curd formation.

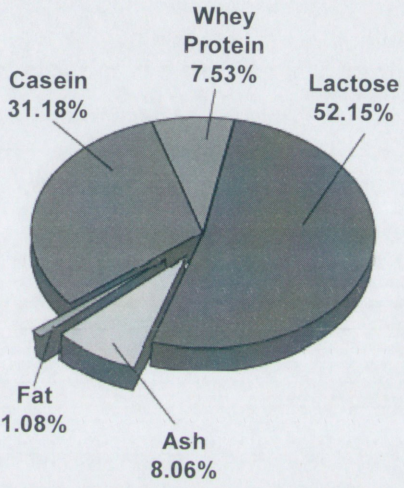



**Fig. 6.3. Composition of nonfat solids of skim milk.**

Whey proteins are located in solution in the *serum phase* of milk along with minor proteins and enzymes, including lactoperoxidase, lactotransferrin, lysozyme, glycoprotein, serum transferrin, and degradation products derived from casein.

Caseins and whey proteins are distinguishable from each other by their physical and chemical characteristics (Table 6.2). Caseins have a distinct, disordered molecular structure that lacks stabilizing disulfide bridges. This characteristic structure makes the casein fraction precipitate in acidic conditions as well as in the presence of di- and poly-valent ions of various salts. Casein molecules exist naturally in an open and extended state. Therefore, heat has little or no effect on them. However, severe heat treatment results in detachment of phosphate groups as well as formation of brown pigments by interaction with milk sugar (the Maillard reaction).

Whey proteins have a relatively more ordered molecular structure, which contains disulfide linkages. Accordingly, they are not vulnerable to precipitation under acidic conditions or by polyvalent ions. Like other globular proteins, they can be heat-denatured, resulting in gel formation. β-Lactoglobulin complexes with κ-casein in milk subjected to rigorous heat treatment. In food systems, milk proteins contribute to properties of the final product.

### Milkfat

The fat in milk occurs in microscopic globules in an oil-inwater type of emulsion. The globules vary in size from 0.1 to 22 μm in diameter. The *lipid* content of milkfat is 97–98% triacylglycerols, 0.2–1% phospholipids, 0.2–0.4% sterols, and traces of fatty acids, as well as vitamins A, D, E, and K. Milkfat is made up of 65% saturated, 32% monounsaturated, and 3% polyunsaturated fatty acids. It contains 7% short-chain fatty acids (C4–C8), 15–20% medium-chain fatty acids (C10–C14),

and 73–78% long-chain fatty acids (C16 or higher). The cholesterol content of whole milk (3.3% fat) and skim milk is 14 mg/100 ml and 2 mg/100 ml, respectively. The functional properties of milkfat are attributed to its fatty acid make-up.

**Table 6.2. Major Physical and Chemical Differences in Milk Proteins**

| Caseins | Whey Proteins |
|---|---|
| Strong hydrophobic regions | Both hydrophobic and hydrophilic regions |
| Little cysteine content | Both cysteine and cystine present |
| Random coil structure | Globular structure with helical contents |
| Heat stable | Easily heat denatured and insolubilized |
| Precipitate in acidic conditions and insoluble at pH 4.6 | Stable in mild acidic environment |
| Precipitated by di- and polyvalent ions | |

### Milk Sugar

Lactose is the major carbohydrate of milk, occurring at a 4.5–4.9% level. It consists of two forms (α and β) that differ in their sweetness and solubility. The α-form is less soluble (70 g/L at 15°C) than the β-form. Crystallization is important in the manufacture and utilization of several dairy ingredients. An equilibrium mixture of α- and β-lactose, formed by mutarotation, exhibits a solubility of 170 g/L of water. β-Lactose is slightly sweeter than the α-form. Overall, in sweetening power, lactose is only 16–33% as sweet as sucrose. The sweetening power is concentration dependent.

## MINOR AND TRACE CONSTITUENTS

The typical composition of major milk minerals is shown in Table 6.3.

Mineral concentration in milk is related to physical-chemical equilibria, which are important in processing, nutritive value, and shelf life of dairy products. Minerals include chloride, $PO_4^{2+}$, and citrates of $K^+$, $Na^+$, $Ca^{2+}$, and $Mg^{2+}$. Their concentration is <1% in milk, but they are involved in heat stability and alcohol coagulation of milk, age-thickening of sweetened condensed milk, *feathering* of coffee cream, rennin coagulation, and clumping of fat globules upon *homogenization*. The calcium level of milk influences the firmness of curd during cheesemaking. Ash, the white residue after incineration of a given weight of milk, is used as a measure of the mineral content of milk. It is not identical to milk mineral level because of decomposition and volatilization of certain minerals due to heat. Ash contains carbonates derived from organic constituents; sulfates from proteins; phosphate, partly from casein, which may contain approximately 1.62% phosphate; and chloride, which is partly lost (45–50%). Citric acid is completely lost. The average ash content is 0.70%, which is equivalent to 0.90% minerals.

**Table 6.3. Major Mineral Composition of Milk**

| Milk Salt Constituents | Concentration (mg/100 ml whole milk) | Concentration Range (mg/100 ml) |
|---|---|---|
| Calcium | 123 | 110–130 |
| Magnesium | 12 | 9–14 |
| Phosphorus (total) | 95 | 90–100 |
| Phosphorus (inorganic) | 75 | 70–80 |
| Sodium | 58 | 35–90 |
| Potassium | 141 | 110–170 |
| Chloride | 99 | 90–110 |
| Sulfate | 10 | ... |
| Carbonate (as $CO_2$) | 20 | ... |
| Citric acid | 160 | ... |

All the minerals considered essential for human nutrition are found in milk. They exist in milk in different states. Sodium, potassium, and chloride are ionic forms and in true solution. They permeate freely across the membrane during *ultrafiltration* and *electrodialysis* of milk and whey. Calcium, magnesium, inorganic phosphorus, and citrate exist in both colloidal and diffusible forms, depending on the pH of milk. Approximately 20–30% of diffusible calcium and magnesium exists as free ions and the remainder as salts of citrate and phosphate. As the pH of milk drops, the colloidal form is converted progressively to the ionic form. At pH 4.4, most of the minerals are in diffusible form.

By lowering the pH of milk or whey, protein concentrates of low mineral content and different mineral ratios can be produced. Trace elements are those constituents found in the parts per million level in milk. Table 6.5 shows their levels. In addition, several nonprotein nitrogen compounds, vitamins, and some organic materials are present in milk (Table 6.5).

**Table 6.4. Trace Minerals of Milk**

| Constituent | Concentration (μg/100 ml of milk) |
|---|---|
| Iron | 30–60 |
| Zinc | 200–600 |
| Copper | 10–60 |
| Manganese | 2–5 |
| Iodine | 2–6 |
| Fluoride | 3–22 |
| Selenium | 0.5–6.7 |
| Cobalt | 0.05–0.13 |
| Chromium | 0.8–1.3 |
| Molybdenum | 1.8–12 |
| Nickel | 0–5 |
| Silicon | 75–700 |
| Vanadium | 0–31 |
| Tin | 4–50 |
| Arsenic | 2–6 |

## PHYSICAL PROPERTIES OF MILK

### Color

The color of milk is perceived by consumers to be indicative of purity and richness. Its white color is due to the scattering of reflected light by the inherent ultramicroscopic particles, fat globules, colloidal casein micelles, and calcium phosphate. The intensity of white color is directly proportional to the size and number of particles in suspension. Homogenization increases the surface area of fat globules significantly as a result of the breakup of larger globules. Accordingly, homogenized milk and cream are whiter than their unhomogenized counterparts. Lack of fat globules gives skim milk a blue tinge. Cow's milk contains the pigments carotene and xanthophyll, which tend to give a golden yellow color to the milkfat.

**Table 6.5. Other Trace Components of Milk**

| Constituent | Concentration per 100 ml of Milk |
|---|---|
| Vitamins | |
| A | 40 µg retinol equivalent |
| B | |
| $B_1$ | 45 µg |
| $B_2$ | 175 µg |
| Niacin | 90 µg |
| $B_6$ | 50 µg |
| Pantothenic acid | 350 µg |
| Biotin | 3.5 µg |
| Folic acid | 5.5 µg |
| $B_{12}$ | 0.45 µg |
| C | 2 mg |
| D | 4 IU |
| E | 100 µg |
| K | 5 µg |
| Nonprotein nitrogen compounds (NPN) | |
| Total NPN | 22.9–30.8 mg |
| Urea N | 8.4–13.4 mg |
| Creatine N | 0.6–2 mg |
| Uric acid N | 0.5–0.8 mg |
| Orotic acid N | 1.2–1.3 mg |
| Choline | 4.3–28.5 mg |
| *N*-acetylneuraminic acid | 12–27 mg |
| Miscellaneous | |
| Lactic acid | 3.4–10.4 mg |
| Acetic acid | 0.3–5 mg |
| Formic acid | 1–8.5 mg |

### Flavour

The flavor of milk is a property difficult to define, but there is no doubt that taste and aroma are critical to the assessment of milk. Flavor constitutes a critical criterion of quality for the consumer. It is a sensory property in which odor and taste interact. The sweet taste of lactose is balanced against the salty taste of chloride, and both are somewhat moderated by proteins. This balance is maintained over a fairly wide range of milk composition even when the chloride ion level varies from 0.06 to 0.12%. Saltiness can be detected by sensory tests in samples containing 0.12% or more of chloride ions and becomes marked in samples containing 0.15%. Some workers attribute the characteristic rich flavor of dairy products to the lactones, methylketones, certain aldehydes, dimethyl sulfide, and certain short-chain fatty acids.

Although milk has a clean, pleasantly sweet flavor, it is quite bland, and therefore any off-flavors are readily discernible. Off-flavors result when the balance

of flavor compounds is altered by microbiological action, dairy farm or processing conditions, or chemical or biochemical reactions.

### Density and Specific Gravity

The density of milk with 3-5% fat averages 1.032 g/cm$^3$. Accordingly, the weight of 1 L of milk is 1.03 kg. To convert the weight of milk from kilograms per liter to pounds per gallon, the number is multiplied by 8.34.

The average specific gravity of milk at 15.5°C (60°F) is 1.032. It varies from 1.028 to 1.035 in commercial milk. The specific gravities of milkfat (0.93), MSNF (1.62), and water (1.0) determine the specific gravity of milk. Specific gravity is increased by the removal of fat and lowered by the addition of water.

### Surface Tension

Surface properties are involved in adsorption phenomena and the formation and stability of emulsions. They are relevant to creaming, fat globule membrane function, foaming, and emulsifier use in dairy products. Normal cow's milk has an inherent surface activity. Its surface tension approximates 70% of that of water. The surface tension of whole milk is usually about 50–52 mN/m (or dyn/cm) at 20°C. The surface tension of skim milk is 55–60 mN/m. For cream, it is approximately 46–47 mN/m. Casein, along with the proteolysis products *protease*-peptones, is largely responsible for the surface activity. Whey proteins make little contribution. Fat reduces surface tension by a physical effect. Lactose and most of the salts tend to raise it when they are present in true solution.

Surface tension decreases as milk temperature rises. Processing treatments such as heating, homogenization, and shear tend to increase surface tension.

### Foaming

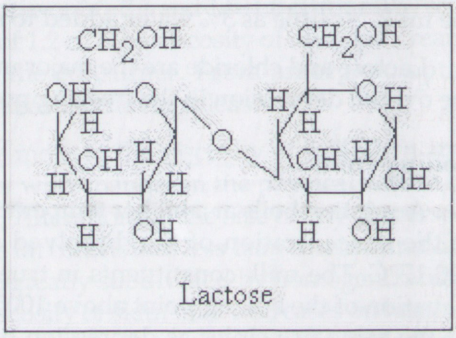
The formation of stable foam depends upon two main factors. First, the lowering of the surface tension allows the gathering and spreading of the surface-active components into thin films. Second, the films must be sufficiently elastic and stable to prevent the coalescence of the gas cells. A stable foam is thus formed when the surface tension of the liquid is not great enough to withdraw the film from between the gas cells and when the stabilizing agent has great internal viscosity.

Foaming of milk is at a minimum at 30–35°C. At 60°C, the foam volume is independent of the fat content. Below 20°C and above 30°C, the foaming tendency appears to increase. Fat tends to stabilize the foam formed below 20°C, for instance, during churning. Skim milk produces slightly more stable foam above 30°C than whole milk or light cream.

Foaming properties affect handling of milk products and how dairy-based ingredients are incorporated into other products.

### Milk Carbohydrate Chemistry

Milk contains approximately 4.9% carbohydrate that is predominately lactose with trace amounts of monosaccharides and oligosaccharides. Lactose is a disaccharide of glucose and galactose. The structure of lactose is:




### Lactose Physical Properties

Lactose is dissolved in the serum (whey) phase of fluid milk. Lactose dissolved in solution is found in 2 forms, called the α-anomer and β-anomer, that can convert back and forth between each other. The solubility of the 2 anomers is temperature dependent and therefore the equilibrium concentration of the 2 forms will be different at different temperatures. At room temperature (70°F, 20°C) the equilibrium ratio is approximately 37% α- and 63% β-lactose. At temperatures above 200°F (93.5°C) the β-anomer is less soluble so there is a higher ratio of α- to β-lactose. The type of anomer present does not affect the nutritional properties of lactose.

Lactose crystallization occurs when the concentration of lactose exceeds its solubility. The physical properties of lactose crystals are dependent on the crystal type and can greatly influence their use in foods. Temperature affects the equilibrium ratio of the α- and β-lactose anomers, as described above. Lactose crystals formed at temperatures below 70°F (20°C) are mainly α-lactose crystals. The α-monohydrate lactose crystals are very hard and form, for example, when ice cream goes through numerous warming and freezing cycles. This results in an undesirable gritty, sandy texture in the ice cream. Gums are often used in ice cream to inhibit lactose crystallization. The crystal form of β-lactose is sweeter and more soluble than the α-monohydrate lactose and may be preferred in some bakery applications. When a lactose solution is rapidly dried it does not have time to crystallize and forms a type of glass. Lactose glass exists in milk powders and causes clumping. The clumping is desirable because it results in a milk powder that dissolves instantly in water.

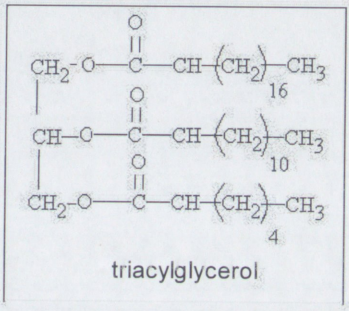
### Influence of Heat Treatments on Lactose Properties

The normal pasteurization conditions used for fluid milk have no significant effect on lactose. The higher temperatures used for ultra high temperature (UHT) pasteurization of extended shelf life products and spray drying can cause browning and isomerization reactions, which may affect product quality and nutritional properties. The browning reaction, called the Maillard reaction, occurs between the lactose and protein in milk and produces undesirable flavors and color, and decreases the available content of the amino acid lysine in milk protein. The isomerization reaction is a molecular rearrangement of lactose to lactulose. Lactulose is produced for use by the pharmaceutical industry in pill production.

## MILK FAT

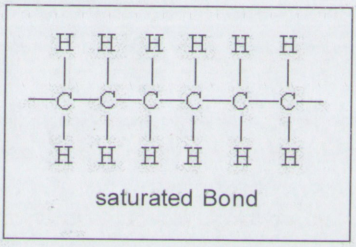
### General Fat Definition and Chemistry

Fats are made from individual fatty acid molecules attached to glycerol, a 3-carbon backbone. The most common type of fat is called a triglyceride, or triacylglycerol, which contains 3 fatty acids attached to the backbone and resembles a fork without the handle.

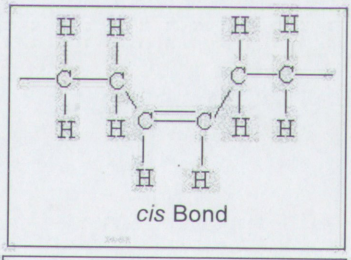


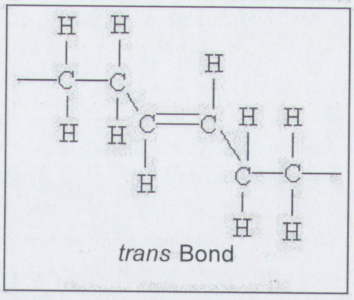
triacylglycerol

Because there are many different fatty acids that can be attached to the backbone, there are many different types of triglycerides or fats. Fat compounds can also be diglycerides that have 2 fatty acids or monoglycerides that have 1 fatty acid on the glycerol backbone. Mono- and diglycerides are used as emulsifiers, compounds that keep the fat and water from separating in foods such as ice cream.

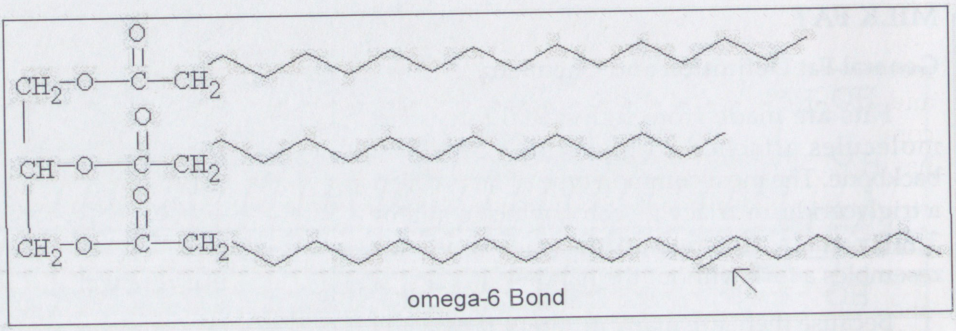

saturated Bond

Individual fatty acids can range in length from 4 to 22 carbons, and may be straight or branching chains. Carbon atoms have 4 bonding sites. Fatty acids may be saturated, which means that each carbon has a single bond to another carbon and 2 hydrogen atoms, or fatty acids may be unsaturated, which means that a carbon has two bonds to the adjacent carbon, called a double bond, and a single bond to another carbon and a hydrogen atom. A monounsaturated fat has 1 double bond and a polyunsaturated fat has 2 or more double bonds in the carbon chain. The bonds in unsaturated fatty acids can be either *cis* or *trans*, depending on the direction of the continuing carbon chain on each side of the double bond. A *cis* bond means that the fatty acid chain continues on the same side of the bond, forming a U shape, and a *trans*bond means that the fatty acid chain continues on opposite side of the bond, forming a Z shape.


*cis* Bond


*trans* Bond

The shorthand description for fatty acids is to list the number of carbons followed by a colon and the number of double bonds. For example, 4:0 is a 4 carbon chain with no double bonds, and 18:1 is an 18 carbon chain with 1 double bond. For unsaturated fatty acids the designation *c* is used for *cis*bonds and *t* is used for *trans* bonds. For example, C18:2 *c,c* is an 18 carbon chain with 2 double bonds that are both *cis*. The terms omega-3 or omega-6 refer to the carbon, either the 3rd or 6th carbon starting at the end of chain not attached to the glycerol backbone, at




which the first double bond appears. The Greek letter omega, Ù , is used to identify the omega position.

Other fatty compounds include phospholipids and sterols. The phospholipids have a basic triglyceride type structure but there is a phosphate group at the 3rd position on the carbon backbone. The phosphate group, a combination of phosphorus and oxygen, provides phospholipids with surface properties that are active at the interface between compounds soluble in water and those that are not, like fats. Phospholipids are important components of cell membranes. Phospholipids make up approximately 1% of the fat in milk. The two most abundant phospholipids are phosphotidyl choline and sphingomyelin. Sphingomyelin has been shown to have a protective effect in some cancers. Sterols, such as cholesterol, are complex chemical compounds that are important components of hormones.

**Milk Fat Chemistry**

Milk contains approximately 3.4% total fat. Milk fat has the most complex fatty acid composition of the edible fats. Over 400 individual fatty acids have been identified in milk fat. However, approximately 15 to 20 fatty acids make up 90% of the milk fat. The major fatty acids in milk fat are straight chain fatty acids that are saturated and have 4 to 18 carbons (4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0), monounsaturated fatty acids (16:1, 18:1), and polyunsaturated fatty acids (18:2, 18:3). Some of the fatty acids are found in very small amounts but contribute to the unique and desirable flavor of milk fat and butter. For example, the C14:0 and C16:0 β-hydroxy fatty acids spontaneously form lactones upon heating which enhance the flavor of butter.

The fatty acid composition of milk fat is not constant throughout the cow's lactation cycle. The fatty acids that are 4 to 14 carbons in length are made in the mammary gland of the animal. Some of the 16 carbon fatty acids are made by the animal and some come from the animal's diet. All of the 18 carbon fatty acids come from the animal's diet. There are systematic changes in milk fat composition that are due to the stage of lactation and the energy needs of the animal. In early lactation, the animal's energy comes largely from body stores and there are limited fatty acids available for fat synthesis, so the fatty acids used for milk fat production

are obtained from the diet and tend to be the longer chain 16:0, 18:0, 16:1 and 18:2 fatty acids. Later in lactation more of the fatty acids in milk are formed in the mammary gland so that the concentration of the short chain fatty acids such as 4:0 and 6:0 are higher than they are in early lactation. These changes in fatty acid composition do not have a great impact on milk's nutritional properties, but may have some effect on processing characteristics for products such as butter.

Milk fat contains approximately 65% saturated, 30% monounsaturated, and 5% polyunsaturated fatty acids. From a nutritional perspective, not all fatty acids are created equal. Saturated fatty acids are associated with high blood cholesterol and heart disease. However, short chain fatty acids (4 to 8 carbons) are metabolized differently than long chain fatty acids (16 to 18 carbons) and are not considered to be a factor in heart disease. Conjugated linoleic acid is a *trans* fatty acid in milkfat that is beneficial to humans in many ways.

The fatty acids are arranged on the triglyceride molecule in a specific manner. Most of the short chain fatty acids are at the bottom carbon position of the triglyceride molecule, and the longer fatty acids tend to be in the middle and top positions. The distribution of the fatty acids on the triglyceride backbone affects the flavor, physical, and nutritional properties of milk fat.

### Milk Fat Physical Properties

Milk fat melts over a wide temperature range, from approximately -40°F to 104°F (40°C). This is best illustrated by the firmness of butter at refrigerator temperature versus room temperature. At refrigerator temperature butter is approximately 50% solid, but is only about 20% solid at room temperature, which is why it spreads more easily as the temperature increases. The melting properties of milk are a result of the melting points of the individual fatty acids that make up milk fat and their arrangement on the triglyceride molecule.

The triglycerides of milk fat are in the form of globules. The globules are surrounded by a protein and phospholipid membrane that stabilizes the globules in the serum (water) phase of milk. The native globules range in size from less than 1 μm to over 10 μm. The uneven size distribution allows the larger globules to float in a process called creaming, thus resulting in a "cream line" at the top of the container. Milk is homogenized to reduce the size of the large globules to less than 1 μm, create a uniform distribution of globules throughout the serum phase, and minimize creaming.

### Deterioration of Milk Fat

Milk fat can be degraded by enzyme action, exposure to light, and oxidation. Each of these processes proceeds through different mechanisms. For further information see the references cited at the top of this page.

Enzymes that degrade fat are called lipases, and the process is called lipolysis. Milk lipases come from several sources: the native milk, airborne bacterial contamination, bacteria that are added intentionally for fermentation, or somatic

cells present in milk. Lipases remove fatty acids from the glycerol backbone of the triglyceride. Usually the action of lipase causes undesirable rancid flavors in milk. Pasteurization inactivates lipases and increases the shelf life of milk. However, in some cheeses, such as blue cheese and provolone, a small amount of lipolysis is needed to achieve the characteristic flavor.

Light induced degradation can happen fairly rapidly in milk and produces a characteristic off-flavor. The majority of this off-flavor is caused by protein degradation. Storing milk in opaque containers minimizes this process. Milk fat can also be degraded by a classical chemical oxidation mechanism, the attack on double bonds in the fatty acids by oxygen. Oxidation of the unsaturated phospholipids in milk produces off-flavors that are described as painty, fishy, or metallic.

### Influence of Heat Treatments on Milk Fat

Milk fat has a wide melting range, and is fully melted at 104°F (40°C). Typical high temperature short time (HTST) pasteurization conditions do not affect the functional and nutritional properties of milk fat. Higher heat treatments may stimulate oxidation reactions and cause fat deterioration and off-flavors. High heat treatments such as ultra high temperature (UHT) pasteurization can disrupt the milk fat globule membrane proteins and destabilize the globules, resulting in their coagulation.

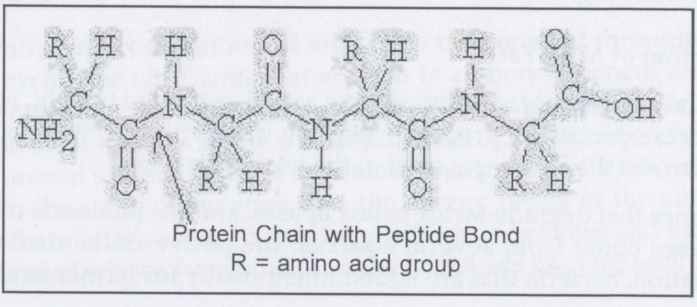
## MILK PROTEIN

### General Protein Definition and Chemistry

Proteins are chains of amino acid molecules connected by peptide bonds.

There are many types of proteins and each has its own amino acid sequence (typically containing hundreds of amino acids). There are 22 different amino acids that can be combined to form protein chains. There are 9 amino acids that the human body cannot make and must be obtained from the diet. These are called the essential amino acids.

The amino acids within protein chains can bond across the chain and fold to form 3-dimensional structures. Proteins can be relatively straight or form tightly compacted globules or be somewhere in between. The term "denatured" is used when proteins unfold from their native chain or globular shape. Denaturing



proteins is beneficial in some instances, such as allowing easy access to the protein chain by enzymes for digestion, or for increasing the ability of the whey proteins to bind water and provide a desirable texture in yogurt production.

**Milk Protein Chemistry**

Milk contains 3.3% total protein. Milk proteins contain all 9 essential amino acids required by humans. Milk proteins are synthesized in the mammary gland, but 60% of the amino acids used to build the proteins are obtained from the cow's diet. Total milk protein content and amino acid composition varies with cow breed and individual animal genetics.

There are 2 major categories of milk protein that are broadly defined by their chemical composition and physical properties. The casein family contains phosphorus and will coagulate or precipitate at pH 4.6. The serum (whey) proteins do not contain phosphorus, and these proteins remain in solution in milk at pH 4.6. The principle of coagulation, or curd formation, at reduced pH is the basis for cheese curd formation. In cow's milk, approximately 82% of milk protein is casein and the remaining 18% is serum, or whey protein.

The casein family of protein consists of several types of caseins ($\alpha$-$_{s1}$, $\alpha$-$_{s2}$, $\beta$, and $\kappa$) and each has its own amino acid composition, genetic variations, and functional properties. The caseins have a relatively random, open structure due to the amino acid composition (high proline content). The high phosphate content of the casein family allows it to associate with calcium and form calcium phosphate salts. The abundance of phosphate allows milk to contain much more calcium than would be possible if all the calcium were dissolved in solution, thus casein proteins provide a good source of calcium for milk consumers. The $\kappa$-casein is made of a carbohydrate portion attached to the protein chain and is located near the outside surface of the casein micelle. In cheese manufacture, the $\kappa$-casein is cleaved between certain amino acids, and this results in a protein fragment that does not contain the amino acid phenylalanine. This fragment is called milk glycomacropeptide and is a unique source of protein for people with phenylketonuria.

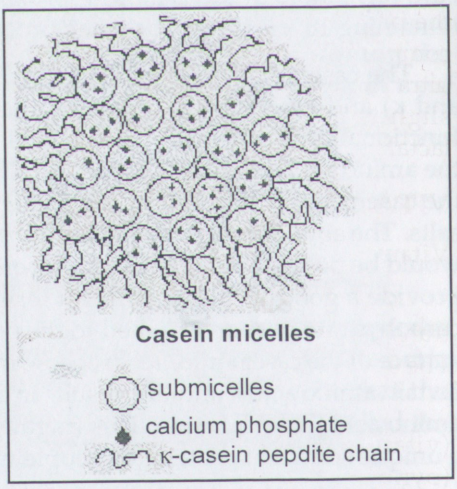
The serum (whey) protein family consists of approximately 50% $\beta$-lactoglobulin, 20% $\alpha$-lactalbumin, blood serum albumin, immunoglobulins, lactoferrin, transferrin, and many minor proteins and enzymes. Like the other major milk components, each whey protein has its own characteristic composition and variations. Whey proteins do not contain phosphorus, by definition, but do contain a large amount of sulfur-containing amino acids. These form disulfide bonds within the protein causing the chain to form a compact spherical shape. The disulfide bonds can be broken, leading to loss of compact structure, a process called denaturing. Denaturation is an advantage in yogurt production because it increases the amount of water that the proteins can bind, which improves the texture of yogurt. This principle is also used to create specialized whey protein ingredients with unique functional properties for use in foods. One example is the use of whey proteins to bind water in meat and sausage products.

The functions of many whey proteins are not clearly defined, and they may not have a specific function in milk but may be an artifact of milk synthesis. The function of β-lactoglobulin is thought to be a carrier of vitamin A. It is interesting to note that β-lactoglobulin is not present in human milk. α-Lactalbumin plays a critical role in the synthesis of lactose in the mammary gland. Immunoglobulins play a role in the animal's immune system, but it is unknown if these functions are transferred to humans. Lactoferrin and transferrin play an important role in iron absorption and there is interest in using bovine milk as a commercial source of lactoferrin.

### Milk Protein Physical Properties

The caseins in milk form complexes called micelles that are dispersed in the water phase of milk. The casein micelles consist of subunits of the different caseins ($\alpha_{s1}$, $\alpha_{s2}$ and β) held together by calcium phosphate bridges on the inside, surrounded by a layer of κ-casein which helps to stabilize the micelle in solution.

Casein micelles are spherical and are 0.04 to 0.3 μm in diameter, much smaller than fat globules which are approximately 1 μm in homogenized milk. The casein micelles are porous structures that allow the water phase to move freely in and out of the micelle. Casein micelles are stable but dynamic structures that do not settle out of solution. They can be heated to boiling or cooled, and they can be dried and reconstituted without adverse effects. β-casein, along with some calcium phosphate, will migrate in and out of the micelle with changes in temperature, but this does not affect the nutritional properties of the protein and minerals.




The whey proteins exist as individual units dissolved in the water phase of milk.

### Deterioration of Milk Protein

Proteins can be degraded by enzyme action or by exposure to light. The predominant cause of protein degradation is through enzymes called proteases. Milk proteases come from several sources: the native milk, airborne bacterial contamination, bacteria that are added intentionally for fermentation, or somatic cells present in milk. The action of proteases can be desirable, as in the case of yogurt and cheese manufacture, so, for these processes, bacteria with desirable proteolytic properties are added to the milk. Undesirable degradation (proteolysis) results in milk with off-flavors and poor quality. The most important protease in milk for cheese manufacturing is plasmin because it causes proteolysis during ripening which leads to desirable flavors and texture in cheese.

Two amino acids in milk, methionine and cystine are sensitive to light and may be degraded with exposure to light. This results in an off-flavor in the milk and loss of nutritional quality for these 2 amino acids.

### Influence of Heat Treatment on Milk Proteins

The caseins are stable to heat treatment. Typical high temperature short time (HTST) pasteurization conditions will not affect the functional and nutritional properties of the casein proteins. High temperature treatments can cause interactions between casein and whey proteins that affect the functional but not the nutritional properties. For example, at high temperatures, β-lactoglobulin can form a layer over the casein micelle that prevents curd formation in cheese.

The whey proteins are more sensitive to heat than the caseins. HTST pasteurization will not affect the nutritional and functional properties of the whey proteins. Higher heat treatments may cause denaturation of β-lactoglobulin, which is an advantage in the production of some foods (yogurt) and ingredients because of the ability of the proteins to bind more water. Denaturation causes a change in the physical structure of proteins, but generally does not affect the amino acid composition and thus the nutritional properties. Severe heat treatments such as ultra high pasteurization may cause some damage to heat sensitive amino acids and slightly decrease the nutritional content of the milk. The whey protein α-lactalbumin, however, is very heat stable.

## VITAMINS AND MINERALS IN MILK

### Vitamins in Milk

Vitamins have many roles in the body, including metabolism co-factors, oxygen transport and antioxidants. They help the body use carbohydrates, protein, and fat. The specific content of vitamins in milk is listed in the Nutrient Content Table.

Milk contains the water soluble vitamins thiamin (vitamin $B_1$), riboflavin (vitamin $B_2$), niacin (vitamin $B_3$), pantothenic acid (vitamin $B_5$), vitamin $B_6$ (pyridoxine), vitamin $B_{12}$ (cobalamin), vitamin C, and folate. Milk is a good source of thiamin, riboflavin and vitamin $B_{12}$. Milk contains small amounts of niacin, pantothenic acid, vitamin $B_6$, vitamin C, and folate and is not considered a major source of these vitamins in the diet.

Milk contains the fat soluble vitamins A, D, E, and K. The content level of fat soluble vitamins in dairy products depends on the fat content of the product. Reduced fat (2% fat), lowfat (1% fat), and skim milk must be fortified with vitamin A to be nutritionally equivalent to whole milk. Fortification of all milk with vitamin D is voluntary. Milk contains small amounts of vitamins E and K and is not considered a major source of these vitamins in the diet.

### Minerals in Milk

Minerals have many roles in the body including enzyme functions, bone formation, water balance maintenance, and oxygen transport. The specific content of minerals in milk is listed in the Nutrient Content Table 6.6.

**Table 6.6: Nutrient Content Tables**

| Nutrient | Milk (1 cup) | | | | |
|---|---|---|---|---|---|
| | Fat-free | Low-fat 1% | Reduced 2% | Whole | 1% Choc |
| Kilocalories | 83 | 102 | 122 | 149 | 158 |
| Protein (g) | 8 | 8 | 8 | 8 | 8 |
| Carbohydrate (g) | 12 | 12 | 12 | 12 | 26 |
| Sugars (g) | 12 | 13 | 12 | 12 | 25 |
| Fiber (g) | 0 | 0 | 0 | 0 | 1 |
| Total Fat (g) | 0 | 2.4 | 4.8 | 7.9 | 2.5 |
| Calories from Fat | 0 | 22 | 43 | 71 | 23 |
| Saturated Fat (g) | 0 | 1.5 | 3.1 | 4.6 | 1.5 |
| Cholesterol | 5 | 12 | 20 | 24 | 8 |
| Cholesterol (%DV) | 2% | 4% | 7% | 8% | 3% |
| Sodium (mg) | 103 | 107 | 115 | 105 | 152 |
| Sodium (%DV) | 4% | 5% | 5% | 4% | 6% |
| Potassium (mg) | 382 | 366 | 342 | 322 | 425 |
| Potassium (%DV) | 11% | 11% | 10% | 9% | 12% |
| Vitamin A (IU) | 500 | 478 | 464 | 395 | 490 |
| Vitamin A (%DV) | 10% | 10% | 13% | 11% | 14% |
| Vitamin B12 (ug) | 1.2 | 1 | 1.3 | 1.1 | 0.8 |
| Vitamin B12 (DV) | 20% | 17% | 22% | 18% | 13% |
| Vitamin C (mg) | 0 | 0 | 1 | 0 | 2.2 |
| Vitamin C (%DV) | 0% | 0% | 2% | 0% | 4% |
| Vitamin D (IU) | 115 | 117 | 120 | 124 | 108 |
| Vitamin D (DV) | 29% | 30% | 30% | 31% | 27% |
| Calcium (mg) | 299 | 305 | 293 | 276 | 290 |
| Calcium (%DV) | 30% | 31% | 29% | 28% | 29% |
| Folate (ug) | 12 | 12 | 12 | 12 | 12 |
| Folate (%DV) | 3% | 3% | 3% | 3% | 3% |
| Iron (mg) | 0.1 | 0.1 | 0.1 | 0.1 | 0.7 |
| Iron (%DV) | 1% | 1% | 1% | 1% | 4% |
| Magnesium (mg) | 27 | 27 | 27 | 24 | 32 |
| Magnesium (% DV) | 7% | 7% | 7% | 6% | 8% |
| Phosphorus (mg) | 247 | 232 | 224 | 205 | 258 |

(contd.)

| Nutrient | Milk (1 cup) | | | | |
|---|---|---|---|---|---|
| | Fat-free | Low-fat 1% | Reduced 2% | Whole | 1% Choc |
| Phosphorus (%DV) | 25% | 23% | 22% | 21% | 26% |
| Thiamin (mg) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Thiamin (%DV) | 7% | 7% | 7% | 7% | 7% |
| Riboflavin (mg) | 0.45 | 0.45 | 0.45 | 0.41 | 0.42 |
| Riboflavin (%DV) | 26% | 26% | 26% | 24% | 25% |
| Niacin (mg) | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 |
| Niacin (%DV) | 1% | 1% | 1% | 1% | 2% |

Vitamin D fortified dairy products can be an excellent source of vitamin D, however, levels vary considerably. Read the food label or contact manufacturer for specific levels.

The sugars listed on the Nutrition Facts label include naturally occurring sugars (like those in fruit and milk) as well as those added to a food or drink.

% DV = Daily Value, based on energy and nutrient recommendations for a general 2000-calorie diet.

**Units**: g=grams, mg=milligrams, µg=micrograms and IU=International Units.

Milk is a good source of calcium, magnesium, phosphorus, potassium, selenium, and zinc. Many minerals in milk are associated together in the form of salts, such as calcium phosphate. In milk approximately 67% of the calcium, 35% of the magnesium, and 44% of the phosphate are salts bound within the casein micelle and the remainder are soluble in the serum phase. The fact that calcium and phosphate are associated as salts bound with the protein does not affect the nutritional availability of either calcium or phosphate.

Milk contains small amounts of copper, iron, manganese, and sodium and is not considered a major source of these minerals in the diet.

### Effects of Heat Treatments & Light Exposure on the Vitamin & Mineral Content in Milk

The mild heat treatment used in the typical high temperature short time (HTST) pasteurization of fluid milk does not appreciably affect the vitamin content. However, the higher heat treatment used in ultra high temperature (UHT) pasteurization for extended shelf combined with the increased storage life of these products does cause losses of some water-soluble vitamins. Thiamin is reduced from 0.45 to 0.42 mg/L, vitamin $B_{12}$ is reduced from 3.0 to 2.7 µg/L, and vitamin C is reduced from 2.0 to 1.8 mg/L. Riboflavin is a heat stable vitamin and is not affected by severe heat treatments.

Calcium phosphate will migrate in and out of the casein micelle with changes in temperature. This process is reversible at moderate temperatures. This does not affect the nutritional properties of milk minerals. At very high temperatures the

calcium phosphate may precipitate out of solution which causes irreversible changes in the casein micelle structure.

Exposure to light will decrease the riboflavin and vitamin A content in milk. Milk should be stored in containers that provide barriers to light (opaque plastic or paperboard) to maximize vitamin retention.

## MILK ENZYMES

Each enzyme has a specific site of action on its target molecule, and optimal conditions (pH and temperature). There are a large number of enzymes in milk and the functions of many are not well-defined. It should be noted that the enzymes in milk do not make a major contribution to the digestion of milk in humans, which is accomplished by enzymes in the human stomach and small intestine.

Lipases are enzymes that degrade fats. The major lipase in milk is lipoprotein lipase. It is associated with the casein micelle. Agitation during processing may bring the lipase into contact with the milk fat resulting in fat degradation and off-flavors. Pasteurization will inactivate the lipase in milk and increase shelf life.

Proteases are enzymes that degrade proteins. The major protease in milk is plasmin. Some proteases are inactivated by heat and some are not. Protein degradation can be undesirable and result in bitter off-flavors, or it may provide a desirable texture to cheese during ripening. Proteases are important in cheese manufacture, and a considerable amount of information is available in the cheese literature.

Alkaline phosphatase is a heat sensitive enzyme in milk that is used as indicator of pasteurization. If milk is properly pasteurized, alkaline phosphatase is inactivated.

Lactoperoxidase is one of the most heat-stable enzymes found in milk. Lactoperoxidase, when combined with hydrogen peroxide and thiocyanate, has antibacterial properties. It is suggested that the presence of lactoperoxidase in raw milk inhibits the disease causing microorganisms (pathogens) present in milk. However, since there is no hydrogen peroxide or thiocyanate present in fresh milk, these compounds would have to be added to milk in order to achieve the antibacterial benefits. Lysozyme is another enzyme that has some antibacterial activities, although the amount of lysozyme present in milk is very small.

# 7

# Milk Microbiology

*Topics*

*Introduction • Bacterial Growth • Moulds • Yeasts • Viruses • Milk Microbiology • Microorganisms in Milk • HACCP • Bacteriophage • Starter Culture Preparation • Milk Pasteurisation • Milk Sterilisation • Methods of Milk Sterilisation and Preservation • Milk as a Substrate for Microorganisms • Contamination in Milk • Microbial Contamination from within the Udder • Microbial Contamination from the Exterior of the Udder • Milk hygiene during and after Milking*

## INTRODUCTION

Milk is virtually sterile when it is synthesized in a healthy cow's udder (mammary gland). Cows, like humans, are natural reservoirs of bacteria. Many of these bacteria are not harmful to humans, but some may be harmful to humans even though the cows are not affected and appear healthy.

Milk may become contaminated with bacteria during or after milking. The mammary glands of cows (and humans) can become inflamed due to a bacterial infection called mastitis. During a mastitis infection, very high numbers of bacteria present can be in the udder and in the milk. Some disease causing organisms (pathogens) can be shed through cow feces and may contaminate the outside of the udder and teats, the farm environment (bedding, for example) and the milking equipment. Although optimal growth conditions for bacteria are different for different organisms, milk contains important nutritional components for mammal growth, and, therefore, it is also an ideal medium for the growth of many different bacteria. Temperature plays an important role in bacterial growth. Many bacteria prefer to grow at body temperature (86-98°F, 30-37°C), but will grow at lower temperatures (such as refrigerator temperature) at slower rates.

The area of dairy microbiology is large and diverse. The bacteria present in dairy products may cause disease or spoilage. Some bacteria may be specifically

added to milk for fermentation to produce products like yogurt and cheese. A detailed discussion of fermentation bacteria is outside the scope of this web site, although these organisms are discussed briefly in the section on yogurt production and cheese production. This section of the web site is dedicated to the discussion of pathogens because of their importance in human health.

Human illness from milkborne pathogens is usually associated with consumption of raw milk or products made from raw milk such as fresh cheeses. In the past 20 years, foodborne illnesses from dairy product consumption have been predominantly associated with *Salmonella enterica, Listeria monocytogenes, Campylobacter jejuni,* and *Escherichia coli* O157:H7. These organisms have been isolated from bulk tank samples at rates ranging from 0.87% of samples taken for E. coli O157:H7 analysis in Ontario, Canada to 10% for *E. coli* O157:H7 in Wisconsin. Because there is a risk of pathogen contamination in milk produced from healthy cows under sanitary milk conditions, pasteurization of milk prior to consumption will destroy pathogens and provide protection for illness associated with consumption of dangerous microbes. Occasionally, human illness has been linked to pasteurized milk products but these cases usually have been a result of contamination of the product after pasteurization or improper pasteurization.

Micro-organism is the term applied to all microscopically small living organisms. We tend to associate micro-organisms with disease. Micro-organisms which cause disease are called pathogens. However, few micro-organisms are pathogens and micro-organisms play a crucial part in the life of our planet. For example, they provide food for fish, they occur in soil where they provide nutrients for plants and they play an important role in ruminant digestion.

In dairying some micro-organisms are harmful, e.g. spoilage organisms, pathogens, while others are beneficial-cheese and yoghurt starters, yeasts and moulds used in controlled fermentations in milk processing. The micro-organisms principally encountered in the dairy industry are bacteria, yeasts, moulds, and viruses.

**Bacteria**

Bacteria are single-celled organisms. They are present in air, water and on most solid materials. Bacterial cells are very small and can only be seen with the aid of a microscope.

When observed under a microscope the cells can be seen to differ in shape and in conformation of groups of cells. Cells are either spherical or rod-shaped (Fig. 7.1). Spherical bacteria are called cocci; those that are rod shaped are called bacilli. This is the first basis for differentiating between bacterial cells.

- Bacteria are also classified according to cell cluster formation:
- Diplococci - two cocci cells paired
- Staphylococci - a number of cells clustered together
- Streptococci - a number of cells arranged in a chain

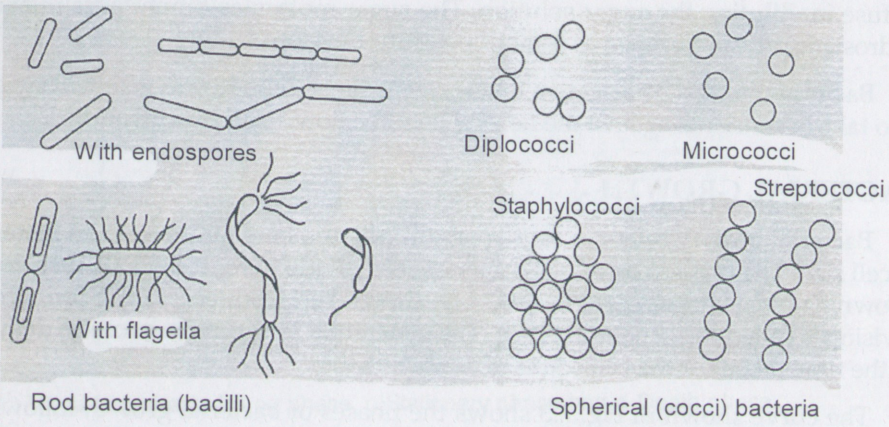



**Fig. 7.1. Rod-shaped (bacilli) and spherical (cocci) bacteria.**

Some bacteria are capable of locomotion by means of flagellae-long, hair-like appendages growing out of the cell. Some rod-shaped bacteria contain spores. These are formed when the cells are faced with adverse conditions, such as high temperature: once suitable conditions are reestablished the spores germinate to form new cells.

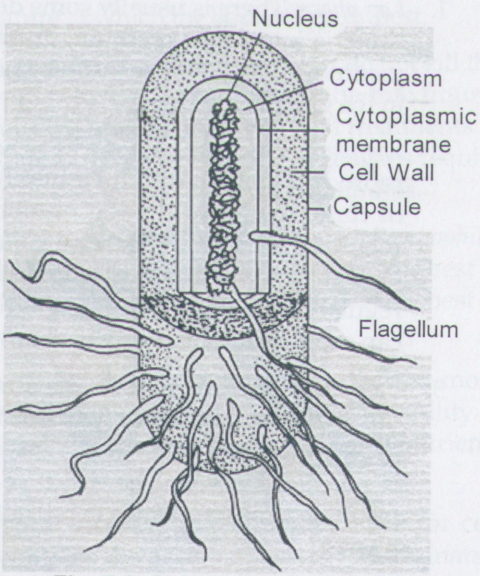



**Fig. 7.2. Schematic illustration of bacterial structure**

Close examination of the simple cell reveals that it is composed of the following components (Fig. 7.2):

- Cell wall-this gives the cell its shape and retains the constituents;
- Cell membrane-used for filtering in food constituents and discharging waste products;
- Nucleus-where the genetic material of the cell is stored;
- Cytoplasm-a semiliquid proteinaceous substance which contains starch, fat and enzymes.

The cell membrane is semi-permeable and allows the cell to feed by osmosis, i.e. the exchange of water between the cytoplasm of a living cell and the surrounding watery material. Only small molecules can pass in and out of the cell, e.g. with a sugar solution on one side of a semipermeable membrane and water on the other, water will

diffuse in, diluting the sugar solution. The sugar molecules cannot pass out so a hydrostatic pressure, known as osmotic pressure, develops.

Bacteria can feed by selective intake of nutrients dissolved in water. They can also take in nutrients against the normal osmotic flow—active transport.

## BACTERIAL GROWTH

Bacterial growth refers to an increase in cell numbers rather than an increase in cell size. The process by which bacterial cells divide to reproduce themselves is known as binary transverse fission. The time taken from cell formation to cell division is called the generation time. The generation time can therefore be defined as the time taken for the cell count to double.

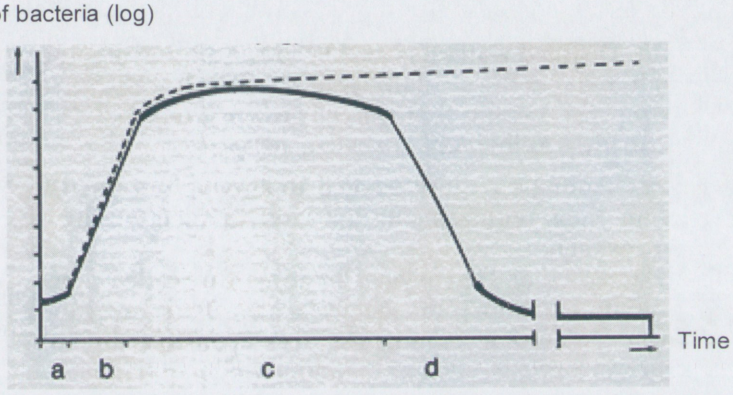
The curve shown in Fig. 7.3 shows the phases of bacterial growth following inoculation of bacteria into a new growth medium. The following phases can be identified:

1. *Lag phase:* There is usually some delay in growth following inoculation of bacteria into a new medium, during which time the bacteria adapt to the medium and synthesise the enzymes needed to break down the substances in the growth medium.
2. *Log phase:* Once the bacteria have adapted to the new medium they start to reproduce quickly and their numbers multiply evenly for each increment of time. A plot of the log number of cells against time gives a linear relationship: this is therefore called the log phase. The cells are at their greatest activity in this phase. Transferring cultures to a fresh medium at regular intervals can maintain the cells in an active state. An active culture can rapidly dominate any new environment.
3. *Stationary phase:* As the bacteria dominate the growth medium, they deplete the available nutrients or toxic waste products accumulate, slowing the rate of reproduction. At the same time, cells are dying off: A state of equilibrium is reached between the death of old cells and formation of new cells, resulting in no net change in cell numbers. This phase is called the stationary phase.
4. *Death phase:* In the next phase the formation of new cells ceases and the existing cells gradually die off: This is called the death phase.
5. The log phase can be prolonged by removing toxic waste, by adding more nutrients or both.

### Factors affecting bacterial growth

Bacterial growth is affected by (1) temperature, (2) nutrient availability, (3) water supply, (4) oxygen supply, and (5) acidity of the medium.

1. *Temperature:* Theoretically, bacteria can grow at all temperatures between the freezing point of water and the temperature at which protein or




a Lag phase, b Log phase, c Stationary phase and d Death phase

**Fig. 7.3. The four phases of bacterial growth.**

protoplasm coagulates. Somewhere between these maximum and minimum points lies the optimum temperature at which the bacteria grow best.

Temperatures below the minimum stop bacterial growth but do not kill the organism. However, if the temperature is raised above the maximum, bacteria are soon killed. Most cells die after exposure to heat treatments in the order of 70°C for 15 seconds, although spore-forming organisms require more severe heat treatment, e.g. live steam at 120°C for 30 minutes.

Bacteria can be classified according to temperature preference: *Psycrophilic* bacteria grow at temperatures below 16°C, *mesophilic* bacteria grow best at temperatures between 16 and 40°C, and *thermophilic* bacteria grow best at temperatures above 40°C.

2. ***Nutrients:*** Bacteria need nutrients for their growth and some need more nutrients than others. Lactobacilli live in milk and have lost their ability to synthesise many compounds, while *Pseudomonas* can synthesise nutrients from very basic ingredients.

   Bacteria normally feed on organic matter; as well as material for cell formation organic matter also contains the necessary energy. Such matter must be soluble in water and of low molecular weight to be able to pass through the cell membrane. Bacteria therefore need water to transport nutrients into the cell.

   If the nutrient material is not sufficiently broken down, the micro-organism can produce exo-enzymes which split the nutrients into smaller, simpler components so they can enter the cell. Inside the cell the nutrients are broken down further by other enzymes, releasing energy which is used by the cell.

3. ***Water:*** Bacteria cannot grow without water. Many bacteria are quickly killed by dry conditions whereas others can tolerate dry conditions for

months; bacterial spores can survive dry conditions for years. Water activity ($A_W$) is used as an indicator of the availability of water for bacterial growth. Distilled water has an $A_W$ of 1. Addition of solute, e.g. salt, reduces the availability of water to the cell and the $A_W$ drops; at $A_W$ less than 0.8 cell growth is reduced. Cells that can grow at low $A_W$ are called *osmophiles.*

4. ***Oxygen:*** Animals require oxygen to survive but bacteria differ in their requirements for, and in their ability to utilise, oxygen.

   Bacteria that need oxygen for growth are called *aerobic.* Oxygen is toxic to some bacteria and these are called *anaerobic.* Anaerobic organisms are responsible for both beneficial reactions, such as methane production in biogas plants, and spoilage in canned foods and cheeses.

   Some bacteria can live either with or without oxygen and are known as *faculative anaerobic* bacteria.

5. ***Acidity*:** The acidity of a nutrient substrate is most simply expressed as its pH value. Sensitivity to pH varies from one species of bacteria to another. The terms pH optimum and pH maximum are used. Most bacteria prefer a growth environment with a pH of about 7, i.e. neutrality.

   Bacteria that can tolerate low pH are called *aciduric.* Lactic acid bacteria in milk produce acid and continue to do so until the pH of the milk falls to below 4.6, at which point they gradually die off. In canning citrus fruits, mild heat treatments are sufficient because the low pH of the fruit inhibits the growth of most bacteria.

### Bacteria in Milk

Milk fresh from a healthy cow contains few bacteria, but contamination during handling can rapidly increase bacterial numbers. Milk is an ideal food and many bacteria grow readily in it.

Some bacteria are useful in milk processing, causing milk to sour naturally, leading to products such as *irgo.* However, milk can also carry pathogenic bacteria, such as *Salmonella, Tuberculosis bovis* and *Brucella,* and can thus transmit disease. Other bacteria can cause spoilage of the milk, and spoilage and poor yields of products.

## MOULDS

Moulds are a heterogeneous group of multicelled organisms which reproduce asexually either by spore formation or by fragmentation. They can grow on a wide variety of substrates and are generally regarded as spoilage organisms. However, moulds are used in the production of antibiotics and in certain cheese varieties. Moulds are aerobic organisms and their growth on foods can be retarded by excluding air through careful packaging. They can be killed by relatively mild heat treatments, but mould spores are more resistant to heat. The structure of moulds is shown in Fig. 7.4.

## YEASTS

Yeasts are unicellular organisms which reproduce asexually by budding. They are used industrially to ferment carbohydrates to such products as alcohol and citric acid. Yeasts are not usually used in milk processing and are normally regarded as spoilage organisms in dairy products. The structure of yeasts is shown in Fig. 7.5.

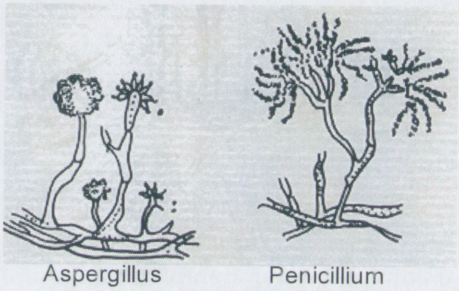



**Fig. 7.4. Structure of moulds.**

## VIRUSES

Viruses are extremely small organisms comprising a spherical head containing the genetic material and a cylindrical tail. They cannot reproduce themselves, and must invade other cells in order to reproduce. Viruses that attack bacterial cells are known as bacteriophages: bacteriophages that attack acid-producing bacteria inhibit acid production in milk.

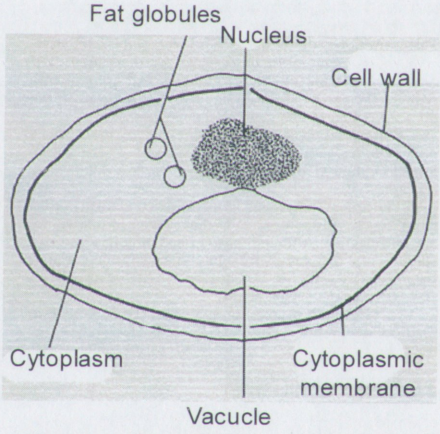



**Fig. 7.5. Structure of a yeast cell.**

## MILK MICROBIOLOGY

In addition to being a nutritious food for humans, milk provides a favourable environment for the growth of microorganisms. Yeasts, moulds and a broad spectrum of bacteria can grow in milk, particularly at temperatures above 16°C.

Microbes can enter milk via the cow, air, feedstuffs, milk handling equipment and the milker. Once microorganisms get into the milk their numbers increase rapidly. It is more effective to exclude micro-organisms than to try to control microbial growth once they have entered the milk. Milking equipment should be washed thoroughly before and after use—rinsing is not enough.

Bacterial types commonly associated with milk are given in Table 7.1.

Microbial growth can be controlled by cooling the milk. Most micro-organisms reproduce slowly in colder environments. Cooling milk also slows chemical deterioration.

The temperature of freshly drawn milk is about 38°C. Bacteria multiply very rapidly in warm milk and milk sours rapidly if held at these temperatures. If the milk is not cooled and is stored in the shade at an average air temperature of 16°C, the temperature of the milk will only have fallen to 28°C after 3 hours. Cooling the milk with running water will reduce the temperature to 16°C after 1 hour. At this

temperature bacterial growth will be reduced and enzyme activity retarded. Thus, milk will keep longer if cooled.

**Table 7.1. Bacterial types commonly associated with milk.**

| | |
|---|---|
| *Pseudomonas* | Spoilage |
| Brucella | Pathogenic |
| Enterobacteriaceae | Pathogenic and spoilage |
| **Staphylococci** | |
| *Staphylococcus aureus* | Pathogenic |
| Streptococcus | |
| *S. agalactiae* | Pathogenic |
| *S. thermophilus* | Acid fermentation |
| *S. lactis* | Acid fermentation |
| *S. lactis-diacetyllatic* | Flavour production |
| *S. cremoris* | Acid fermentation |
| *Leuconostoc lactis* | Acid fermentation |
| *Bacillus cereus* | Spoilage |
| **Lactobacillus** | |
| *L. lactis* | Acid production |
| *L. bulgaricus* | Acid production |
| *L. acidophilus* | Acid production |
| *Propionibacterium* | Acid production |
| *Mycobacterium tuberculosis* | Pathogenic |

Natural souring of milk may be advantageous: for example, in smallholder butter-making, the acid developed assists in the extraction of fat during churning. The low pH retards growth of lipolytic and proteolytic bacteria and therefore protects the fat and protein in the milk. The acidity of the milk also inhibits the growth of pathogens. It does not, however, retard the growth of moulds.

Naturally soured milk is used to make many products, e.g. *irgo*, yoghurt, sour cream, ripened buttermilk and cheese. These products provide ways of preserving milk and are also pleasant to consume. They are produced by the action of fermentative bacteria on lactose and are more readily digested than fresh milk.

The initial microflora of raw milk reflects directly microbial contamination during production. The microflora in milk when it leaves the farm is determined by the temperature to which it has been cooled and the temperature at which it has been stored.

The initial bacterial count of milk may range from less than 1000 cells/ml to 106/ml. High counts (more than 105/ml) are evidence of poor production hygiene. Rapid tests are available for estimating the bacterial quality of milk.

## MICROORGANISMS IN MILK

Milk is sterile at secretion in the udder but is contaminated by bacteria even before it leaves the udder. Except in the case of mastisis, the bacteria at this point are harmless and few in number. Further infection of the milk by microorganisms can take place during milking, handling, storage, and other pre-processing activities.

### Lactic Acid Bacteria

This group of bacteria are able to ferment lactose to lactic acid. They are normally present in the milk and are also used as starter cultures in the production of cultured dairy products such as yogurt. Note: many lactic acid bacteria have recently been reclassified; the older names will appear in brackets as you will still find the older names used for convenience sake in a lot of literature. Some examples in milk are:

- lactococci
  - *L. delbrueckii* subsp. *lactis* (*Streptococcus lactis*)
  - *Lactococcus lactis* subsp. *cremoris* (*Streptococcus cremoris*)
- lactobacilli
  - *Lactobacillus casei*
  - *L. delbrueckii* subsp. *lactis* (*L. lactis* )
  - *L. delbrueckii* subsp. *bulgaricus* (*Lactobacillus bulgaricus*)
- Leuconostoc

### Coliforms

Coliforms are facultative anaerobes with an optimum growth at 37°C. Coliforms are indicator organisms; they are closely associated with the presence of pathogens but not necessarily pathogenic themselves. They also can cause rapid spoilage of milk because they are able to ferment lactose with the production of acid and gas, and are able to degrade milk proteins. They are killed by HTST treatment, therefore, their presence after treatment is indicative of contamination.Escherichia coli is an example belonging to this group.

### Significance of Microorganisms in Milk

- Information on the microbial content of milk can be used to judge its sanitary quality and the conditions of production
- If permitted to multiply, bacteria in milk can cause spoilage of the product
- Milk is potentially susceptible to contamination with pathogenic microorganisms. Precautions must be taken to minimize this possibility and to destroy pathogens that may gain entrance
- Certain microorganisms produce chemical changes that are desirable in the production of dairy products such as cheese, yogurt.

### Spoilage Microorganisms in Milk

The microbial quality of raw milk is crucial for the production of quality dairy foods. Spoilage is a term used to describe the deterioration of a foods' texture, colour, odour or flavour to the point where it is unappetizing or unsuitable for human consumption. Microbial spoilage of food often involves the degradation of protein, carbohydrates, and fats by the microorganisms or their enzymes.

In milk, the microorganisms that are principally involved in spoilage are psychrotrophic organisms. Most psychrotrophs are destroyed by pasteurization temperatures, however, some like *Pseudomonas fluorescens, Pseudomonas fragi* can produce proteolytic and lipolytic extracellular enzymes which are heat stable and capable of causing spoilage.

Some species and strains of *Bacillus, Clostridium, Cornebacterium, Arthrobacter, Lactobacillus, Microbacterium, Micrococcus,* and *Streptococcus* can survive pasteurization and grow at refrigeration temperatures which can cause spoilage problems.

### Pathogenic Microorganisms in Milk

Hygienic milk production practices, proper handling and storage of milk, and mandatory pasteurization has decreased the threat of milkborne diseases such as tuberculosis, brucellosis, and typhoid fever. There have been a number of foodborne illnesses resulting from the ingestion of raw milk, or dairy products made with milk that was not properly pasteurized or was poorly handled causing post-processing contamination. The following bacterial pathogens are still of concern today in raw milk and other dairy products:

- *Bacillus cereus*
- *Listeria monocytogenes*
- *Yersinia enterocolitica*
- *Salmonella spp.*
- *Escherichia coli* O157:H7
- *Campylobacter jejuni*

It should also be noted that moulds, mainly of species of *Aspergillus, Fusarium,* and *Penicillium* can grow in milk and dairy products. If the conditions permit, these moulds may produce mycotoxins which can be a health hazard.

### HACCP

Raw and end-products may be tested for the presence, level, or absence of microorganisms. Traditionally these practices were used to reduce manufacturing defects in dairy products and ensure compliance with specifications and regulations, however, they have many drawbacks:

1. destructive and time consuming
2. slow response
3. small sample size
4. delays in the release of the food

In the 1960's, the Pillsbury Company, the U.S. Army, and NASA introduced a system for assuring pathogen-free foods for the space program. This system, called Hazard Analysis and Critical Control Points (HACCP), is a focus on critical food safety areas as part of total quality programs. It involves a critical examination of the entire food manufacturing process to determine every step where there is a possibility of physical, chemical, or microbiological contamination of the food which would render it unsafe or unacceptable for human consumption. These identified points are the critical control points (CCP). There are seven prinicples to HACCP:

1. analyze hazards
2. determine CCPs
3. establish critical limits
4. establish monitoring procedures
5. establish deviation procedures
6. establish verification procedures
7. establish record keeping procedures

Before these principles can be put into place, a prerequisite program and preliminary setup is necessary.

*Prerequisite Programme*

- premise control
- receiving and storage control
- equipment performance and maintenance control
- personnel training
- sanitation
- recall procedure

*Preliminary Setup:*

- assemble team
- describe the product
- identify intended use

- construct flow diagram and plant schematic
- verify the diagram on-site

**Starter Cultures**

Starter cultures are those microorganisms that are used in the production of cultured dairy products such as yogurt and cheese. The natural microflora of the milk is either inefficient, uncontrollable, and unpredictable, or is destroyed altogether by the heat treatments given to the milk. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. The primary function of lactic starters is the production of lactic acid from lactose. Other functions of starter cultures may include the following:

- flavour, aroma, and alcohol production
- proteolytic and lipolytic activities
- inhibition of undesirable organisms

There are two groups of lactic starter cultures:

1. simple or defined: single strain, or more than one in which the number is known
2. mixed or compound: more than one strain each providing its own specific characteristics

Starter cultures may be categorized as mesophilic, for example:

- *Lactococcus lactis* subsp. *cremoris*
- *L. delbrueckii* subsp. *lactis*
- *L. lactis* subsp. *lactis* biovar *diacetylactis*
- *Leuconostoc mesenteroides* subsp. *cremoris*

or thermophilic:

- *Streptococcus salivarius* subsp. *thermophilus (S. thermophilus)*
- *Lactobacillus delbrueckii* subsp. *bulgaricus*
- *L. delbrueckii* subsp. *lactis*
- *L. casei*
- *L. helveticus*
- *L. plantarum*

Mixtures of mesophilic and thermophilic microorganisms can also be used as in the production of some cheeses.

## BACTERIOPHAGE

Bacteriophages are viruses that require bacteria host cells for growth and reproduction. Initially, the bacteriophage attaches itself to the bacteria cell wall and

injects nuclear substance into the cell. Inside the cell, the nuclear substance produces shells, or phage coats, for the new bacteriophage which are quickly filled with nucleic acid. The bacterial cell ruptures and dies as the new bacteriophage are released.

Bacteriophages are ubiquitous but generally enter the milk processing plant with the farm milk. They can be inactivated heat treatments of 30 min at 63 to 88°C, or by the use of chemical disinfectants.

Bacteriophages are of most concern in cheese making. They attack and destroy most of the lactic acid bacteria which prevents normal ripening known as slow or dead vat.

## STARTER CULTURE PREPARATION

Commercial manufacturers provide starter cultures in lyophilized (freeze-dryed), frozen or spray-dried forms. The dairy product manufacturers need to inoculate the culture into milk or other suitable substrate. There are a number of steps necessary for the propagation of starter culture ready for production:

1. Commercial culture
2. Mother culture - first inoculation; all cultures will originate from this preparation
3. Intermediate culture - in preparation of larger volumes of prepared starter
4. Bulk starter culture - this stage is used in dairy product production

## MILK PASTEURISATION

Pasteurisation is the most common process used to destroy bacteria in milk. In pasteurisation, the milk is heated to a temperature sufficient to kill pathogenic bacteria, but well below its boiling point. This also kills many non-pathogenic organisms and thereby extends the storage stability of the milk.

Numerous time/temperature combinations are recommended but the most usual is 72°C for 15 seconds followed by rapid cooling to below 10°C. This is normally referred to as High Temperature Short Time (HTST) treatment. It is carried out as a continuous process using a plate heat-exchanger to heat the milk and a holding section to ensure that the milk is completely pasteurised. Milk is normally pasteurised prior to sale as liquid milk. Pasteurisation is used to reduce the microbial counts in milk for cheese-making, and cream is pasteurised prior to tempering for butter-making in some factories.

Batch pasteurisation is used where milk quantities are too small to justify the use of a plate heat-exchanger. In batch pasteurisation, fixed quantities of milk are heated to 63°C and held at this temperature for 30 minutes. The milk is then cooled to 5°C and packed.

The lower temperature used for batch pasteurisation means that a longer time is required to complete the process - 30 minutes at 63°C, compared with 15 seconds a 72°C.

### The Purpose of Pasteurization

1. To increase milk safety for the consumer by destroying disease causing microorganisms (pathogens) that may be present in milk.
2. To increase keeping the quality of milk products by destroying spoilage microorganisms and enzymes that contribute to the reduced quality and shelf life of milk.

### Pasteurization Conditions

The conditions were determined to be the minimum processing conditions needed to kill *Coxiella burnetii*, the organism that causes Q fever in humans, which is the most heat resistant pathogen currently recognized in milk. Milk can be pasteurized using processing times and temperatures greater than the required minimums.

Pasteurization can be done as a batch or a continuous process. A vat pasteurizer consists of a temperature-controlled, closed vat. The milk is pumped into the vat, the milk is heated to the appropriate temperature and held at that temperature for the appropriate time and then cooled. The cooled milk is then pumped out of the vat to the rest of the processing line, for example to the bottling station or cheese vat. Batch pasteurization is still used in some smaller processing plants. The most common process used for fluid milk is the continuous process. The milk is pumped from the raw milk silo to a holding tank that feeds into the continous pasteurization system. The milk continuously flows from the tank through a series of thin plates that heat up the milk to the appropriate temperature. The milk flow system is set up to make sure that the milk stays at the pasteurization temperature for the appropriate time before it flows through the cooling area of the pasteurizer. The cooled milk then flows to the rest of the processing line, for example to the bottling station. There are several options for temperatures and times available for continuous processing of refrigerated fluid milk. Although processing conditions are defined for temperatures above 200°F, they are rarely used because they can impart an undesirable cooked flavor to milk

### Effects of Pasteurisation on Milk

Pasteurisation reduces the cream layer, since some of the fat globule membrane constituents are denatured. This inhibits clustering of the fat globules and consequently reduces the extent of creaming. However, pasteurisation does not reduce the fat content of milk.

Pasteurisation has little effect on the nutritive value of milk. The major nutrients are not altered. There is some loss of vitamin C and B group vitamins, but this is insignificant.

The process kills many fermentative organisms as well as pathogens. Micro-organisms that survive pasteurisation are putrefactive. Although pasteurised milk has a storage stability of 2 to 3 days, subsequent deterioration is cause by putrefactive organisms. Thus, pasteurised milk will putrefy rather than develop acidity.

In rural milk processing, many processes depend on the development of acidity, and hence pasteurisation may not be appropriate.

## MILK STERILISATION

In pasteurisation, milk receives mild heat treatment to reduce the number of bacteria present. In sterilisation, milk is subjected to severe heat treatment that ensures almost complete destruction of the microbial population. The product is then said to be commercially sterile. Time/temperature treatments of above 100°C for 15 to 40 minutes are used. The product has a longer shelf life than pasteurised milk.

Another method of sterilisation is ultra-heat treatment, or UHT. In this system, milk is heated under pressure to about 140°C for 4 seconds. The product is virtually sterile. However, it retains more of the properties of fresh milk than conventionally sterilised milk.

## METHODS OF MILK STERILISATION AND PRESERVATION

### Sterilised Milk

The sterilisation of milk is accomplished by heating it up to the boiling point, 212° F. In a vacuum this may be accomplished by a temperature a few degrees lower.

The construction of steam sterilisers for milk, beef tea, or other foods is based upon a very simple plan. It is a familiar fact that the steam which rises from water heated in a tin or copper can condenses in drops on the lid, and this condensed vapour drips back along the sides of the can. If the lid is raised a little and is made somewhat larger than the can, the condensed steam will drip down on the outside of the can instead of the inside. If a second larger can be inverted like a hood or jacket over the first, the steam condensed between the two cans drips back and may be made to rejoin the water from which it started. In this way a kind of perpetual motion is kept up so long as a flame is applied beneath the water to vapourise it. Bottles containing the food to be sterilised are suspended in racks in the upper part of the inner chamber in the atmosphere of steam, and their contents soon acquire the temperature of boiling water, 212° F.

Siebert removes the grosser impurities of milk by placing it on a thick layer of sterilised absorbent cotton in a clean glass funnel. A quart of milk will pass through in ten or fiteen minutes.

Mobrun preserves milk for six months by warming it in a tin receptacle, the only opening of which is through a lead tube. The milk is rendered sterile by the heat, arid all air is driven out through the tube, which is then compressed and soldered.

The taste of sterilised milk is peculiar and resembles that of boiled milk. If put in bottles which have been sterilised by boiling water or steam, by stopping them with pledgets of absorbent cotton which have been baked, the milk will keep fresh for a number of days - long enough to be carried upon a voyage to England or across the continent.

It is suggested that prolonged sterilisation of milk may lessen its antiscorbutic powers for young infants, but scurvy in infants is due also to other causes, such as feeding with artificial foods, and prolonged exclusive plain milk diet for adults reduces them to a condition resembling scurvy.

According to Leeds, the following alterations are produced in milk by sterilisation at 212°F:

1. The amylolytic ferment is destroyed.
2. The casein coagulates less readily by rennin.
3. The digestibility of casein by the gastric and pancreatic juices is somewhat retarded.
4. The fat is less promptly absorbed than normally from the intestine.
5. If the heating is continued for some time, the milk sugar is destroyed.

The mineral salts of milk are also dissociated in some degree from their organic compounds.

It is thus demonstrated that the chief change produced by sterilisation of milk, and even by Pasteurisation at 167° F., is destruction of the enzymes or organic ferments of which this highly complex fluid contains a number. Babcock and Russell of Wisconsin have shown that when milk is rendered aseptic by salicylic acid, sodium fluoride or other germicides, a process of self-digestion goes on, which increases the longer the milk is kept. The result of this natural process is the formation of albumoses at the expense of casein, and the action is due to a ferment which these experimenters named "galactase," resembling trypsin.

Another ferment, an oxydase or anaeroxydase, has been demonstrated in cow's milk by a number of French experimenters. This ferment, as its name implies, causes a peculiar reaction in milk towards oxidising agents, such as turpentine or tincture of guars.

Spolverini identified a new ferment in cow's milk which resembles closely the glycolytic ferment of the blood.

A ferment, called by Bourquelot "lipase," has been identified in both human and cow's milk; its action is hydrolytic, resolving monobutyrin into glycerin and butyric acid.

In churning sterilised milk, butter forms more slowly than from raw milk, a period two or three times longer being required. Researcherattributes this to toughening of the albuminoid envelopes (of lactalbumin) of the fat globules, which is produced by the heat.

For these several reasons an infant to be fully nourished on sterilised milk requires more of it than of raw milk.

Practically, in normal stomachs of either infants or adults these changes are not sufficiently pronounced to seriously interfere with the digestibility of the milk, but

dyspeptic and catarrhal conditions of the stomach, especially in infants, make it highly sensitive to very slight modifications in the composition and reaction of milk.

In quite recent years it has been proved that the method of Pasteurisation is preferable to sterilisation, but there is, at the present time, a decided reaction against the prolonged use of both these types of milk, in distinction from temporary emergency use, and von Behring in common with many American clinicians maintains that when mother's milk is unprocurable, the proper infant food is good, fresh, clean milk, not heated or otherwise modified than by necessary dilution or sweetening, von Behring states that fresh milk contains powerful antibodies which react against the bacillus acidi lactici and colon bacillus. As these are the two types of bacilli commonly present in the infant organism, and as the heat of sterilisation or Pasteurisation promptly destroys the antibodies, by the use of heated milk the infant is deprived of a protective agent against gastro-intestinal disorder.

After all, in selecting a proper milk for infant feeding, the question of immediate digestibility is less important than that of permanent effect upon nutrition. The difficulty with infant feeding among the poor is to obtain really good, fresh, raw milk; hence in a given case the choice of feeding must ultimately depend upon the character of raw milk obtainable. When this is first-class the relative digestibility, as well as relative permanent nutritive value, stands as follows in order of preference: (1) raw, (2) Pasteurised, (3) sterilised.

The directions for predigestion combined with sterilisation of milk are given below:

1. Have thenursing bottlesprepared clean every morning.
2. Takecream, five ounces; milk, two and a half ounces.
3. Put in skillet; add pancreatin powder (pancreatih, two and a half grains; sodium bicarbonate, five grains); heat overalcoholflame for six minutes; stir and sip constantly; do not overheat.
4. Of this mixture put in each bottle six drachms (for a two-ounce bottle). Use funnel.
5. Add to each bottle ten drachms of sugar solution. (Make sugar solution by dissolving an ounce of sugar of milk (one powder) in a pint of warm water).
6. Stopper the mouth of each bottle with dry baked cotton and sterilise for twenty minutes.
7. Set aside to cool.
8. Before use put bottle in warming cup; apply nipple immediately before giving it to infant.

The alimentary canal of the newborn infant is sterile - it contains no bacteria - but after the first few passages of meconium two species of bacilli and one of micrococci are found (Escherich, Bes-lau), which disappear with the meconium. There are but two species of bacteria found in the stools of healthy nursing infants,

the Bacterium lactis aerogenes, obtained from the ileum below the duodenum, and Bacillus coli communis, from the colon.

In diarrhoeal conditions other bacteria, to the number of forty species, have been found (Booker). Their action in different diseases is not yet differentiated, but they are associated more or less directly with malfermentation and the formation within the intestines of gases and irritable or poisonous products. Many of these species thrive and are reproduced in hot weather outside the body in milk, in the atmosphere, and upon diapers and utensils. This explains the importance of absolute aseptic cleanliness in everything that pertains to the nursing and feeding of infants - cleanliness of the breasts and nipples, of the nurse's hands, of all receptacles for milk or other foods, of the child's body, and prompt removal and disinfection of soiled diapers or clothing.

Most of these bacteria grow best in milk, and the diarrhoeal diseases which are caused by and associated with them are absolutely preventable when the methods are understood of avoiding milk infection, and of sterilisation when it is infected. Vaughan says with proper emphasis: " If parents were willing to pay for wholesome uninfected milk half the fancy price which they readily give for some prepared baby food, their children would be better nourished and disease among them would be less frequent".

## MASTITIS AND SOMATIC CELLS

This section briefly defines and describes the relationship between Mastitis infection in cows, and the presence of Somatic Cells in milk.

### Mastitis

Mastitis is an inflammation of the udder, typically caused by a microbiological infection. Mastitis can occur in all mammals, including humans. Many types of microbes can cause infection and they can be transmitted from both environmental sources (for example, contaminated water, soil, bedding) and from contagious sources (from other infected cows). The microbes can enter the udder and multiply. The microbes can enter milk as it passes through the udder during the milking process. Mastitis in cows (or other dairy animals) can be a source of disease causing bacteria (pathogens) and spoilage organisms in milk.

Milk from cows infected with mastitis generally have higher total bacteria counts and somatic cell counts than milk from uninfected cows. Therefore, bacterial counts and somatic cells counts are used by dairy farmers and processors as indicators of milk quality. In general, the higher the counts, the lower the milk quality. Milk from mastitic cows may have off-flavors and may undergo deterioration of the milk fat and protein more quickly than milk from healthy cows.

There are regulatory standards for microbial numbers (total bacteria count) as well as quality control and human health parameters (somatic cell count, and antibiotic drug residues) in milk. Cows with mastitis are usually separated from the herd to help control the spread of infection and to ensure the quality of the milk

produced on that farm. On some farms, cows with mastitis are treated for the infection with antibiotics. The milk from the treated cows is either discarded or diverted to a separate tank to prevent contamination of milk collected from healthy cows. Milk from cows treated with antibiotics is not used for human consumption.

### Somatic Cells

Somatic cells are cells from the cow (predominantly white blood cells, otherwise known as leukocytes) that are normally present in milk. During most mastitis infections, the number of somatic cells present in the udder increases to help the cow fight the infection. There are several types of somatic cells that have different functions in fighting infection. Somatic cells can contain lipolytic and proteolytic enzymes, which degrade fats and proteins, respectively. An increase in somatic cells count during a mastitis infection increases the amount of destructive enzymes present in the milk, which increases the rate of deterioration of the milk fat and protein.

## ANTIBACTERIAL PROPERTIES OF MILK

This section describes the antibacterial properties of 2 minor proteins in milk, Lactoferrin and Lactoperoxidase.

### Lactoferrin

Lactoferrin is an iron-binding protein that is found in the milk, saliva, and other body fluids of mammals. Purified lactoferrin has been shown in research studies to have some antibacterial activity against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and other foodborne pathogens and spoilage organisms.

Purified lactoferrin (>95%) is produced on a commercial scale from skim milk and cheese whey. Although the natural lactoferrin content of milk is low, the availability of large quantities of milk and whey provide a good source of materials for lactoferrin production. The purification technique uses a high heat pasteurization process (194-212°F (90-100°C) for 5 to 10 min) to inactivate bacteria and viruses that may be present in raw milk. Consequently, the pasteurization conditions used for beverage milk do not destroy the activity of lactoferrin.

Purified lactoferrin is used commercially in infant formula, milk, yogurt, and nutritional supplements. The typical concentration of lactoferrin naturally present in beverage milk is 0.1 g/kg. There are currently no reports available in the scientific literature that have evaluated the effectiveness of the natural levels of lactoferrin in milk to prevent against illness from pathogens that may be present in the same milk.

### Lactoperoxidase

Lactoperoxidase is one of most heat stable enzymes found in milk. Lactoperoxidase has antibacterial activity when it is combined with hydrogen

peroxide and thiocyanate. The lactoperoxidase system has been used to reduce spoilage and extend the shelf-life of raw milk in countries where refrigeration may be unavailable (e.g., India). The lactoperoxidase system has been shown to be effective in reducing the growth of *Listeria monocytogenes* in raw milk at refrigerator temperatures.

It has been suggested that the presence of lactoperoxidase in raw milk inhibits the disease causing microorganisms (pathogens) present in milk. However, as hydrogen peroxide and thiocyanate must be added to milk in order to activate the system to achieve antibacterial benefits, (since the latter compounds are not naturally present in raw milk), it is unlikely that the lactoperoxidase system contributes significantly to control of pathogens in fresh raw milk.

## MICROBIAL GROWTH AND ITS CONTROL

### Non Sterilizing Methods to Control Microbial Growth

Many physical and chemical technologies are employed by our civilization to control the growth of (certain) microbes, although sterility may not the desired end-point. Rather, preventing spoilage of food or curing infectious disease might be the desired outcome.

### Applications of Heat

The lethal temperature varies in microorganisms. The time required to kill depends on the number of organisms, species, nature of the product being heated, pH, and temperature. Autoclaving, which kills all microorganisms with heat, is commonly employed in canning, bottling, and other sterile packaging procedures. This is an ultimate form of preservation against microbes. But, there are some other uses of heat to control growth of microbes although it may not kill all organisms present.

**Boiling**: 100° for 30 minutes (more time at high altitude). Kills everything except some endospores. It also inactivates viruses. For the purposes of purifying drinking water, 100° for five minutes is a "standard" in the mountains" though there have been some reports that Giardia cysts can survive this process. Longer boiling might be recommended for Mississippi River water the closer to the Gulf.

**Pasteurization** is the use of mild heat to reduce the number of microorganisms in a product or food. In the case of pasteurization of milk, the time and temperature depend on killing potential pathogens that are transmitted in milk, i.e., staphylococci, streptococci, *Brucella abortus* and *Mycobacterium tuberculosis*. But pasteurization kills many spoilage organisms, as well, and therefore increases the shelf life of milk especially at refrigeration temperatures (2°C).

Milk is usually pasteurized by heating, typically at 63°C for 30 minutes (batch method) or at 71°C for 15 seconds (flash method), to kill bacteria and extend the milk's usable life. The process kills pathogens but leaves relatively benign microorganisms that can sour improperly stored milk.

During the process of **ultrapasteurization**, also known as ultra high-temperature (UHT) pasteurization, milk is heated to temperatures of 140 °C. In the direct method, the milk is brought into contact with steam at 140°C for one or two seconds. A thin film of milk falls through a chamber of high-pressure steam, heating the milk instantaneously. The milk is flash cooled by application of a slight vacuum, which serves the dual purpose of removing excess water in the milk from condensing steam. In the indirect method of ultrapasteurization, milk is heated in a plate heat exchanger. It takes several seconds for the temperature of the milk to reach 140°C, and it is during this time that the milk is scalded, invariably leading to a burned taste. If ultrapasteurization is coupled with aseptic packaging, the result is a long shelf life and a product that does not need refrigeration.

A review of protocols and recommendations for the use of heat to control microbial growth is given in Table 7.2.

**Table 7.2. Recommended use of heat to control bacterial growth**

| Treatment | Temperature | Effectiveness |
|---|---|---|
| Incineration | >500° | Vaporizes organic material on nonflammable surfaces but may destroy many substances in the process |
| Boiling | 100° | 30 minutes of boiling kills microbial pathogens and vegetative forms of bacteria but may not kill bacterial endospores |
| Intermittent boiling | 100° | Three 30-minute intervals of boiling, followed by periods of cooling kills bacterial endospores |
| Autoclave and pressure cooker (steam under pressure) | 121°/15 minutes at 15 # pressure | kills all forms of life including bacterial endo-endospores. The substance being sterilized must be maintained at the effective T for the full time |
| Dry heat (hot air oven) | 160°/2 hours | For materials that must remain dry and which are not destroyed at T between 121° and 170° Good for glassware, metal, not plastic or rubber items |
| Dry heat (hot air oven) | 170°/1 hour | Same as above. Note increasing T by 10 degrees shortens the sterilizing time by 50 percent |
| Pasteurization (batch method) | 63°/30 minutes | kills most vegetative bacterial cells including pathogens such as streptococci, staphylococci and Myco-bacterium tuberculosis |
| Pasteurization (flash method) | 72°/15 seconds | Effect on bacterial cells similar to batch method; for milk, this method is more conducive to industry and has fewer undesirable effects on quality or taste |

(contd.)

| Treatment | Temperature | Effectiveness |
|---|---|---|
| Ultrapasteurization (direct method) | 140°/2 seconds | Effect on most bacterial cells is lethal. For milk, this method creates a product with relatively long shelf life at refrigeration temperatures. |

*Low Temperature (refrigeration and freezing)*

Most organisms grow very little or not at all at 0°C. Perishable foods are stored at low temperatues to slow rate of growth and consequent spoilage (e.g. milk). Low temperatures are not bactericidal. Psychrotrophs, rather than true psychrophiles, are the usual cause of food spoilage in refrigerated foods. Although a few microbes will grow in supercooled solutions as low as minus 20°C, most foods are preserved against microbial growth in the household freezer.

*Drying (removal of $H_2O$)*

Most microorganisms cannot grow at reduced water activity ($A_w < 0.90$). Drying is often used to preserve foods (e.g. fruits, grains, etc.). Methods involve removal of water from product by heat, evaporation, freeze-drying, and addition of salt or sugar.

*Irradiation (UV, x-ray, gamma radiation)*

Irradiation destroys microorganisms as described under "sterilization". Many spoilage organisms are readily killed by irradiation.

In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent. The practice has not been accepted in the U.S. UV light can be used to pasteurize fruit juices by flowing the juice over a high intensity ultraviolet light source. UV systems for water treatment are available for personal, residential and commercial applications and may be used to control bacteria, viruses and protozoan cysts.

The FDA has approved irradiation of poultry and pork to control pathogens, as well as foods such as fruits, vegetables, and grains to control insects, and spices, seasonings, and dry enzymes used in food processing to control microorganisms. Food products are treated by subjecting them to radiation from radioactive sources, which kills significant numbers of insects, pathogenic bacteria and parasites.

According to the FDA, irradiation does not make food radioactive, nor does it noticeably change taste, texture, or appearance. Irradiation of food products to control food-borne disease in humans has been generally endorsed by the United Nation's World Health Organization and the American Medical Association. Two important Disease-causing bacteria that can be controlled by irradiation include *Escherichia coli* 0157:H7 and *Salmonella* species.

**Table 7.3. Common antiseptics and disinfectants**

| Chemical | Action | Uses |
|---|---|---|
| Ethanol (50-70%) | Denatures proteins and solubilizes lipids | Antiseptic used on skin |
| Isopropanol (50-70%) | Denatures proteins and solubilizes lipids | Antiseptic used on skin |
| Formaldehyde (8%) | Reacts with $NH_2$, SH and COOH groups | Disinfectant, kills endospores |
| Tincture of Iodine (2% I2 in 70% alcohol) | Inactivates proteins | Antiseptic used on skin<br>Disinfection of drinking water |
| Chlorine ($Cl_2$) gas | Forms hypochlorous acid (HClO), a strong oxidizing agent | Disinfect drinking water; general disinfectant |
| Silver nitrate ($AgNO_3$) | Precipitates proteins | General antiseptic and used in the eyes of newborns |
| Mercuric chloride | Inactivates proteins by reacting with sulfide groups | Disinfectant, although occasionally used as an antiseptic on skin |
| Detergents (e.g. quaternary ammonium compounds) | Disrupts cell membranes | Skin antiseptics and disinfectants |
| Phenolic compounds (e.g. carbolic acid, lysol, hexylresorcinol, hexachlorophene) | Denature proteins and disrupt cell membranes | Antiseptics at low concentrations; disinfectants at high concentrations |
| Ethylene oxide gas | Alkylating agent | Disinfectant used to sterilize heat-sensitive objects such as rubber and plastics |
| Ozone | Generates lethal oxygen radicals | Purification of water, sewage |

## Control of Microbial Growth by Chemical Agents

**Antimicrobial agents** are chemicals that kill or inhibit the growth microorganisms. Antimicrobial agents include chemical preservatives and antiseptics, as well as drugs used in the treatment of infectious diseases of plants and animals. Antimicrobial agents may be of natural or synthetic origin, and they may have a static or cidal effect on microorganisms.

## Types of Antimicrobial Agents

- **Antiseptics**: microbicidal agents harmless enough to be applied to the skin and mucous membrane; should not be taken internally. Examples include alcohols, mercurials, silver nitrate, iodine solution, alcohols, detergents.
- **Disinfectants**: agents that kill microorganisms, but not necessarily their spores, but are not safe for application to living tissues; they are used on inanimate objects such as tables, floors, utensils, etc. Examples include, hypochlorites, chlorine compounds, lye, copper sulfate, quaternary ammonium compounds, formaldehyde and phenolic compounds.

  Common antiseptics and disinfectants and their uses are summarized in Table 7.3. Note: disinfectants and antiseptics are distinguished on the basis of whether they are safe for application to mucous membranes. Often, safety depends on the concentration of the compound.
- **Preservatives**: static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic. Examples are calcium propionate, sodium benzoate, formaldehyde, nitrate and sulfur dioxide. Table 7.4a and 7.4b are lists of common preservative and their uses.

**Table 7.4a. Some common preservatives added to processed foods**

**Salt** - retards bacterial growth. Not good for blood pressure.

**Nitrates** - can be found in some cheeses, adds flavor, maintains pink color in cured meats and prevents botulism in canned foods. Can cause adverse reactions in children, and potentially carcinogenic.

**Sulfur Dioxide and Sulfites** - are used as preservatives and to prevent browning in alcoholic beverages, fruit juices, soft drinks, dried fruits and vegetables. Sulfites prevent yeast growth and also retard bacterial growth in wine. Sulfites may cause asthma and hyperactivity. They also destroy vitamins.

**Benzoic Acid and Sodium Benzoate** - are used to preserve oyster sauce, fish sauce, ketchup, non-alcoholic beverages, fruit juices, margarine, salads, confections, baked goods, cheeses, jams and pickled products. They have also been found to cause hyperactivity.

**Propionic Acid and Propionates** - used in bread, chocolate products, and cheese for lasting freshness.

**Sorbic Acid and Sorbates** - prevent mold formation in cheese and flour confectioneries

**Table 7.4b. Common food preservatives and their uses**

| Preservative | Effective Concentration | Uses |
|---|---|---|
| Propionic acid and propionates | 0.32% | Antifungal agent in breads, cake, Swiss cheeses |
| Sorbic acid and sorbates | 0.2% | Antifungal agent in cheeses, jellies, syrups, cakes |
| Benzoic acid and benzoates | 0.1% | Antifungal agent in margarine, cider, relishes, soft drinks |
| Sodium diacetate | 0.32% | Antifungal agent in breads |
| Lactic acid | unknown | Antimicrobial agent in cheeses, buttermilk, yogurt and pickled foods |
| Sulfur dioxide, sulfites | 200-300 ppm | Antimicrobial agent in dried fruits, grapes, molasses |
| Sodium nitrite | 200 ppm | Antibacterial agent in cured meats, fish |
| Sodium chloride | unknown | Prevents microbial spoilage of meats, fish, etc. |
| Sugar | unknown | Prevents microbial spoilage of preserves, jams, syrups, jellies, etc. |
| Wood smoke | unknown | Prevents microbial spoilage of meats, fish, etc. |

## MILK AS A SUBSTRATE FOR MICROORGANISMS

The substrates are nutrients required for growth, metabolism and activity of microbial cells. Microbes, especially bacteria can grow on variable growth substrates and hence possess versatile metabolic activities. Nutritional versatility makes bacteria adaptable to different types of habitats and environments. That is why they are cultured in laboratory; a particular strain of the genus can be cultured on various nutrient media. Depending upon growth and energy substrate requirement, bacteria are classified as organotrophs and lithotrophs. Organotrophs grow on organic compounds and lithotrophs need inorganic substrates for growth. They can be chemotrophic (chemical compounds) or phototrophic (light energy) depending upon their source of energy. All microorganisms require carbon as substrate for building up cell components; autotrophic microbes use carbon dioxide as sole source of carbon while as heterotrophs require organic compounds as carbon source. Obligate parasites like viruses and rickettsias fulfill their carbon and energy requirement by growing and living in host tissue. Taking into consideration, the type of nutrition of particular species, bacteria are cultured on laboratory conventional nutrient media. These media are composed of known concentrations of essential growth elements like carbon, nitrogen, phosphorus, potassium in utilizable form; trace elements like salts of Fe, Mg, Mn, Mo, Cu, Ni, B, Co, Ca

dissolved in water. Nutrient medium is sometimes supplied with growth factors like amino acids and vitamins $B_1$, $B_6$, $B_{12}$, K, C, biotin, niacin, riboflavin and pantothenic acid. Conventional media are also called as defined media as their composition is known. Bacteria can also be cultured on chemically undefined media; such media contain substrates whose chemical composition is unknown. Such substrates are added as source of protein, carbohydrates, vitamins and fatty acids. They are generally derived from plant or animal tissues and routinely used in microbiology laboratories for growing the heterotrophic microorganisms. Some chemically undefined substrates are extracts of beef, yeast, meat, malt, corn meal, soil, leaf, root or seed; rumen fluid, blood serum, whole blood, casein hydrolysate and peptone.

**Unusual Substrates**

Microbes produce adaptive enzymes in response to the presence of substrates; therefore many bacteria are able to utilize substances that are not usually preferred by other organisms. Metabolic versatility is hence the function of enzymes; hydrolases especially, which confer an extraordinary ability to grow on unusual substrates. These substrates are wastes from kitchen, agriculture, heavy industries, dairy, poultry, food processing, paint and varnish, paper and pulp, fish market, vegetable market, waste treatment or compositing units and least but not the last from hydroelectric and nuclear power stations. To name some, carbon monoxide and other toxic environmental pollutants, lignin, cellulose, lignocelluloses, pectin, spent wash, molasses, Corn steep liquor, sewage, sludge, human and animal excreta, night soil, organic solvents, waste vegetable oils, petroleum byproducts, raw milk and whey are unusual substrates used by bacteria for the growth.

**Substrates in Natural Environment**

Among natural growth environments, soil is enriched habitat favoring the growth of diverse group of microorganisms. Soil and rhizosphere regions are plentiful with substrates such as root exudates consisting of sugars, amino acids, vitamins, fatty acids; humus, lignin, cellulose, pectin, compost derivatives, organic matter from dead remains of plants, animals or microbes, inorganic salts and minerals. Water bodies contain organic matter, trace elements, metal salts and pollutants received from different sources. Substrate composition of water is always dynamic and changes during flow and rainy season. Marine aquatic system consists of higher concentration of various salts and is also subjected to fluctuations in substrate composition. Air does not contain any growth substrate but moisture and dust in air harbor many forms of microbes including their spores. Microbial cells or spores can remain suspended in air in the form of droplet nuclei or aerosols for longer time.

**Substrate Related Phenomena**

1. **Diauxie growth** - When two substrates are provided in the growth medium, microbes are known to consume the substrates sequentially. The cells will

first consume the 'preferred substrate' over less preferred substrate. The second substrate will be consumed only when the first preferred one is completely utilized. Microbial cells thus are not greedy but very wise and patient! This sequential utilization of two substrates in growth medium was first time observed by Scientist Monod who called it 'Diauxie' (2 growth phases in Greek) phenomenon.

2. **Extracellular digestion** - Some substrates are macromolecules (cellulose) or highly charged molecules (DNA) and cannot pass cell membrane. To utilize such substrates, microbes degrade them outside the cell with the help of hydrolytic enzymes. This extracellular digestion breaks down larger molecules into smaller which can enter into cell to be metabolized endogenously. In *Bacillus* spp. starch, pectin and RNA are digested to glucose, galacturonic acid, and ribonucleocides by production of enzymes amylase, pectinase and ribonuclease respectively.

3. **Substrates in limiting and excess quantity** - Nutrient substrate limitation or excess is a kind of stress condition that microbes always encounter in natural environment. However, limiting or starvation conditions are more prevalent. Excess nutrients generally do not promote increased growth response as one might expect but instead they can be toxic and deleterious for survival. Excess amount of particular nutrient may also lead to scarcity of other nutrient/s. Microbes have adapted themselves to survive under substrate limiting conditions by one or other means. The most common strategy is reduced endogenous metabolism but it is not applicable for requirement of trace elements. Normally essential trace elements Fe, Ni, Cu, Mo and Zn are always present in limiting amount in particular niche which is necessary for proper metabolic functioning of microbe. In complex microbial community such as in soil, there is always competition to acquire these metal ions and microbes have developed sequestration systems (siderophores) to chelate these ions for growth. Increase in concentration of even one of the metal ions is highly toxic for growth and hence the survival of microorganisms. When nutrients are in excess, some microbes store them in cytoplasm as inclusions in polymeric forms such as PHB, glucan or polyphosphate granules. They are utilized during nutrient stress conditions. Some microbes like soil actinomycetes may transform into physiologically inactive state like spores to sustain unfavorable nutrient and environmental conditions.

**Substrate Utilization for Purpose**

Microbial ability to grow on unusual substrates has been exploited for the production of secondary metabolites like antibiotics, vitamins and biosurfactants, enzymes, amino acids and SCP. They are also used for production of compost feed and fertilizer mixture. Microbe's potential to use different conventional and unconventional substrates is being used in industrial-domestic waste treatment, recycling processes, sewage treatment and biogas generation, in bioremediation and

oil spill cleanup strategies. It seems that microbial substrate utilization is not limited to growth but it is very productive, meant for scavenging deadly and dirty and also the best substitute for environmental cleanup.

## CONTAMINATION IN MILK

Milk is natural and essential food for human being worldwide. The adulteration and contamination in milk are rampant in India and it is facing shortage of milk. More especially clean, hygienic & quality milk is not at all available, further more certified organic cow's fat free milk. To meet growing demand, the touts are coming up with spurious/synthetic milk. Some fraudulent milk handlers are caught for deliberately contaminating milk with low quality, cheap, non-edible, toxic substances. Consumption of such white substance with the name of milk/milk products is dangerous to the life of human being.

Water, salt, sugar, wheat flour, ground rice, blotting paper, baking soda, washing soda, caustic soda, urea, hydrogen peroxide, formalin are common adulterants used in milk. Apart from that It is general tendency of the milking animal owners to inject stimulus chemicals into the body of cattle in order to fetch more milk. The milk so contaminated with stimulus chemicals may hazardous to health.

The milk secreted into an uninfected cow's udder is sterile. Invariably it becomes contaminated during milking, cooling and storage, and milk is an excellent medium for bacteria, yeasts and moulds that are the common contaminants. Their rapid growth, particularly at high ambient temperatures can cause marked deterioration, spoiling the milk for liquid consumption or manufacture into dairy products. This can be avoided by adopting the simple, basic rules of clean milk production.

### Possible Reasons of Contamination

In India the organized sector carries out only procurement and processing and marketing/distribution of milk. In fact, in India "dairying" means only procurement & processing and marketing /distribution of milk. The most critical functions of dairying viz. animal rearing and milk production are carried out by un-organized farmers.

80% of milk comes from small, even marginal farmers, who own a single head of cattle In India, there is no trustworthy cattle market, no honest cattle traders nor any reliable dairy animals. Simply because, absolutely no records whatsoever are maintained by anyone about the animals; be it pedigree, yield or any other matter.

The possible reasons for such a high bacteria count of raw milk could be attributed to -

- Absence of cleanliness and hygienic conditions of animal housing, animals, milkmen, milking utensils/equipments, animal health

- Indefinite delay (5 to 10 hours) in chilling and processing of raw milk
- Farmers still consider cattle rearing as a supplementary activity to farming
- Do not look at dairying as an independent profession.
- Primitive Animal Husbandry norms practiced.
- Not only the milk producers, but also some veterinarians themselves do not know the basic information related to animals, i.e. average weight of adult animals, their daily feed ration requirement, accurate lactation yield, daily quantity of cow manure, etc.

**Other Sources of Contamination**

Under normal grazing conditions, cows' udders will appear clean and therefore washing and drying will be unnecessary. Otherwise, any visible dirt must be removed using clean, running water, individual paper towels or cloths in clean water to which a disinfectant has been added (e.g. sodium hypochlorite at 300 ppm). If udder cloths are used, provide a clean cloth for each cow. After each milking, wash and disinfect them and hang up to dry. Disposable paper towels are preferable and more effective for drying after washing. When cows are housed or graze in heavily stocked paddocks, external udder surfaces are usually grossly contaminated with bacteria even when they appear visibly clean, therefore routine udder preparation procedures should be followed. Whenever udders are washed they should be dried.

Fore milking has little affect on the total bacterial count of the milk but is an effective way of detecting clinical symptoms of mastitis. Filtering or straining the milk removes visible dirt but not the bacteria in the milk because they pass through the filter. Aerial contamination of milk by bacteria is insignificant under normal production conditions.

The milk contact surfaces of milking and cooling equipment are a main source of milk contamination and frequently the principal cause of consistently high bacterial counts. Simple, inexpensive cleaning and disinfecting routines can virtually eliminate this source of contamination.

## MICROBIAL CONTAMINATION FROM WITHIN THE UDDER

Raw milk as it leaves the udder of healthy cows normally contains very low numbers of microorganisms and generally will contain less than 1,000 total bacteria per ml. In healthy cows, the teat cistern, teat canal, and the teat apex may be colonized by a variety of microorganisms although microbial contamination from within the udder of healthy animals is not considered to contribute significantly to the total numbers of microorganisms in the bulk milk or to the potential increase in bacterial numbers during refrigerated storage. Natural flora of the cow generally have little influence on SPCs.

While the healthy udder should contribute very little to the total bacteria count of bulk milk, a cow with mastitis has the potential to shed large numbers of

microorganisms into the milk supply. The influence of mastitis on the total bacteria count of bulk milk depends on the strain of infecting microorganism(s), the stage of infection, and the percentage of the herd infected. Infected cows have the potential to shed in excess of 107 bacteria per ml. If the milk from one cow with 107 bacteria per ml comprises 1% of the bulk tank milk, the total bulk tank count, disregarding other sources, would be 105 per ml.

Mastitis organisms found to most often influence the total bulk milk count are *Streptococcus* spp., most notably *S. agalactiae* and *S. uberis* although other mastitis pathogens have the potential to influence the bulk tank count as well. Staphylococcus aureus is not thought to be a frequent contributor to total bulk tank counts although counts as high as 60,000/ml have been documented. Detection of implied pathogens does not necessarily indicate that they originated from cows with mastitis. Potential environmental mastitis pathogens and/or similar organisms can occur in milk as a result of other contributing factors such as dirty cows, poor equipment cleaning, and/or poor cooling. An increase in SCC can sometimes serve as supportive evidence that a mastitis bacterium may have caused an increase in the bulk milk bacteria count. This seems to hold true more for *Streptococcus* spp. than for *S. aureus*, which appears to be shed into the milk in lower numbers. Correlations of somatic cell responses and environmental mastitis organisms, including coliform bacteria, streptococci, and certain coagulase-negative *Staphylococcus* spp., were found to be poor as well. These organisms are by nature associated with the cow's environment and may influence bulk milk bacteria counts through other means. *S. agalactiae* and *S. aureus* are not thought to grow significantly on soiled milking equipment or under conditions of marginal or poor cooling. Their presence in bulk tank milks is considered strong evidence that they originated from infected cows.

## MICROBIAL CONTAMINATION FROM THE EXTERIOR OF THE UDDER

The exterior of the cow's udder and teats can contribute microorganisms that are naturally associated with the skin of the animal as well as microorganisms that are derived from the environment in which the cow is housed and milked. In general, the direct influence of natural inhabitants as contaminants in the total bulk milk count is considered to be small, and most of these organisms do not grow competitively in milk. Of more importance is the contribution of microorganisms from teats soiled with manure, mud, feeds, or bedding.

Teats and udders of cows inevitably become soiled while they are lying in stalls or when allowed in muddy barnyards. Used bedding has been shown to harbor large numbers of microorganisms. Total counts often exceed $10^8$-$10^{10}$ per gram. Organisms associated with bedding materials that contaminate the surface of teats and udders include streptococci, staphylococci, spore-formers, coliforms, and other Gram-negative bacteria. Both thermoduric (bacteria that survive pasteurization) and psychrotrophic (bacteria that grow under refrigeration) strains of bacteria are commonly found on teat surfaces indicating that contamination from the exterior

of the udder can influence Lab Pasteurization Counts (LPCs) and Preliminary Incubation Counts (PICs).

The influence of dirty cows on total bacteria counts depends on the extent of soiling of the teat surface and the wash procedures used immediately before milking. For example, if one gram of teat soil containing $10^8$ bacteria is allowed into the milk of one cow giving approximately 30 lb (~13,400 g) of milk, the total bacteria count for that cow's milk, excluding other sources, would be in excess of 7,000 per ml. Milking heavily soiled cows could potentially result in bulk milk counts exceeding $10^4$ per ml. Several studies have investigated premilking udder hygiene techniques in relation to the bacteria count of milk. Generally, thorough cleaning of the teat with a sanitizing solution (spray, wet towel, or dip) followed by thorough drying with a clean towel is effective in reducing the numbers of microorganisms in milk contributed from soiled teats. Counts of coliform bacteria, although highly associated with manure, barnyard mud, and used bedding, were relatively low in these studies, even for the untreated cows, suggesting that higher coliform counts in bulk milk are more likely to occur due to other factors (i.e., equipment, mastitis).

## MILK HYGIENE DURING AND AFTER MILKING

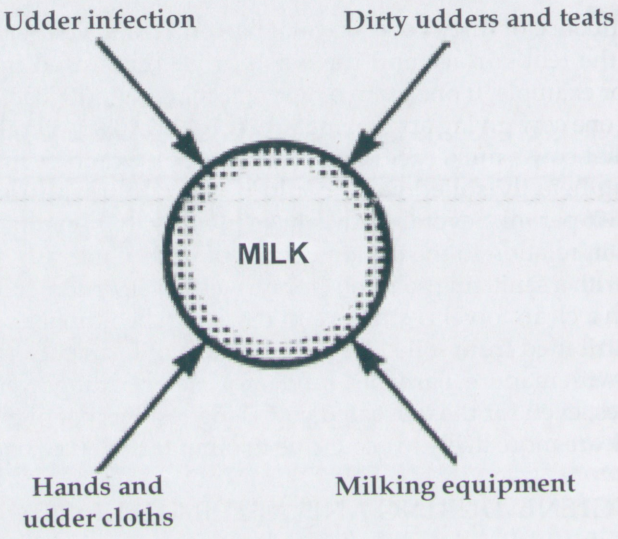
Milk sterile when secreted into an uninfected udder. Contamination occurs during and after milking. Exclude milk from clinical mastitis cases to avoid high bacterial counts. Use mastitis control routines at each milking to reduce the proportion of infected cows and clinical mastitis cases.

The milk secreted into an uninfected cow's udder is sterile. Invariably it becomes contaminated during milking, cooling and storage, and milk is an excellent medium for bacteria, yeasts and moulds that are the common contaminants. Their rapid growth, particularly at high ambient temperatures can cause marked deterioration, spoiling the milk for liquid consumption or manufacture into dairy products. This can be avoided by adopting the simple, basic rules of clean milk production.

### Udder Infection

The essential requirements are to maintain udders free from infection (eg. mastitis); manage cows so that their udders and teats are clean; milk them in such a way that minimises bacterial contamination; store the milk in clean containers and, wherever possible, at temperatures which discourage bacterial growth until collected. Simple and low-cost husbandry practises enable milk to be produced with a bacterial count of less than 50,000 per ml. The golden rule of clean milk production is that prevention is better than cure.

It is impossible to prevent mastitis infection entirely but by adopting practical routines it can be kept at low levels. Most mastitis is subclinical and although not readily detected by the stockman, it will not normally raise the bacterial count of herd milk above 50,000 per ml. Once the clinical stage is reached, the count may increase to several millions/ml and one infected quarter may result in the milk from




the whole herd being unacceptable. It is important to detect clinical cases and exclude their milk from the bulk.

**Other Sources of Contamination**

Under normal grazing conditions, cows' udders will appear clean and therefore washing and drying will be unnecessary. Otherwise, any visible dirt must be removed using clean, running water, individual paper towels or cloths in clean water to which a disinfectant has been added (eg. sodium hypochlorite at 300 ppm). If udder cloths are used, provide a clean cloth for each cow. After each milking wash and disinfect them and hang up to dry. Disposable paper towels are preferable and more effective for drying after washing. When cows are housed or graze in heavily stocked paddocks, external udder surfaces are usually grossly contaminated with bacteria even when they appear visibly clean, therefore routine udder preparation procedures should be followed. Whenever udders are washed they should be dried.

Foremilking has little affect on the total bacterial count of the milk but is an effective way of detecting clinical symptoms of mastitis. Filtering or straining the milk removes visible dirt but not the bacteria in the milk because they pass through the filter. Aerial contamination of milk by bacteria is insignificant under normal production conditions.

The milk contact surfaces of milking and cooling equipment are a main source of milk contamination and frequently the principal cause of consistently high bacterial counts. Simple, inexpensive cleaning and disinfecting routines can virtually eliminate this source of contamination.

### Milking Equipment

Milking equipment must have smooth milk contact surfaces with minimal joints and crevices. Renew rubber components at regular intervals.

### Water For Dairy Use

Water for dairy use must be either an approved, piped supply or chlorinated (50 ppm) before use. In hard water areas, milking and ancillary equipment must be de-scaled periodically.

### Detergents

Detergents are necessary to clean milking and ancillary equipment effectively before disinfection. Effectiveness is increased with solution temperature, concentration and time of application.

### Cleaning Milk Production Equipment

It is virtually impossible with practical cleaning systems to remove all milk residues and deposits from the milk contact surfaces of milking equipment. Except in very cold, dry weather, bacteria will multiply on these surfaces during the interval between milkings, so that high numbers (e.g. 106 per $m^2$) can be present on visually clean equipment. A proven cleaning and disinfectant routine is required so that with the minimum of effort and expense, the equipment will have low bacterial counts as well as being visually clean.

The essential requirements are, to use milking equipment with smooth milk contact surfaces with minimal joints and crevices, an uncontaminated water supply, detergents to remove deposits and milk residues and a method of disinfection to kill bacteria.

### Water Supplies

Unless an approved piped supply is available it must be assumed that water is contaminated and therefore hypochlorite must be added at the rate of 50 ppm to the cleaning water. Hard water (i.e. high levels of dissolved calcium and other salts) will cause surface deposits on equipment and reduce cleaning effectiveness. In such cases, it is necessary to use de-scaling acids such as sulphamic or phosphoric, periodically.

### Detergents and Disinfectants

Detergents increase the 'wetting' potential over the surfaces to be cleaned, displace milk deposits, dissolve milk protein, emulsify the fat and aid the removal of dirt. Detergent effectiveness is usually increased with increasing water temperature, and by using the correct concentration and time of application. Detergents contain inorganic alkalis (e.g. sodium carbonate and silicates and tri-sodium phosphate), surface-active agents (or wetting agents), sequestering (water-

softening) agents (eg. polyphosphates) and acids for de-scaling. Many proprietary, purpose-made detergents are usually available, but otherwise, an inexpensive mixture can be made to give a concentration in solution of 0.25% sodium carbonate (washing soda) and 0.05% polyphosphate (Calgon). Disinfectants are required to destroy the bacteria remaining and subsequently multiplying on the cleaned surfaces. The alternatives are either heat applied as hot water or chemicals. Heat penetrates deposits and crevices and kills bacteria, providing that correct temperatures are maintained during the process of disinfection. The effectiveness of chemicals is increased with temperature but even so, they do not have the same penetration potential as heat and they will not effectively disinfect milk contact surfaces which are difficult to clean.

**Milking Premises**

The milking premises should have a dairy or suitable place equipped with a piped hot and cold water supply, a washtrough, brushes, a work surface, storage racks and cupboards and, if necessary, a vacuum pipeline connection. In addition, it is advisable to have a dairy thermometer (0°C - 100°C), rubber gloves and goggles for use when handling chemicals.

**Daily Routines**

Daily routines for cleaning and disinfecting vary with the size and complexity of the milking installation but will include methods of removing dirt and milk from the equipment followed by disinfection. For hand milking, bucket and direct-to-can milking machines, basic manual methods of cleaning and sterilizing are adequate and effective. For pipeline milking machines in-situ (in-place) systems are necessary.

Milk can become grossly contaminated from bacteria on ancillary equipment which must also be cleaned and disinfected effectively. Coolers, either the corrugated surface or the turbine in-can, can best be cleaned and disinfected manually and stored in the dairy to drain. Refrigerated bulk milk tanks can be cleaned either manually using cold or warm detergent/disinfectant solutions, or for the larger tanks, by automatic, programmed equipment. In either case, a cold water chlorinated (50 ppm) rinse preceeds and follows the washing solution. Foremilk cups can be a potent source of bacterial contamination and need to be cleaned and disinfected after each milking. They should then be stored in the dairy to drain.

It is important with any method of cleaning that the equipment is drained as soon as possible after washing for storage between milkings. Bacteria will not multiply in dry conditions but water lodged in milking equipment will, in suitable temperatures, provide conditions for massive bacterial multiplication. Equipment with poor milk contact surfaces, crevices and large number of joints, remaining wet between milkings in ambient temperatures above 20°C, should receive a disinfectant rinse (50 ppm available chlorine) before milking begins.

# 8

# Dairy Fermentations and Bacteriophages

***Topics***

*Introduction • Performance of Starter Cultures in Dairy Fermentations • Phage entry into dairy environments • Recycling of milk by-products • Classification of Dairy Bacteriophages • Detection and Quantification of Dairy Bacteriophages • Control Strategies in Dairy Plants •*

## INTRODUCTION

In a fermentative dairy process, lactic acid bacteria (LAB) growth and metabolic activities are needed to assure a high-quality final product. These microorganisms produce lactic acid via lactose fermentation, which leads to a rapid decrease in pH. Cheese and fermented milk manufacture depends, largely, on this factor, which is also crucial for ensuring control of pathogenic and spoilage microorganisms.

Bacteriophages or "phages" are viruses that infect bacteria. They are now believed to represent the most abundant biological entities with an estimated range of $10^{30}$ to $10^{32}$ total phage particles on earth, assuming that they outnumber bacteria about 10-fold. These bacterial viruses are present in ecosystems where bacteria have been found, including man-made ecological niches such as food fermentation vats. The industry has been dealing with this biological phenomenon for many years now and has relied on a variety of practical approaches to control phages, which include adapted factory design, improved sanitation, adequate ventilation, process changes, improved starter medium, and culture rotation. Despite extensive efforts, however, phage infection of starter LAB cultures remains the most common cause of slow or incomplete fermentation in the dairy industry, and both researchers and industrial technologists are aware of regular, although unpublished, cases where phage infections actually cause product downgrading. Thus, the goal of this review is to make the reader aware of the relevance and implication of phage attacks in dairy fermentations, with special emphasis on the daily and practical aspects related to this problem in the dairy fermentative industry.

## PERFORMANCE OF STARTER CULTURES IN DAIRY FERMENTATIONS

The growth of dairy starter cultures can be influenced by a number of factors including the raw milk quality, presence of antibiotics or sanitizers, bacterial interactions, and phages.

### Raw Milk Composition

LAB have generally complex nutritional requirements. As a consequence, most LAB species can growth only in media where constituents like amino acids and vitamins are freely available. Even if milk provides this ideal growing environment, other components may act as inhibitors of LAB. The lactoperoxidase-thiocyanate-hydrogen peroxide system, as well as immunoglobulins naturally present in milk are among the known inhibitors affecting LAB activity. If hydrogen peroxide is present in milk as a metabolite of some microorganism, it combines with the lactoperoxidase to oxidize thiocyanate into products (sulfate, carbon dioxide, ammonia, and water) that will inhibit some LAB. Some bacteria, including LAB species, may also agglutinate in raw milk. Antibodies found in the globulin fraction of milk cause this effect. As a consequence bacteria can form clumps and sediment on the bottom of vats, causing slow or heterogeneous acid production. However, the inhibitory role of these compounds is mainly relevant when raw milk is used in cheese manufacture, since agglutinins and immunoglublins are inactivated by heat treatments or homogenization process.

Leucocytes and lysozyme, also present in milk, have antimicrobial properties, the last being particularly resistant to thermal treatments. Normally, their levels are very low in milk, but increases due to mastitis and high somatic cell counts. An antibacterial activity is also frequently associated with lactoferrin, an iron-binding glycoprotein present in milk. Finally, antibiotics, which may enter milk due to the treatment of cows for bacterial infection of the udder, can also affect LAB growth and activity. Good quality raw milk should not contain antibiotic residues, but some reports point to these molecules as responsible for slow acidification during milk fermentation processes. The sensitivity of dairy starters to antibiotics will vary although in general, *Lactococcus* spp are much more resistant to penicillin, *Lactobacillus* spp to tetracycline and *Streptococcus thermophilus* to streptomycin.

### Bacterial Interactions and Phages

The acid production rate of some LAB strains might be increased in the presence of other microorganisms, such as *Micrococcus* spp, which either remove $H_2O_2$ or produce stimulating metabolites. In contrast low concentrations of free fatty acids may also be inhibitory to several LAB strains. Low levels of these organic acids are present in fresh raw milk, with increasing concentration as consequence of the activity of psychrotrophic bacteria, such as *Pseudomonas* spp Even if the ability of some dairy starters to produce bacteriocins is generally considered as a positive attribute for food safety reasons, this feature may be problematic when the antimicrobial spectrum includes LAB species.

Despite of the above, phage infection represents the most significant biological factor affecting industries that rely on bacterial growth and metabolic activities. Depending on the process stage in which the infection proceeds, consequences may vary from slow acid production to completely lost batches. High pH values, high residual lactose concentration and insufficient lactic acid content are the result of phage attacks occurring during the early stages of the fermentation. In particular, the residual lactose might be the substrate for the growth and metabolic activity of spoilage bacteria that negatively affect the quality of the product. Besides the inadequate overall product quality, all these factors may constitute an optimum ecosystem for the growth of pathogens, with the serious consequences on the consumer health.

The recognized ubiquity of phages in dairies is the basis for studies aimed to control rather than to eradicate them. For several reasons, cheese manufacture is the most affected process. Worldwide, large volumes of raw milk are daily fermented by LAB starters, with *Lactococcus lactis* being the most extensively used. Consequently, *Lactococcus lactis* phages are the best studied and documented over the world, followed by *S. thermophilus* phages. The number of reported *Lactobacillus* (*Lb.*) phages is notably lower, possibly due to the characteristics of processes involving this genus. However, several phages affecting fermentation processes driven by *Lb. helveticus*, *Lb. delbrueckii* subsp *bulgaricus* or *Lb. delbrueckii* subsp *lactis* were isolated and documented. Lastly, emerging data suggests an increasing occurrence of phages for specific probiotic LAB strains, especially *Lb. plantarum*, *Lb. acidophilus*, *Lb. casei* and *Lb. paracasei*, which are increasingly used in several fermented products.

## PHAGE ENTRY INTO DAIRY ENVIRONMENTS

### Raw Milk

It is now acknowledged that the most permanent source of new phages within dairy environments is through raw milk, with their concentration ranging between $10^1$ and $10^4$ phages per ml. Madera et al. reported that almost 10% of raw milk samples collected from different dairies in Spain contained infectious lactococcal phages. Several research groups have also reported that many dairy phages are able to survive milk pasteurization. Moreover, the concentration of phages is even higher if only thermized or raw milk are used to manufacture fermented milk products. Consequently, phages might enter the manufacturing process and accumulate rapidly during fermentation if phage-sensitive strains are used, reaching concentrations up to $10^9$ phages per ml of cheese whey or per g of product, up to $10^8$ plaque-forming units (PFU) per ml in brine, and up to $10^8$ PFU per $m^3$ in air. Taken altogether, a great diversity of phages is naturally present in the raw milk ecosystem, thus the absence of phages in dairies is unreachable.

### Factory Environment

Although raw milk is the most logical source of phages in the industrial environment, several dispersion pathways may be occur in dairies. Aerosolization

is currently recognized as an important route of dispersion. Personnel movements or transport of equipment and/or raw materials might cause the dispersion of phage particles as an aerosol. The consequences of this aerosolization are even worse if dispersion is unrestricted between contaminated and uncontaminated zones. In addition, phages present in recycled by-products may also spread to the entire factory environment, since bioaerosols can remain in the air for long periods. Additional underestimated sources of phage contamination are the working surfaces in the dairy facilities. In a recent study, a qPCR assay found evidence for the presence of genetic material from c2-like and 936-like lactococcal phages on a variety of surfaces, such as floors, walls, stairs, door handles, office tables, equipment, cleaning materials and pipes. Although it is unclear whether these phages were active or inactive at sampling, these data emphasize the relevance of correct sanitation measures as well as personal training to diminish the risks of phage infection.

## RECYCLING OF MILK BY-PRODUCTS

The dairy industry, particularly cheese manufacturing, recycles whey protein concentrates (WPC) to increase product yield and/or enhance attributes of the final product. However, such a process is risky due to the possible presence of phages in these ingredients. Indeed, phage remained present in liquids (whey, WPC, etc) subjected to pasteurization and even stronger heat treatments, such as 95°C for several minutes.Moreover, salts, fat, saccharides, and whey proteins may protect phages from thermal damage, thus increasing the risk of this recycling practice. To compound the risk associated with WPC, whey is frequently concentrated (ultrafiltration or microparticulation), thereby increasing the phage levels due to the possible retention of virions by the membranes. A general recommendation to minimize problems associated with WPC should consider its addition only to a fermentation involving the use of significantly different starter cultures, such as mesophilic and thermophilic bacteria. It must also be noted that whey products derived from manufactures using natural undefined (unknow composition) starters should not be added to processes driven by defined (known composition) strain cultures. Natural starters often contain phages and those viruses represent a serious threat to the limited number of strains composing the defined starter cultures.

### Prophages

Genome sequencing projects has confirmed that many LAB strains contains prophages. In fact, lysogeny is widely distributed among dairy lactococci and lactobacilli. A significant lower incidence of lysogeny was demonstrated in *S. thermophilus* species, as only a few strains (1 - 2%) were induced by mitomycin C, although others reported much higher frequencies (25%). A recent study showed that 25 out of a collection of 30 probiotic strains of Lactobacillus contained inducible prophages. Putting these lysogenic LAB under certain environmental conditions such as heat, salt, antimicrobials, or starvation, may activate the induction prophages that will replicate, leading to the release of new virions. The latter can potentially infect sensitive strains if present in starter cultures. Capra et al. isolated

two lytic phages for the strain *Lb. paracasei* A from pure cultures, indicating that both phages could most probably have evolved from a lysogenic state. Whenever possible, the presence of prophages as well as the risk of their spontaneous induction should be carefully investigated when selecting strains and designing cultures for specific industrial fermentation processes.

It should be noted that detecting the presence of inducible prophages in lysogenic strains might involves several assays. Ideally, culture treatment with an inductor leading to cell lysis and the subsequent plaque formation is the first evidence of lysogeny. However, suitable indicator strains may be hard to find and thus, a negative result is not proof for the absence of inducible prophages. Observation under an electron microscope to visualize induced phages in a lysate may be an option. Even prophage remnants that have lost most of the phage genome, are not inert entities within bacterial chromosomes. Indeed, defective (and functional) prophages are source of genes to recombine with infecting virulent phages.

Interestingly, lysogenic strains may not always result in detrimental consequences. Studies of controlled lysis of lysogenic bacteria have shown positive effects, such as a decrease in bitterness for some ripened cheeses, where the hydrolysis of casein-derived hydrophobic peptides is performed by intracellular bacterial peptidases released by phage lysis. Prophages might also be responsible for the resistance of a lysogenic strain against infection by virulent phages. The protection is conferred by prophage genes, particularly superinfection exclusion genes, which might encode repressor molecules.

## CLASSIFICATION OF DAIRY BACTERIOPHAGES: AN OVERVIEW

According to the International Committee on Taxonomy of Viruses (ICTV), all known phages infecting LAB are tailed phages and members of the *Caudovirales* order. Tailed phages are, in turn, organized into three families: *Podoviridae*, *Myoviridae*, and *Siphoviridae*. *Podoviridae* members have short and noncontractile tails; myophages have tails with a contractile sheath and a central tube while siphophages have noncontractile tails.

As previously stated, *Lactococcus* is the most extensively LAB used by the dairy industry and phages infecting this genus are the most studied. Lactococcal phages belong mainly to the *Siphoviridae* family, with a few being *Podoviridae*. Lactococcal phages are currently classified into 10 groups based on morphology and genomic sequence analyses. At least one genome from each lactococcal phage group is available.

A recent review of *Lactobacillus* phages reported 231 phages, 186 of them morphologically characterized.A total of 109 were siphophages, 76 were myophages, and only one belonged to the family *Podoviridae*. Before the availability of genomic sequences, the classification of *Lactobacillus* phages was based mainly on morphological observations and DNA homology, *Lb. delbrueckii* phages being the first to be classified in the 1980s. Later, several completely sequenced *Lactobacillus*

genome phages were assigned to a classification scheme based on the organization of the structural gene module of the siphophages. Further proposals for classification of *Lactobacillus* phages were based on the deduced proteomic trees, disregarding phage morphology, but the under-representation of *Lactobacillus* phages in these schemes might distort the impact of those phylogenetic trees.

All *S. thermophilus* phages reported to date are members of the *Siphoviridae* family, and can be assembled into two distinct groups according to their DNA packaging mechanism (*cos* or *pac*) and the number of major structural proteins. Although a third group of *S. thermophilus* phages may have been uncovered recently.A strict correlation exists between the presence of a particular set of major structural phage proteins and the mechanism of DNA packaging, demonstrating that *cos*-containing phages possess two major structural proteins in contrast to the *pac*-containing phages, which possess three major structural proteins. Moreover, a great diversity of streptococcal phages is often observed in cheese making, in contrast with a more homogeneous phage population in yogurt production facilities. The diversity of phage populations in cheese making may be due to the rotation of multiple strains of *S. thermophilus* in starter cultures, as compared with yogurt starters. Genomic sequences are available for a few isolates of each group.

*Leuconostoc* strains are present in some dairy mesophilic starters, most often mixed with lactococci. This combination is essential for most applications since *Leuconostoc* grows slowly in milk as compared with lactococci but its addition still provides specific dairy flavors. Very scarce information is available on the biology of *Leuconostoc* phages, possibly because few phage problems have been reported in the literature. On the other hand, *Leuconostoc* phages have been isolated during in coffee fermentationa nd in sauerkraut fermentation brines. Globally, most of these phages were assigned to the *Siphoviridae*and to the *Myoviridae* families. More recently, the first complete phage genome sequence from a*Leuconostoc* phage was reported. Bioinformatic analysis revealed low similarity with other phage genomes, pointing out that this phage is a rather unique.

## DETECTION AND QUANTIFICATION OF DAIRY BACTERIOPHAGES: A BRIEF SURVEY

Early phage detection in raw milk, ingredients or the dairy environment is designed to diminish and control phage attacks during the fermentation processes. Two general types of phage detection methods are available: direct and indirect. Direct detection methods focus on detecting the presence of lytic phage particles or their components (DNA, proteins) in a sample. Standard microbiological methods, i.e., plaque assays, spot tests and activity tests, are usually applied to milk or fermented products (cheese whey and fermented milks). One of the advantages of this type of technique is discrimination between phage and non-phage inhibitors. Disadvantages include the requirement for a sensitive indicator strain and the relatively long time needed to obtain results. Therefore, molecular detection is a preferred method, especially because the assay time is much shorter. Several assays based on the polymerase chain reaction (PCR) have been designed and successfully

applied to detect, or even classify, *Lactobacillus*, *Lactococcus* and *Streptococcus* phages in different dairy matrixes, including cheese whey, cheese whey starters, and milk samples. The detection limit of a classical one-step PCR method usually ranges from $10^4$-$10^7$ PFU $ml^{-1}$, depending on the phage type and sample, but an additional phage concentration step will allow detection of as little as $10^3$ PFU $ml^{-1}$. qPCR-based methods provide highly sensitive, rapid and real time monitoring of specific phages during the fermentation process. Rapid detection assays (no more than 30 min) of *Lb. delbrueckii* and *S. thermophilus*phages were recently reported, with $10^4$ PFU $ml^{-1}$ and $10^5$ PFU $ml^{-1}$ of milk as quantification limits, respectively. In a recent study, this methodology allowed the detection and enumeration of three groups (c2, 936 and P335) of lactococcal phages in goat's raw milk and whey with a low detection limit ($10^2$ UFP/ml) in about 2 h. However, it must be noted that these molecular methods do not discriminate between active and non-active phage particles since they detect phage DNA. Molecular detection techniques can also be too expensive and too specific for routine monitoring. Moreover, another major inconvenience common to all DNA-based detection methods is that they can only detect phages whose genome sequences are available. To overcome these limitations, PCR-based methods and classical microbiological assays might be used together to obtain more data about the phages contained in the sample (titers, host range, phage type).

Of the traditional indirect methods, the activity test is one of the most commonly implemented for routine analysis in dairy plants. The presence of phages in a sample is assessed as a decrease in acid production (compared with a phage-free control sample) by a starter or strain culture in sterile, steamed or pasteurized milk. Important limitations must be considered for this assay, particularly for mixed strain starters, since phage-insensitive strains will continue to grow and acidify. Other detection methods, called indicator tests, are based on the reduction of an indicator compound (generally, methylene blue or bromochresol purple), due to culture acidification in presence and absence of sample filtrate. If phages are present in the sample, a time delay or a failure in the color change is observed. As in the activity test, mixed cultures may mask the presence of phages, producing false-negative results.

Another indirect method proposed for monitoring the fermentation process involves flow cytometric analysis. Flow cytometry is based on the detection of cells with low mass that are found late in the lytic cycle. Detection of lactococcal phage infection by flow cytometry was recently reported with limits comparable to classical PCR methods ($10^5$ PFU/ml).

In this case, the authors observed that during phage infection the typical lactococcal chains are broken up while cells with low-density appeared and could be detected. Results of the study demonstrated that phage infection of *L. lactis* is fast and efficiently detected, in a real time and even at the first signs of phage attack. The detection was evidenced as early as 1 to 2% of the lactococcal cells were infected. On the other hand, the authors argued that large particles such as eukaryotic cells and fat globules should be removed in order to avoid the blocking of the flow cytometer.

Another indirect method is based on the detection of changes in the electrical impedance or conductance of the milk, due to a decrease in lactic acid production when a phage infection occurs. In a recent study, Researchers developed a rapid phage detection method based on the evaluation of impedance changes during infection of a host-biofilm established onto metal (platinum and gold) microelectrodes. The infection and subsequent host cell lysis was monitored by non-faradaic impedance spectroscopy in milk samples. In this case, an *Escherichia coli* phage and its host were chosen as models, but the methodology would be applicable to any dairy phage, as long as a suitable bacterial host can grow on the microelectrode surface. The simplicity of the assay and the possibility of miniaturization of the system are among the advantages.

Recently, a method combining epifluorescense microscopy and Atomic Force Microscopy (AFM) was reported to monitor the presence of phages. Specifically, epifluorescence microscopy allows *Lb. helveticus*phage particles to be enumerated from phage-infected cultures, while AFM allow monitoring changes in phage and bacteria population during the infection process. Phage particles to be enumerated with epifluorescence microscopy require SYBR Green I staining, then the emitting green light results in a bright particle larger than the actual size of the virion, enabling it to be counted with a fluorescence microscope. As disadvantages, authors have highlighted that both virulent and non-virulent phage particles are counted by the epifluorescence microscopy. Considering these facts, the authors suggested a combined phage count approach, including plaque assay and epifluorescence, in order to determine the total viral abundance and host specificity in dairy samples.

As acknowledged the early detection of bacteriophage in milk, raw ingredients or by-products at any point during fermentation, is extremely helpful minimizing the detrimental consequences of phage attacks in dairy factories. Nevertheless, several characteristics must be taken into account when selecting a particular phage detection method, including the volume of milk transformed each day, the type of fermentation process, the starter culture used, the diversity of the phage population, and the risk or frequency of phage infections. Additional considerations include the requirement for rapid results, the quantification limit, and, finally, the cost of the assay.

## CONTROL STRATEGIES IN DAIRY PLANTS

Since the presence of phages is unavoidable in dairy plant environments, phage control strategies are designed to control rather than eradicate them. Culture rotation programs, direct vat-inoculation of starters, careful handling and disposal of whey, use of phage-inhibitory media, optimized sanitation, and use of starter cultures with increased phage resistance are some of the approaches applied to minimize phage spreading in dairy plants.

### Plant Design, Airflow, and Equipments

The layout of a dairy factory is one of the critical factors for preventing phage infection. Contact between raw materials and waste (whey or water) should be

avoided. Some examples include the physical separation of the milk reception from other plant areas, as phage-containing aerosols can be generated during tanker emptying and raw milk spillage. The starter preparation room should be sealed off from the manufacturing area and maintained under a positive pressure of filtered air. Another concern involves the whey tanks and separators, which should be placed in a separate area situated as far as possible from the starter room and the cheese manufacturing vats.

Avoiding the generation of bioaerosols as well as limiting the air microbial count by using spray systems with appropriate disinfectants should help in controlling infections. Additionally, the air used for positive pressure applications must be filtered to remove dust particles, which may bind phages, and the air inlet for the filters should be located as far as possible from the milk silos and whey tanks. Finally, the efficiency of the filters should be checked regularly.

Stainless steel with a high grade of polish is the ideal material for all the equipment used in the fermentation processes, as it should be subjected to an efficient cleaning and sanitizing before and after use. The fermentation tanks must be closed, sterilized by heat or sanitizers, with positive pressure filtered air in the headspace; the tanks should be monitored periodically for cracks.

**Factory sanitation**

A strict system for cleaning and disinfection of equipment and utensils used during processing are mandatory for maintaining phage levels as low as possible, and minimizing the risk of phage infection and dissemination within the dairy. No compromise should be made here. Several factors should be considered when selecting a sanitizer, including a fast antimicrobial activity, ease of application, low cost, lack of negative impact on the final product, and degradation into harmless final compounds. The effectiveness on phage inactivation is a criterion taken into consideration only recently, which is reflected by an increasing number of studies on their viral effectiveness.

Peracetic acid-containing products are often the most effective, assuring fast and efficient inactivation of phage particles. Sodium hypochlorite, ethanol and isopropanol, typically used for cleaning laboratory surfaces and utensils, are notably less effective in the inactivation of viruses. Recently, the effectiveness of several classic biocides used by the dairy industry was evaluated on phages infecting *Lb. delbrueckii*, *Lb. casei* and *Lb. paracasei*. Biocides at extreme pH, such as alkaline chloride foam or ethoxylated nonylphenol with phosphoric acid (pH values $> 12$ and $< 2$, respectively), were exceptionally efficient, although pH level is not the only factor to take into consideration when choosing a biocide. While quaternary ammonium chloride was efficient, p-toluensulfonchloroamide showed no reduction in phage numbers.

As phage particles can remain in the air for long period of time, bioaerosols are one of the most important dispersion routes of virions. Very few studies have addressed this issue. For example, little data are available on the viral efficiency of

fumigation/fogging systems, ozone treatment, and UV light irradiation in industrial facilities. The photocatalytic properties of $TiO_2$ have been investigated, but mainly for the photochemical pollutant oxidation. Several advantages of photocatalysis, such as low cost, high abundance and safety of $TiO_2$, the absence of residues, treatment of pollutant mixtures, broad range and ease of operation, suggest this methodology as an alternative to the traditional chemical disinfection. Semiconductor $TiO_2$ generates highly oxidizing species (O2- and .OH) when photoexcited by UV radiation, thus catalyzing various chemical reactions, including the decomposition of organic compounds. Photocatalysis application has been mostly intended to destroy fungi, bacteria and spores in the air, but its efficiency for inactivating viruses in bioaerosols has been explored only recently. Kakita et al. and Kashige et al. have reported the inactivation of LAB phage PL-1 (*Lb. casei*) liquid suspensions using a ceramic preparation coated with a mixture of oxides ($TiO_2$ and AgO) and black-light (BL) (300 - 400 nm). Reduction of 6 logs were reproted for *Lb. delbrueckii* and *Lb. plantarum* phages after photocatalysis exposure for less than an hour while two hours was needed for *Lb. casei* and *L. lactis* phages.

**Ingredients Treatment and Recycling of Products**

From the time of collection, immediate refrigerated storage of milk is required to diminish the risk of microbial propagation including bacterial viruses. Depending on the type of product to be manufactured, the milk undergoes different heat treatments to reduce microbial load (pathogens and spoilage). These heat treatments also indirectly reduce viral titers. However, a remarkably high thermal resistance has been reported for some phages infecting *L. lactis*, *S. thermophilus*, *Lb. casei* and *Lb. paracasei*, even up to 5 min at 95°C. High levels of thermo-resistant phages ($10^9$ PFU / ml) have also been found in whey, brine and cream. Thus, the recycling of these by-products should be avoided since the return of even small quantities of phages can lead to constant propagation. Consequently, adequate heat treatment of by-products, prior to recycling, is recommended in order to reduce the problems associated with recontamination. It is recognized, however, that the physical properties and function of whey proteins can be severely affected by treatments to minimize phage load in by-products.

Inactivation of dairy phages using technologies involving high pressure has been explored. The most studied and applied pressure-based processes are high hydrostatic pressure (HHP) and high pressure homogenization (HPH). Moroni et al. demonstrated a significant difference in sensitivity to HPH between the two morphological types of lactococcal phages: prolate-headed (c2-like) were less stable than isometric-headed (936- P335-like). Others observed reduction of 2 to 6 logs for phages of *Lb. paracasei*, *Lb. casei*, *Lb. delbrueckii*, *Lb. plantarum*, *Lb. helveticus*, *S. thermophilus* and *L. lactis*, after 5 passes at 100 MPa in reconstituted skim milk. The phage inactivation rate was proportional to both applied pressure and number of passes. The influence of suspension media (milk, whey permeate, buffer) was variable, with the results dependent on the phage tested and authors.

Similarly, HHP has been proposed as an alternative to the thermal treatments applied in food preservation.Little data are available on HHP, although inactivation

of some dairy phages has been reported and seems variable from one phage to another. Specifically, *L. lactis* phages P001 (c2-like) and P008 (936-like), suspended in enriched M17-broth, were treated at up to 600 MPa. The isometric phage P008 was considerably more resistant, with a 5-log reduction in concentration after treatment for 2 h at 600 MPa, whereas the same titer reduction was obtained for prolate phage P001 during the pressure-build-up time. Only an exhaustive analysis of costs, involving the estimated yield and the desired product characteristics, would help dairies to select alternative treatments to be applied to raw materials and the dairy environment in order to diminish the risk of phage infections.

**Phage Inhibitory Media**

Culture media might be designed to contain components that inhibit or delay phage propagation. For example, one strategy use culture media containing chelating agents, such as phosphates or citrates, capable of binding divalent cations, which are often needed to successfully complete the phage lytic cycle. The use of sodium tripolyphosphate-high solubility (TAS) at low concentrations (0.3 to 0.5%) in milk was effective at inhibiting the lytic cycle of LAB phages. However, some bacterial strains showed a delayed growth and acidification profile, possibly due to the buffer ability of the added phosphates. Another technology used purified phage peptides as an additive to protect a lactococcal culture, though phages were not inactivated.The peptides were able to extend the growth of *Lactococcus* culture in phage-containing L-M17 medium and milk. The culture was even protected from phage infection through renneting and ripening stages when the starter culture bulk was prepared in a medium containing the phage peptides.

**Starter cultures**

The use of natural starters composed of an undefined mixture of different strains and/or species is still the key for the production of many traditional cheeses in various countries. These artisanal starters are considered to be highly tolerant to phage infection because they are grown in the presence of phages, which lead to the dominance of resistant or tolerant strains. However, the limited reproducibility of their technological performance has led to the replacement of these traditional starters by direct multi strain cultures (DSC) in the production of many industrial large-scale cheese varieties. The strain and/or species in DSC are perfectly defined and their technological performance is highly reproducible. However, as a consequence of the limited number of strains used, a phage infection may cause the disruption of lactic acid fermentations. The use of concentrated DSC, added directly to the vat (Direct Vat Inoculation cultures - DVI) constitutes an alternative without need for on-site starter propagation, therefore, diminishing the risk of infection by phages from the cheese factory environment. Moreover, rotation of these cultures is probably the main basis for an efficient phage control program: avoiding recontamination by the same phage and build-up of high phage levels in a cheese plant. Although this strategy is not suitable for all dairy manufacturing processes, it provides a relatively simple way to minimize fermentation failures due to phages. As a consequence, recent efforts have been made to search for potential new starter

bacteria from the pool of wild strains recoverable from raw milk, undefined cultures, or traditional dairy fermented products. Hence, LAB strains with dairy-grade (e.g., antibiotic susceptibility) or pro-technological (e.g., broad phage resistance, high acidification activity, lack off-flavor development) traits are highly valued.

The extended co-survival of LAB and phages in the same environment has prompted the strains to acquire a variety of native phage defense systems. These mechanisms include inhibition of phage adsorption, blocking of DNA injection, restriction/modification systems, CRISPR-Cas systems and abortive infection (for a list of reviews on this subject see Garneau and Moineau). In lactococci, these mechanisms may be encoded by chromosomal or plasmid genes. Interestingly, natural gene transfer by conjugation of plasmid DNA is a common feature of lactococci. So, the conjugation of native phage resistant plasmids has been a profitable strategy for genetically improving dairy LAB for over 20 y, yielding multiple dairy starter cultures that have been in commercial use for many years, many of them under worldwide patent. However, although the conjugal transfer of phage resistance plasmids represents one of the most convenient, simple, and "natural" strategies to improve starter strains, The isolation of bacteriophage-insensitive mutants (BIMs) is an alternative for bacteria without conjugative plasmids. Several studies have described the isolation of spontaneous phage resistant variants from sensitive strains of lactococci, *S. thermophilus*, *Lb. helveticus*, *Lb. delbrueckii*, *Lb. casei* and *Lb. paracasei* strains. Though there are some disadvantages to this methodology (e.g., a high frequency of phenotype reversion and physiological bacterial modifications), the isolation of BIMs has gained recent popularity because it is simple and involves no genetic manipulation, thus there are no regulatory restrictions to applications in industrial environments. The mechanism involved in BIM generation has been attributed to mutations in the phage receptors, even though recent studies have demonstrated that CRISPR-Cas systems or abortive infection systems play a role in the development of BIMs.

Over the past 25 y, the construction of genetically engineered strains has been intensively studied as an alternative to the development or use of transconjugants or phage-resistant mutants. Several genetic tools, based on the characterization and exploitation of the LAB native phage defense mechanisms as well as some phage genetic elements, have been designed. These antiphage approaches include origin-derived phage-encoded resistance, antisense RNA technology, phage triggered suicide systems, overproduction of phage proteins, DARPins, and neutralizing antibody fragments. Nevertheless, despite intensive research and economic support, dairy and starter culture industries have not benefited as expected mainly due to modest progress in the development of legislation regarding Genetically Modified Organisms.

Controlling phage infections of probiotic bacteria is starting to be documented and may become a new challenge. The manufacture of certain types of probiotic products involves propagation of the strains as a starter making them particularly vulnerable to phages. Also, *Lactobacillus* strains have long been known to harbor

prophages, yielding the possibility of spontaneous prophage induction during use, or of prophage DNA involvement in the generation of new virulent phages. For probiotic phages, control strategies are limited as strain rotation is likely not possible and specific health claims may not be directly applicable if a phage-resistant derivative is generated.

## CONCLUDING REMARKS

The risk of phage infection in processes relying on bacterial growth is here to stay. Despite significant progress made over the past decades to reduce the overall problem associated with phage contaminations, improvements are still needed. Ideally, fast and online tools that would detect amplifying phages would be a welcomed addition for most industries relying on bacterial growth. New technologies to remove phages from raw materials and by-products as well as air and equipments are still needed. Finally, a better understanding of phage-host interactions is an ongoing venture to appropriately select or develop bacterial strains for long-term industrial use in phage-contaminated environments.

# 9

# Processing of Milk

**Topics**

*Introduction • Pasteurised milk • Cream • Butter and ghee • Ghee • Cheese • Cultured milks and yoghurt • Yoghurt • Ice cream • Milk confectionery • Quality and testing • Health hazards • Receiving, Transportation and Storage • Standardizing Milk*

## INTRODUCTION

Although cow's milk is the most popular in many ACP countries, milk from goats, horses, camels and sheep is also important in some areas. Milk has a low acidity and a high nutrient content, which make it the perfect breeding ground for bacteria. As a result, it both spoils quickly and can support bacteria that cause food poisoning (pathogens). The main reason for processing milk is to preserve it and make it safe by destroying any bacteria that contaminate it. The bacteria may come from the animal, from insects that fall into the milk, from utensils used in processing, or from the hands of operators. Infections in the animal that cause illness may also be passed directly to consumers through the milk.

Contamination of milk with antibiotics is caused by their overuse for treatment of cattle diseases, particularly mastitis. This has been brought under control in some ACP countries by strict limits on the use of antibiotics, regular testing of milk for antibiotic residues, enforcement of regulations, and education. Milk containing antibiotics should not be used in processes that involve fermentation, e.g. production of yoghurt and cheeses, as they interfere with the fermentation.

The main methods of processing milk are:

- heat treatment, e.g. pasteurisation or boiling to destroy naturally occurring enzymes and contaminating micro-organisms. Canning and UHT processing are other types of heat treatment but they require specialist equipment, expertise, and a high level of investment and are therefore not suitable for small-scale processors

- increasing the acidity by fermentation to produce yoghurt or cultured milks or by addition of an acid such as citric acid or vinegar to control the growth of pathogens
- removing water by concentration (boiling), by separation (churning butter, draining whey from cheese) or by evaporation (drying milk, but remember, the equipment necessary to make dried or evaporated/sweetened condensed milk is likely to be too expensive for small-scale processors)
- adding sugar to make milk confectionery, or salt for cheese or butter production.

Some types of processing involve more than one of these methods.

**Shelf Life**

Most dairy products have a short shelf life and a high public health risk. It is essential that both customers (shops, hotels or institutions) and final consumers be given clear advice regarding sell-by and use-by dates. The calculation of shelf life is based on microbiological tests and the experience of producers. Very few small dairies have the expertise or facilities for microbiological testing, and the shelf life should be calculated using the knowledge and facilities at a university food science department or Bureau of Standards. The shelf life of new products, or those for which the recipe is changed should be assessed as follows:

- prepare several batches of the product
- store the product under the normal storage conditions (e.g. in a refrigerator at 4°C or in a freezer at –18°C). Check the temperature daily
- remove samples of short shelf life products daily or weekly for frozen products. Keep the samples cold or frozen in an insulated box and take them quickly to an analytical laboratory
- the laboratory should check the Total Viable Count and the numbers of *Escherichia coli* and *Salmonella* spp. These are indicator micro-organisms that are commonly used to define microbiological quality.

Over a period of time the microbiological quality of the product declines to a level that is unacceptable for consumption. The time taken to reach this stage is the maximum shelf life. However, the actual shelf life is considerably less than the maximum in order to have a safety factor, for example, if the maximum life for cultured milk is found to be 7 days, the recommended use-by date should be 4 days from the date of manufacture.

These tests assume that the product will be stored exactly as recommended, but this may not always be the case. For this reason 'abuse' tests are done alongside the shelf life tests. These tests involve placing the food in a warm place for a few hours, to simulate conditions when a consumer takes the food home from a shop, and then placing it in a refrigerator at 8°C (the more usual domestic refrigerator temperature). The tests described above are then conducted. Abuse tests give a more accurate

estimate of the likely use-by date as they take into account the conditions that the product faces during distribution and home storage.

## PASTEURISED MILK

Milk is known as an 'oil-in-water' (O/W) emulsion (see technical details in Appendix I) in which tiny droplets of milk fat are dispersed throughout the milk. The fat is solid below 20°C and an oil above 30°C. Milk contains between 3.5% and 8% fat depending on a number of factors including the type of animal from which it came (Table 9.1), the particular breed, the animal's diet and its health.

**Table 9.1 Fat content of milk from different types of animals**

| Animal | Fat (%) |
|---|---|
| Buffalo | 7.4 |
| Camel | 5.4 |
| Cow | 3.7 |
| Goat | 4.2 |
| Horse | 1.6 |
| Llama | 3.2 |
| Sheep | 7.9 |
| Yak | 8.0 |

The processing stages for pasteurised milk include:

- receipt and filtration of raw milk
- separation of all or part of the milk fat (to standardise milk, or produce milk that has a specific fat content for making yoghurt or cheese)
- pasteurisation, followed by cooling to 10°C
- homogenisation (not usually done on a small scale)
- packaging and cold storage
- cold distribution.

### Standardisation

Raw milk can be 'standardised' to produce milk that has a constant butterfat content, by partially skimming off the cream. This makes it possible to standardise the composition of finished products and to set aside part of the cream for butter production. Different types of pasteurised milk include 'full cream', 'skimmed', and 'semi-skimmed' milks. These are described in legislation. Flavoured milks are becoming popular in some ACP countries, and may include chocolate, vanilla and fruit flavours with appropriate colourings that are added before pasteurisation.

The payment system for raw milk is usually based on the volume delivered plus a premium for the percentage of milk fat. If, for example, milk is received with a fat content of 3.5%, but a fat content of 2.5% is required, the amount of skimmed milk (0.5% fat) that should be mixed with the raw milk to lower its fat content is calculated using a Pearson Square (Fig. 9.1) as follows:

- the required % fat is written in the centre of the square
- the percentages of fat in the milk and skimmed milk are written in the two left-hand corners

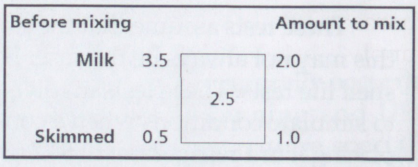



**Fig. 9.1 Pearson Square**

- the two values are subtracted along the diagonals to give the amounts of each to be used.

Thus, 3.0 litres of milk with a 2.5% fat content are obtained by blending 2.0 litres of milk with a 3.5% fat content and 1.0 litre of skimmed milk with 0.5% fat content. If the normal daily reception is, for example, 350 litres of milk with an average fat content of 3.5%, it is necessary to skim 100 litres of milk to standardise the batch. A hand-operated cream separator, having a throughput of 60–100 litres per hour is suitable for doing this. Another method of calculating how much of each ingredient to mix in order to obtain a standardised product is to use a mass balance.

**Pasteurisation**

Milk is properly pasteurised using the following temperature/time combinations, both of which should be followed by rapid cooling to 10°C to ensure it is safe from food-poisoning bacteria:

- 63°C for 30 minutes
- 72°C for 15 seconds.

Other combinations of temperature and time are possible but the law in many ACP countries may specify minimum pasteurisation conditions. In practice, these time/temperature combinations may be exceeded; particularly if there is a risk that raw milk may be contaminated with significant numbers of bacteria. Heating to 80–85°C for up to 5 minutes is often used as this also helps to prevent recontamination of milk before packaging.

At smaller scales of operation, milk can be pasteurised using a simple stainless steel (or less desirably aluminium) pan placed directly over the heat source. However, milk is sensitive to over-heating and likely to burn in this type of pan unless great care is taken to control the heat and thoroughly stir the milk as it is being heated. This equipment also slows down production because the pan must be cleaned between batches. The more usual method in small dairies is to heat milk in a jacketed stainless steel boiling pan. Steam from a boiler heats the space between the outer jacket and inner pan to give more uniform heating and avoid localised burning of the pan contents. A milk stirrer and thermometer are needed to ensure that this is done accurately.

The most appropriate type of heater depends on the cost and availability of different fuels in a particular area. In urban centres, gas or electricity are the preferred options because their use presents no risk of contaminating the product. In rural areas, supplies may not be available or reliable and other types of fuel (e.g. charcoal or kerosene) have to be considered, or a generator used for the electricity supply.

The milk is then cooled, either by immersing milk cans in a tank of cold running water, or by running cold water through the double sides of a cheese vat. In either method, the milk should be stirred constantly to achieve uniform cooling.

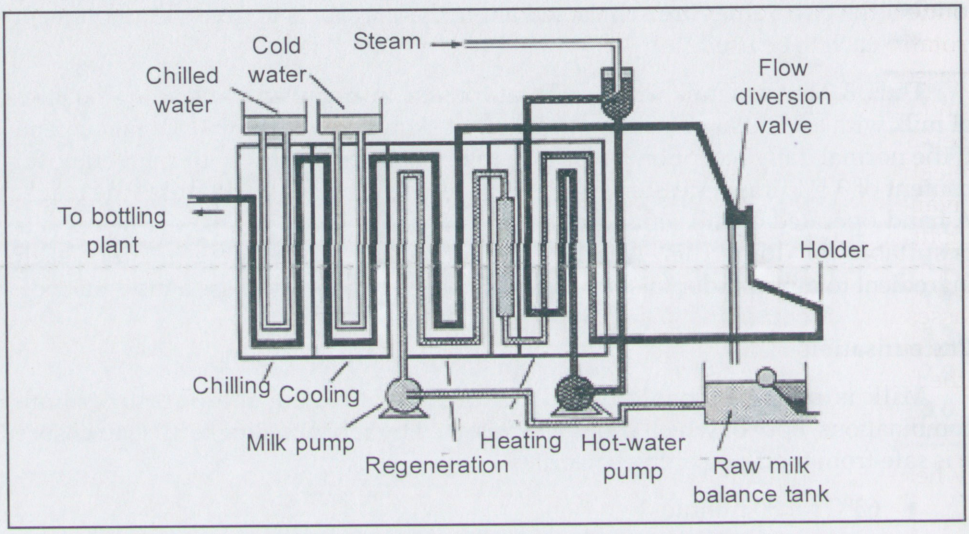



**Fig. 9.2. Operation of a plate heat exchanger**

The higher-temperature, shorter-time pasteurisation (72°C for 15 seconds) cannot be achieved using boiling pans so a plate heat exchanger (Fig. 9.2) is required. These are expensive and may only be affordable in larger dairies in ACP countries, unless second-hand equipment can be located. This equipment also requires pumps, a boiler, a source of chilled water and spare gaskets to seal the plates.

After pasteurisation, milk should be kept covered and cool until it is filled into containers and sealed. Glass bottles sealed with foil are used in countries that have a glass-making industry, but in most ACP countries milk is filled into polythene bags and sealed using a form-fill-seal machine. Alternatively paperboard cartons that have an internal coating of wax or plastic are sometimes used. Increasingly, plastic (PVC or polythene) bottles are becoming available, but these are more expensive than bags, although their filling/sealing equipment is cheaper than a form-fill-seal machine. Pasteurised milk has a shelf-life of a few days when stored under refrigeration.

## CREAM

Creams are rarely used in most ACP households but are more common as a bakery ingredient in some countries. Cream is an O/W emulsion with a fat content of 35-40%. The simplest method of separating cream is to allow it to float to the surface of the milk and then skim it off with a ladle. However, this is time-consuming and even in a small-scale operation a cream separator is preferable. The equipment can either be a tubular bowl centrifuge, or more commonly a disc bowl centrifuge (Fig. 9.3). The tubular bowl centrifuge has a vertical cylinder, which is rotated at 1500-1600 rpm inside a stationary casing. The milk is fed in at the base of the cylinder and cream and skimmed milk are separated, emerging from separate

outlets. The disc bowl centrifuge has a stack of rotating conical discs inside a casing. The milk passes along the surfaces of the spinning discs and cream is separated, again emerging from a separate outlet to the skimmed milk. Manual or electric versions of disc bowl centrifuges are available with capacities from 50-150 litres per hour.

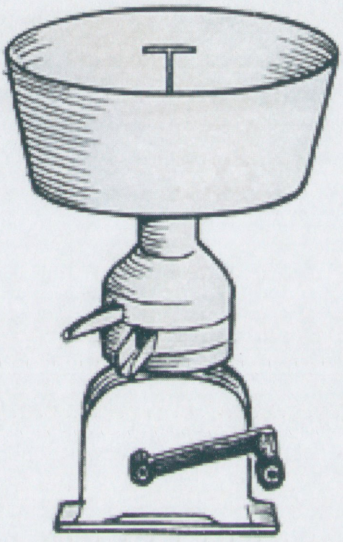


**Fig. 9.3 Disc bowl cream separator**

The efficiency of a cream separator ($E_s$) is assessed by the amount of fat transferred from the milk to the cream. It can be calculated using the following formula:

$$E_s = 1 - f_s/f_m$$

where:

$f_s$ = % fat in the skimmed milk

$f_m$ = % fat in the milk.

There are a range of cream products that have legally defined fat contents, including cream, half cream, whipped cream, heavy whipped cream and double cream.

### Whipped cream

A planetary mixer, fitted with a balloon whisk, is used to whip cream.

**Worked example 1: Mass balance**

A mass balance states that the mass of material going into the process = mass of material going out of the process. If for example, a dairy has 240 kg of 40% cream and this is to be standardised to cream with a fat content of 18% by blending it with skimmed milk:

x = weight of skimmed milk (kg) and y = weight of 18% cream (kg),

The mass balance is: 240 + x = y

The mass balance for fat states that the fat into the process = fat out of the process, i.e. 0.4 (240) = 0.18 (y)

That is 40% of 240 kg comes into the process and 18% of y goes out of the process, i.e. 0.4 (240) = 0.18 (240 + x)

96 = 43.2 + 0.18 x

So:

0.18 x = 96 – 43.2

x = (96 – 43.2)/0.18

= 293 kg skimmed milk

### Sour Cream

Cultured or sour cream uses a similar lactic acid bacteria starter and fermentation conditions to those used for cultured milks, but the fermentation is stopped when acidity reaches 0.6% lactic acid. It has a fat content of 12–30% and is usually pasteurised at 75–80°C.

## BUTTER AND GHEE

Butter is a W/O emulsion of more than 80% milk fat, containing water in the form of tiny droplets, so small that the butter looks dry. It should have a uniform pale yellow/cream colour, be dense and have a smooth consistency so that it spreads easily and melts in the mouth. It is used as a spread, for cooking, or as a baking ingredient. There are five types of butter, which can be made from fresh or fermented milk, or from the whey that remains after making cheese (Table 9.2).

Cream or fermented milk is churned to make the fat droplets join together and form grains of butter. The liquid by-product from butter making is 'buttermilk', which is either drunk or fed to animals. Churning changes the emulsion from the oil-in-water (O/W) emulsion of cream to a water-in-oil emulsion (W/O).

**Table 9.2: Types of butter**

| Type of butter | Description |
|---|---|
| Cultured (or 'lactic') butter | Made from soured milk or cream, may be salted or unsalted, slightly acid taste |
| Fresh (or 'sweet') butter | Made from cream, unsalted and unripened, mild creamy flavour |
| Fresh salted butter | Made from cream, salted (1–8% salt), unripened, mild creamy flavour |
| *Ghee* | A pale golden-brown fat made by heating unsalted butter to remove the moisture |
| Whey butter | Made from whey, salted or unsalted, slightly acid flavour |

Most butters contain about 16% water, and any contaminating micro-organisms are found in the water droplets. When salted butter is made, the salt (1–3%) dissolves in the water droplets, so the effective salt concentration is approximately 10% in the water. It acts as a preservative to improve the shelf life by suppressing the growth of any micro-organisms that are present, and is also used to improve the flavour.

### Fresh (or 'sweet') and salted fresh butter

Traditionally, butter was made from cream that had been allowed to stand and sour naturally. The cream was skimmed from the top of the milk and butter was made by hand using churns. However, natural souring is prone to infection by

contaminating micro-organisms, which spoil the product. In modern butter making (Fig. 9.4), milk is preheated and passed through a cream separator (Fig. 9.3) to produce the cream. The cream should be sweet (with a pH >6.6, and a total acidity of 0.1–0.12%) and not be rancid or oxidised. It is pasteurised to destroy enzymes and micro-organisms that would reduce the shelf life of the butter.

| Stage in process<br>Optional | Notes |
|---|---|
| Cooled raw milk | Milk with a high fat content gives a higher butter yield |
| ↓ | |
| Filter | |
| ↓ | |
| Separate | Pre-heat milk to 36–40°C. Separate into skimmed milk and cream in an electric or manual cream separator. The fat content of the cream is measured using a cream butyrometer and should be standardised to approximately 40% fat using the separated skimmed milk. This optimises the churning efficiency and the butter yield |
| Pasteurise | At 72°C for 15 seconds or 63°C for 30 minutes. To improve the keeping quality of the butter, it is advisable to exceed this minimum heat treatment (e.g. 75°C for one minute). Start timing when it reaches the correct temperature and continually stir to ensure even heating |
| Chill | To below 4°C for several hours (or overnight) to 'age' the cream and improve churning efficiency and butter yield |
| Churn | In a butter churn. Keep the temperature as low as possible during churning. The cream viscosity increases and finally the cream 'breaks' to give a clear separation into butter grains and buttermilk. Churning should be continued until butter grains adhere together into large lumps |
| Separate | Drain off the buttermilk |
| ↓ | |
| Wash ← Salt | Add clean chilled water to the butter and slowly churn to remove residual buttermilk and thus improve its shelf-life For salted butter, add salt (1–3% of butter weight) with continued slow churning to achieve even salt distribution |
| Work/Mould | Work to correct consistency with butter pats and mould into solid blocks |
| Pack/Store | Into greaseproof paper, foil or plastic bags and store below 4°C in a refrigerator |

**Fig. 9.4 Processing fresh and salted butter**

After pasteurisation, cream is cooled to refrigeration temperature and held for several hours to allow the fat to partly crystallise. This helps the churning process. During churning, the cream is agitated violently to make the fat globules coalesce into butter grains. Small butter churns have a paddle that is rotated either manually or using an electric motor. On a larger scale, a specially shaped drum is rotated end-over-end to churn the cream.

After filling the churn, the following steps are taken:

- rotate the churn at 25–35 rpm for 5 minutes
- stop the churn and release the gases
- churn again at 25–35 rpm for 35–45 minutes
- drain the buttermilk into pails
- add the same amount of cold water as the amount of buttermilk removed, and churn at 10–15 rpm for 5 minutes
- drain off the water
- churn at 10–15 rpm for 10–20 minutes
- Remove the butter.
- churn again at 25–35 rpm for 35–45 minutes
- drain the buttermilk into pails
- add the same amount of cold water as the amount of buttermilk removed, and churn at 10–15 rpm for 5 minutes
- drain off the water
- churn at 10–15 rpm for 10–20 minutes
- Remove the butter.

The efficiency of churning (Ec) is assessed by the amount of fat transferred from the cream to the butter, and can be calculated as follows:

$$Ec = 1 - f_{bm} / f_c$$

where

$f_{bm}$ = % fat in the buttermilk

$f_c$ = % fat in the cream.

These can be used to predict the expected yield of butter per kg of milk (Worked example 2).

The efficient operation of a dairy requires that losses of milk and products be minimised at each stage of processing. If churning takes place at too high a temperature, there are higher losses of fat in the buttermilk.

**Worked example 2: Calculation of butter yield**

If there is 3.6% milk fat in milk, 0.05% milk fat in skimmed milk, 40% milk fat in cream and 0.3% milk fat in buttermilk:

The separation efficiency (Es ) = 1 – 0.05/3.6 = 98.6

The churning efficiency (Ec ) = 1 – 0.3/40 = 99.25

The butter is then worked with butter pats to produce an even distribution of the finely dispersed water droplets. It is then either pressed into a butter mould, and packaged in greaseproof paper, plastic or aluminium foil, or placed directly in plastic tubs (usually 250 g or 500 g)

Alternatively butter can be moulded in small butter moulds that may have special shapes, especially for use in hotels. Small plastic single-serve portion pots (10–15 g) of butter are another type of product that is supplied to airlines and hotels in some countries.

If a refrigerator or chilled water is not available, it is possible to make butter but there may be the following problems:

If the cream is not allowed to age at a low temperature, the fat globules do not develop the crystalline structure needed for good separation into butter and buttermilk.

A higher temperature during churning reduces the butter yield, because some of the butterfat liquefies, and is lost with the buttermilk. The water used for washing butter must be potable or else it will recontaminate the butter and reduce its shelf life.

The shelf life of butter is reduced if it is stored without refrigeration. At refrigeration temperatures, butter will keep for several months, but at ambient temperatures off-odours develop after only a few days. However, in many ACP countries, butter with a slightly rancid flavour is acceptable.

The main cause of butter spoilage is the development of rancidity, and in some countries the use of an anti-oxidant such as butylated hydroxy-anisole (BHA) or butylated hydroxy-toluene (BHT) may be permitted by law to prevent this. However, butter is not usually stored for long periods in ACP countries and these chemicals are not needed.

### Cultured or Lactic Butter

Traditional butters, made from fermented milk, are more acidic than products from commercial dairy plants, but may be preferred by people in some countries because of their pronounced flavour. They can be made more easily than fresh butter on a small scale as they do not require chilling or the use of a cream separator. Fresh milk is pasteurised and cooled to about 37°C. A starter culture or a small amount of butter from a previous batch is added, and the milk is fermented to form

yoghurt, for either several hours or up to 3–4 days depending on the ambient temperature. Churning the yoghurt produces the butter. In some areas the containers are coated inside with wood smoke to disinfect them before each batch. The wood smoke imparts a characteristic flavour to the butter and the buttermilk. The resulting yoghurt is cooled, salted (by adding approximately 0.5% salt) and churned manually in gourds, leather bags or wooden churns to produce the lactic butter. The containers are shaken and/or rocked back and forth until the butter grains form. The tightly closed mouth of the container is fitted with a vent to release the air that builds up during the first few minutes of shaking. The butter grains float on the surface of the milk, growing in size as the agitation nears completion. They are collected and washed with water two or three times. The butter is then packed for distribution in containers or in plastic bags.

Cream may also be 'ripened' with mixed cultures of *Streptococcus cremoris, S. lactis diacetyl lactis,* and *Leuconostoc* sp. that increase the flavour of butter. Dried cultures may be obtained from specialist suppliers in some ACP countries, or imported. The cream is ripened for 12–15 hours at 21°C to pH 5.5 and then at 13°C to pH 4.6. The lower the temperature during ripening, the more flavour develops. Alternatively, a culture may be inoculated during churning. Ripened butter is usually not washed or salted. Butters that have their flavour enhanced using this process are termed 'lactic,' 'ripened' or 'cultured' butters. Although the product is claimed to have a superior flavour, its storage life is more limited than sweet/salted butters.

## GHEE

*Ghee* is made by heating cream to break down the emulsion, release the water and boil it off (Fig. 9.5), leaving a clear golden fat with a characteristic flavour. It is made from cow's or buffalo milk and has a high demand in some ACP countries as a domestic cooking oil and as an ingredient for bakeries or confectionery manufacturers. The main quality factors are the colour and clarity of the oil, which depend on proper filtering, and the flavour and odour, which depend on the time and temperature of heating. *Ghee* is preserved by a combination of heat, which destroys enzymes and contaminating micro-organisms, and by removing water from the oil to prevent micro-organisms growing during storage. It has a long shelf life if it is stored in a cool place, using airtight, lightproof and moisture-proof containers to slow down the development of rancidity.

## CHEESE

There are hundreds of varieties of cheese produced in different parts of the world, but each relies on similar principles of coagulating the proteins in milk to form curds, and then separating them from the liquid whey. The different cheese flavours and textures arise from variations in the type of milk, the amount of fat in the milk, bacteria that are used to ferment the milk and variations in the processing conditions. Cheeses may be broadly classified into 'soft', 'semi-hard' and 'hard' cheeses (Table 9.3). Production of soft cheeses is more straightforward than

| Stage in process | Notes |
|---|---|
| Milk ↓ | Fresh, filtered cow or buffalo milk |
| Pre-heat | In an aluminium pan to 36–40°C to optimise the efficiency of the cream separator |
| Separate | Preheated milk is separated into cream and skimmed milk using a cream separator. (Traditionally milk is boiled and cooled several times and the fatty scum is skimmed off) |
| Heat | Cream is boiled with constant stirring to evaporate water. The end point of the boiling stage is shown by the correct colour and texture of the *ghee*. There is no simple test for this and it is judged by experience |
| Cool | After the heating is stopped and the product is left to set, and particles settle at the bottom of the vessel |
| Filter | The product is filtered carefully using cheesecloth so that it is clear without any particles |
| Pack | Into airtight glass or metal containers (iron or copper should not be used because they accelerate the development of rancidity). Containers should be dry and thoroughly cleaned, especially if they are re-used |
| Store | At room temperature away from heat and sunlight. The shelf life can exceed 12 months with proper packaging and storage conditions |

**Fig. 9.5 *Ghee* production**

hard cheeses, and these are the more common traditional types that are in demand in ACP countries. The demand for hard cheeses may be more limited and a careful market analysis is needed before starting their production.

The coagulation of milk proteins can be achieved in a number of different ways (Table 9.4):

- using rennet, an enzyme found in the fourth stomach of ruminant animals, or made synthetically
- fermenting to form lactic acid
- adding acid (e.g. lime juice or vinegar)
- boiling
- using plant extracts.

Milk is coagulated in cheese vats using rennet, or bacterial cultures. Rennet causes the milk proteins to form a semi-firm gel. Lactic acid production by bacterial cultures helps the whey to separate from the curd and also determines the final cheese flavour and texture. The curd is cut into small cubes using vertical and horizontal curd cutters.

**Table 9.3 Types of cheeses**

| Type of cheese | Moisture content (%) | Fat content (%) | Texture | Shelf-life |
|---|---|---|---|---|
| Soft cheeses | 45–75 | <40 | Soft, white, spreadable | A few days |
| Semi-hard cheeses | 35–45 | <35 | Firm, crumbly, can be sliced | A few months |
| Hard cheeses | 30–40 | <30 | Very firm, dense, sometimes grainy | One year or more |

**Table 9.4 Coagulants used in cheese making**

| Coagulating agent | Rate of addition to milk |
|---|---|
| Citric acid (from lemon or lime juice) | 1–1.5% by weight |
| Cut stems of *Bryophylum* species | 4 stems per litre |
| Fig bark (ficin enzyme) | Data not available |
| Lactic acid (from yoghurt) | 2–2.5 g per litre |
| Leaves of *Calotropis procera* | 1 leaf per litre |
| Papaya sap (papain enzyme) | Data not available |
| Pineapple pulp (bromelin enzyme) | Data not available |
| Rennet tablet, powder or liquid | 1 tablet per 100 litres, 1 teaspoon of powder per 40 litres or 20–30 ml liquid per 100 litres |

These may either be motorised and fitted to a cheese vat or hand cutters as shown in Fig. 9.7. Cutters are stainless steel frames strung with thin wires or blades. The vertical cutter (A) is first pulled through the length of the cheese vat until all of the curd is cut in one direction. It is then used to make vertical cuts across the vat. Finally, the horizontal cutter (B) is used to cut the curd in one direction only, to form the curd into cubes. The whey (mostly water and lactose) drains from the curds and the cubes are then poured into cheese moulds and pressed to remove more whey.

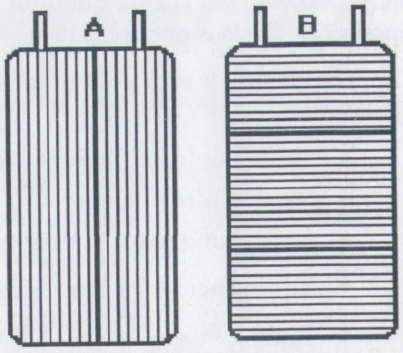



**Fig. 9.7. Curd cutters**

Simple cheese moulds can be made from plastic drainage pipe cut into 10–25 cm lengths with holes drilled to allow drainage. The moulds are fitted with plastic or wooden discs for the lid and base sections. The simplest curd press is made by placing weights or cement blocks on cheese in moulds. Manual cheese presses have

a press plate, which is raised and lowered by a screw. In operation a muslin or cotton bag is placed in the mould and curd is poured in. The bag is closed and the press plate lowered. Whey drains through the holes in the mould, and after a suitable time the screw is raised and the bag removed. To clean the press, the mould is removed, washed thoroughly with detergent and rinsed with clean water. The other components are cleaned while the mould is removed. The press bags should be washed with detergent and then sterilised by boiling for 10–15 minutes before drying in the sun.

Seven types of cheeses can be grouped, according to texture and manufacturing processes, as follows:

**Acid-coagulated fresh cheese (e.g. Cottage, Quark and Cream cheeses)**

'Fresh cheese' is produced by acid coagulation to pH 4.6–4.8 at 30–32°C without added rennet. Acid is produced by lactic acid bacterial cultures but some fresh cheese may also be produced by direct acidification. After cutting, the curd is heated to 52°C to inactivate the culture and prevent further acid development. Washing the curd before salting also reduces the acidity. In many ACP countries, traditionally produced soft cheeses have a sour taste and pronounced flavour. They are made using fermented buttermilk (from traditional butter-making), which is heated gently for about 30 minutes until completely coagulated. It is then cooled, and the whey is drained off using a straw mat or stainless steel mesh, leaving the cheese behind. It is packed in plastic bags and sold fresh, within 24 hours if refrigeration is not available. Alternatively the cheese is pressed into blocks and stored in brine until it is sold from the brine tub.

A similar soft cheese known as Wagashie in West Africa, has a slightly sour, fermented flavour and may be coloured dark red using dried sorghum leaves. It is made by preheating slightly acidified milk (pH 5.6-6.0) to about 40°C, adding juice from leaves or bark of the Sodom Apple tree and heating it to 60-70°C for approximately 1 hour, stirring continuously to avoid charring until it is partially solidified. The curd is worked by hand, washed to remove lactose, salted in boiled water and then boiled with dried sorghum leaves to impart the red colour. After cooling, the curd is dried and cut or moulded into small pieces and fried carefully in oil until it is slightly browned. The product has a shelf life of 1–5 months.

**Rennet-coagulated fresh cheese (e.g. Queso Blanco, Queso Fresco, Halloumi)**

Fresh rennet cheese is produced without bacterial cultures. Without acid production, the cheese pH remains high (6.5-6.7) and for safety, these cheeses must be handled with extra attention to hygiene, sanitation and refrigeration. Such cheeses have a moisture content of 60-70% and a shelf life of 2-3 weeks.

**Heat/acid precipitated cheese (e.g. Ricotta, Paneer)**

Milk proteins are coagulated in one of three ways:

- Coagulation of milk protein (casein) with rennet, and acid production by lactic acid bacteria.
- Coagulation of casein by acid produced using a natural fermentation at 20–35°C. In this temperature range, a pH of less than 4.9 is needed to form the curd.
- Coagulation by lactic or citric acid added to hot milk at 75–100°C, but no fermentation is involved. The whey proteins (Appendix I) bind water so that a firm cheese with a high moisture content (55–80%) is produced. At high temperatures less acidification is needed, so the final cheese is much less acidic (pH 5.2–6.0) than fresh cheese (pH 4.4–4.8). The inclusion of whey proteins prevents cheeses from melting so that they can be used as frying/cooking cheeses.

Heat/acid precipitated varieties are normally consumed fresh. An exception is Mizithra, a type of ricotta that is cured, dried, and eaten as grating cheese.

**Soft-ripened cheese (e.g. Feta, Camembert, Brie)**

Coagulation is by rennet, but compared to cooked and pressed varieties, there is a larger lactic acid bacteria inoculum and the ripening period before renneting is extended. The setting time is increased for greater acidification and curd is broken up with paddles and left to stand in a warm room for several hours. Conversion of lactose to lactic acid continues until the accumulation of lactic acid stops the culture growing. The pH is 4.3–4.6 on the day following manufacture although the pH of mould-ripened varieties (e.g. Camembert and Brie) increases during the curing period (2–8 weeks). The moisture content is typically 45–60%.

**Semi-hard Washed Cheese (e.g. Edam, Gouda, Colby, Montasio, Muenster)**

This is the largest and most diverse group of cheeses, Production of rennet-coagulated cheeses that have moisture contents of >40% requires a washing treatment to remove lactose. The aim is to limit the amount of lactose, to restrict lactic acid development and produce a pH of 5.0–5.2 (but not less than pH 5.0). The amount of moisture expelled from the curd (known as 'syneresis') is controlled by the temperature and time of cooking and by the wash water temperature. Higher temperatures during cooking or washing cause the curd to contract and expel more moisture. Typically, these cheeses have moisture contents of 40–50% after curing for between 2 weeks and 9 months.

**Hard Cheese: Low Temperature (e.g. Cheddar and Pasta Filata)**

Hard cheeses are characterised by lower moisture contents than other types. Low moisture is achieved by high-temperature cooking or by controlled fermentation and curd handling. The moisture content is typically 35–39% for cheddar types and up to 52% for Pasta Filata types. It is controlled by the cooking temperature and time, stirring after draining and the amount of culture and salt used. The method of manufacture is similar in the early stages, but Pasta Filata types

are worked and stretched in hot water and salted using brine. Cheddar types are salted before hooping and pressing. As with semi-hard cheeses, the aim is to have a minimum pH 5.0–5.2 within 1–3 days after manufacture. The lactose and moisture contents are reduced by fermentation during cheddaring and salting. The cheeses are ripened for 1–36 months.

**Hard Cheese: High Temperature (e.g. Parmesan, Swiss, Romano)**

The pH is controlled by the type of culture, the time and temperature of pressing, and lactose removed by syneresis. Moisture is removed by a high renneting temperature and a high cooking temperature and the cheese is ripened for 10–36 months. Some properties of cheese are categorised according to type of coagulation and procedures used for pH and moisture control.

A summary of the processing stages for cheese manufacture is shown in Fig. 9.8.

**Preparation of Starter Cultures**

Starter cultures are used because the naturally occurring lactic acid bacteria in milk are inefficient, uncontrollable or unpredictable, or are destroyed by the heat treatments given to the milk. A starter culture produces a more controlled and predictable fermentation and inhibits undesirable micro-organisms. Milk used for the preparation of a starter culture is held at 63°C for an additional 15–30 minutes longer than the pasteurisation process to ensure that the large majority of bacteria are destroyed. After pasteurisation, it is cooled to 45°C and then poured into 1-litre bottles and a 5-litre covered container. The dried starter culture is added to bottles of the milk, shaken well to dissolve the powder, and grown at 40–45°C for 3–4 hours. This forms the 'mother culture' and it is incubated at 20–22°C for 15–16 hours. All subsequent starter cultures will be made from this. Part of the mother culture is then used to prepare an intermediate culture in 5 litres of milk, and part of the intermediate culture is used to prepare a 'bulk starter culture' for use in production of the batch of cheese. If the cultures are prepared carefully, each packet of commercial culture can be sub-cultured for 1–2 months.

Unlike commercial dried starter cultures, rennet can be made locally if advice from a university laboratory is obtained. Abomasa (the fourth stomachs of calves), preferably from un-weaned calves, are washed and the fat and veins are removed. They are then inflated with air to avoid the two sides touching, and the neck and base are tied with string. The inflated abosama are hung in a well-ventilated area to dry for about 1 month. When dried, the flattened abosama can be kept for up to a year without signs of deterioration. Each abosamum is sliced into 5-mm wide strips, and soaked for 24 hours at 20–25°C in a plastic or stainless steel tank, containing 10% brine with 1% sodium benzoate preservative. After soaking, the liquid is poured into a container. The same abosama strips and brine may be renewed 4–5 times before the enzyme activity diminishes. Commercially produced liquid rennet can also be obtained in some ACP countries.

| Stage in process | Notes |
|---|---|
| Soft and semi-hard cheeses / Hard cheeses | |
| Raw milk | Collect the milk in carefully cleaned, covered vessels. Filter milk and cool immediately to control growth of micro-organisms and enzyme activity. Milk that is likely to contain antibiotics should not be used as they will inhibit the action of lactic acid bacteria |
| Standardise | To approximately 26 g per litre fat content |
| Pasteurise | At 63°C for 30 minutes in a double-walled cheese vat. The milk should not be boiled as this adversely affects the flavour of products |
| Coagulate | By adding acid or vegetable extract while milk is hot |
| Cool | Cool the milk to 35–40°C by running cold water through the vat or by re-circulating refrigerated water in the vat |
| Ferment | Add starter culture and leave for 15–30 minutes |
| Coagulate | With rennet (strength 1/10,000 – see text) at 30°C in the same vat. Rennet added at 20–25 ml per 100 litres of milk. If required, add 5–50 g calcium chloride per 100 litres of milk. Leave for 10–15 minutes |
| Cut | To approximately 1–3 cm cubes using cheese knives. Two sets of knives are used, one with vertical blades and the other with horizontal blades (Figure 4.10a) |
| Stir | For 5–10 minutes |
| Drain | Drain whey from the vat through a tap. Replace with water or brine and stir for 10–20 minutes |
| Drain/wash | |
| Drain | Drain, remove the curd and allow water to drain using cheesecloth on a slatted table. Cheesecloth should be sterilised by boiling for 10 minutes after use |
| Salt | Mix 30 g salt per kg curd |
| Salt (Optional) | |
| Mould | Place curds in wooden or plastic moulds, lined with sterilised cheesecloth |
| Mould | |
| Press | In a cheese press for 1–5 hours. Turn the moulds 2 or 3 times |
| Salt | Soak cheese in brine (200 g salt per litre water) for 8–24 hours to form a rind on the outside (Figure 4.12) |
| Salt (Optional) | |
| Ripen | To develop characteristic flavour and texture and to lose moisture. Use wooden racks in a storeroom at 12–15°C and 85–95% relative humidity for several weeks/ months, depending on the type of cheese. In tropical climates this can be achieved using an underground cellar |
| Smoke | (Optional for speciality cheeses) |
| Pack | Pack soft cheese in pots. Hard cheeses can be dipped in food-grade wax or packed in polythene film, clingfilm or greaseproof paper |
| Milk Solids-Not-Fat (MSNF) = proteins, lactose, minerals, acids, enzymes, vitamins. Total Milk Solids = fat + MSNF | |

**Fig. 9.8 Generalised process for making different types of cheese**

### Processed cheese

Sub-standard cheeses and cheese trimmings can be melted to make saleable products such as processed cheese spread. The cheese rind is removed and the cheese is cut into small pieces, mixed with emulsifying salt and heated for 10 minutes up to 90°C. It is then cooled and packaged while still liquid. Products may be varied by adding a variety of ingredients, including flavourings, pieces of mushroom, nuts, meats, etc. The product can be packaged for sale in plastic tubs or wrapped in aluminium foil.

## CULTURED MILKS AND YOGHURT

Fermented milk products such as cultured milk and yoghurt are produced using lactic acid bacteria that ferment the lactose in milk to form lactic acid. The acid helps to prevent the growth of pathogens, and also inhibits the growth of spoilage bacteria to preserve the milk for a short time. The removal of lactose enables people who suffer from lactose intolerance to consume these dairy products. There are many different types of cultured milks that are liquid or semi-solid and have different flavours depending on the types of bacteria that are present, the type of milk and the incubation conditions (Table 9.6).

**Table 9.6 Examples of cultured milk products**

| Type of product | Bacteria used | Description |
|---|---|---|
| Acidophilus milk | *Lactobacillus acidophilus*, *Streptococcus lactis*, *Torula* yeast, *Betabacterium caucasium* | Liquid, acidic flavour, slightly fizzy and alcoholic |
| Cultured milks | *S. lactis*, *S. cremoris*, *S. diacetilactis*, *Leuconostoc citrovorum* | Liquid, made from skimmed milk or buttermilk (from butter making), strong acidic flavour |
| Yoghurts | *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* in a ratio of 1:1 | Gel-like or highly viscous, creamy, slightly acidic |

### Acidophilus Milk

Acidophilus milk is a traditional milk made from skimmed or whole milk that is fermented with *Lactobacillus acidophilus*, which is believed to have therapeutic effects on the digestive system. The milk is heated to 95°C for 1 hour to reduce the numbers of contaminating micro-organisms and prevent competition for the slow growing *Lactobacillus* culture. Milk is inoculated with 2–5% *Lactobacillus* culture and incubated at 37°C until coagulated. Some acidophilus milks have an acidity as high as 1% lactic acid, but 0.6–0.7% is more common. Sweet acidophilus milk has the *Lactobacillus* culture added but there is no fermentation, thus delivering the therapeutic benefits without the high acidity and strong flavour that are considered undesirable by some people.

### Cultured Milks

Traditional soured milk is thick, clotted and has a stronger flavour and a more acidic taste than yoghurt. It has a shelf life of 3–8 days at ambient temperatures and is used as a drink or as an accompaniment to meals. Raw milk is allowed to sour by naturally occurring bacteria in lidded containers, placed in warm room for 1–2 days. The product may be sold in portions directly from the culture vessel, or alternatively the inoculated milk may be poured into pots and allowed to ferment in them. The returnable or disposable pot plus the contents is then sold. In an upgraded method, the milk is inoculated with a *Lactobacillus* or *Leuconostoc* starter culture in a stainless steel vessel and held at 25–30°C in a thermostatically controlled water bath. It is packed in polythene bags or plastic pots and has a shelf life of up to 1 week under refrigerated storage.

## YOGHURT

Yoghurt is produced by fermenting milk using two types of lactic acid bacteria (Table 9.6) either bought as a dry powder or taken from a portion of a previous batch of product. They produce lactic acid, which causes the characteristic curd to form and restricts the growth of some spoilage bacteria, so that yoghurt can be kept for up to 10 days under refrigerated storage. *Streptococcus* grows faster and produces both acid and carbon dioxide. This stimulates *Lactobacillus* growth. *Lactobacillus* produces peptides and amino acids, which are used by *Streptococcus*. The yoghurt mixture coagulates during fermentation due to the drop in pH. The *Streptococcus* causes the initial pH to drop to approximately 5.0, and then the *Lactobacillus* cause a further decrease to pH 4.0. The taste and texture of yoghurt are determined by the amount of lactic acid produced during the fermentation and this in turn depends on the amount of starter added and the temperature/time of incubation.

In processing (Fig. 9.9), milk is standardised to the required fat content and the different ingredients are blended in a mixing tank. The mixture is pasteurised to both destroy contaminating bacteria, and to denature and coagulate whey proteins to enhance the texture of the final product. The yoghurt is cooled to 40–45°C and fermented for 4–6 hours until the acidity is 0.85–0.90%. It is then cooled and for stirred yoghurt (below) it is stirred to break the gel. The product is then packaged and stored at refrigeration temperatures (5°C) to slow down chemical and microbiological changes that would shorten its shelf life.

Yoghurt incubators can be locally made using a wooden or steel box approximately 75 cm each side that is insulated with approximately 10 cm thick expanded polystyrene or other insulating materials. A 40W light bulb is sufficient to maintain the temperature at 40–45°C inside the cabinet. Alternatives include using a shallow water bath fitted with a small electric heating element and thermostat to keep the culture warm . Indentations can also be made into a block of 10-cm thick polystyrene to hold plastic pots, with a polystyrene lid placed on top. The insulating effect of the polystyrene prevents heat loss sufficiently to maintain the temperature of the product during a 4–6 hour fermentation. On a very small scale, the warm yoghurt mix can also be filled into large commercial thermos flasks to keep it warm.

| Stage in process | | Notes |
|---|---|---|
| | Optional | |
| Milk | | Collect the milk in carefully cleaned, covered vessels. Filter milk to remove visible dirt and 'ropiness', and cool immediately to control growth of micro-organisms and enzyme activity. Milk which is likely to contain antibodies should not be used as they will inhibit the action of lactic acid bacteria |
| Pasteurise | | At 63°C for 30 minutes or at 72°C for 15 seconds |
| Cool | | To 40–45°C |
| Ferment | | Add a starter culture of the yoghurt bacteria. Keep the milk at the same temperature for 4–6 hours while the fermentation takes place |
| | Fill | If set yoghurt is required, fill the pots with the culture after inoculating with bacteria |
| | Fill | For liquid yoghurt, stir the yoghurt after fermentation to break the gel and then pour liquid yoghurt into pots |
| Cool | | To 5–10°C |
| Seal | | Seal pots with a foil or plastic lid |
| Cool/store | | In a cool place away from sunlight and preferably in a refrigerator |

**Fig. 9.9 Process for making yoghurt**

The correct balance of the two types of bacteria is important for good quality yoghurt. Dried yoghurt cultures can be obtained in most large ACP cities, and they can be grown in pasteurised milk and kept in a refrigerator. A part of this 'mother culture' is then used each day for a week. The last part is re-inoculated into milk to form a new mother culture. With good hygiene, this method can be continued for several months, but eventually undesirable bacteria will contaminate the culture and it must be replaced. If a refrigerator is not available, it is possible to add one or two teaspoonfuls per litre of milk of commercial yoghurt (which has not been pasteurised) as the starter culture. Alternatively, part of the yoghurt production can be used as a starter and added to a new batch of milk the following day. However, there is a greater risk of contamination using this method and it is not recommended unless the other methods are not possible.

There are two potential problems in making cultured milk products:

1. Spoilage by bacteria or moulds. This is caused by unclean equipment, contaminated milk or poor hygiene of production staff. It is prevented by thoroughly cleaning and sterilising all equipment and utensils with chlorine solution or boiling water. This should be done before and after processing, and followed by thorough rinsing in clean water. Other causes of spoilage are the use of old milk and poor hygiene by production workers.

2. Maintenance of correct incubation temperature.

**Product Variations**

Although milk of different animals is used to make yoghurt in various parts of the world, most yoghurt production uses whole cow's milk, partially skimmed milk, skimmed milk or cream. The raw milk must have a low bacteria count and be free from antibiotics, viruses, cleaning chemicals, mastitis milk, colostrum, and rancid milk. Other yoghurt ingredients may include:

- non-fat dried milk or whey to increase the non-fat solids content. Thicker stirred yoghurt is made by adding dried skimmed milk (at approximately 50 g per litre) to the milk before pasteurising sweeteners, such as glucose, sucrose or aspartame
- flavours, colourings or fruit pulps, including natural and artificial flavouring
- thickeners/stabilisers, such as gelatine, carboxymethyl cellulose, locust bean gum, guar gum, alginates, carrageen, starch or pectin The use of thickeners adds to the cost of the product and they are not necessary.

Other yoghurt products include 'fruit-on-the-bottom' style, in which a layer of fruit pulp at the base of the pot is covered by inoculated yoghurt and incubation occurs in the sealed cups. Fruits or chopped nuts can be added to stirred or set yoghurt, but care is needed to ensure that they are thoroughly cleaned, and that nuts are blanched to avoid contamination by moulds or bacteria. Less-acidic fruits such as melon or pawpaw are more successful because they do not react with the milk. Acidic fruits (lemon, lime, pineapple etc.) may cause excessive curdling and separation of the yoghurt. In some countries a layer of fruit syrup on the top of set yoghurt is a popular alternative.

Drinking yoghurt is stirred yoghurt with added flavouring and colouring, and has a total solids content not exceeding 11%. It may be homogenised to further reduce the viscosity. Cultured buttermilk is the fermented by-product of butter manufacture, but it is also produced as a product from skimmed or whole milk using a culture of *S. lactis* or *S. cremoris* sp. Milk is heated to 95°C and cooled to 20-25°C before adding the starter culture at 1-2%. The fermentation is continued for 16-20 hours, to an acidity of 0.9% lactic acid. This product may be used as an ingredient in bakeries as well as being packaged for retail sale. There are a great many other fermented dairy products, including kefir, koumiss, labneh, and others that are specific to regional areas and have various flavours, textures, and gas or ethanol from the fermentation process, depending on the starter organisms used.

## ICE CREAM

Ice cream is both a foam (air bubbles in the water phase), and an oil-in-water (O / W) emulsion of tiny globules of milk fat in a complex water phase containing ice crystals, concentrate, unfrozen solution of sugars, milk solids, flavouring,

colouring, emulsifiers and stabilisers. It is made by freezing, and at the same time aerating, a liquid mix that has all the required ingredients (Fig. 9.10). The incorporation of air increases the softness and lightness of the product and allows it to be easily scooped. The increase in volume of the product due to the air is known as the 'overrun' and the percentage overrun in commercial ice creams varies from 60–100%.

$$\text{Overrun \%} = \frac{100 \times \text{weight of a given volume of mix} - \text{weight of same volume of ice cream}}{\text{weight of same volume of ice cream}}$$

If, for example, 3 kg of mix fills a container and 1.6 kg of ice cream fills the same container, the overrun

$= 100 \times (3 - 1.6)/1.6$

$= 100 \times 1.4/1.6$

$= 87.5\%$

| Stage in process Optional | Notes |
|---|---|
| Weigh | Weigh all main ingredients into pasteurisation vessel, premix minor dry ingredients with 3 or 4 times their weight of sugar |
| Heat | To 50°C and add any solid fats |
| Pasteurise—Homogenise | At 65°C for 30 minutes or 72°C for 10 minutes with thorough mixing |
| Cool | For a minimum of 4 hours at 3-5°C to allow fats to crystallise and the viscosity to increase |
| Freeze | To –5°C as quickly as possible |
| Pack | Fill into pots or cardboard cartons |
| Harden | At below –20°C |
| Cold store | At –18° to –20°C |

**Fig. 9.10 Production of ice cream**

Manual or electric ice cream makers have a stainless steel bowl that is immersed in a freezing liquid (e.g. an ice/salt mixture), placed in a freezer, or surrounded by refrigeration coils. Two types of larger ice cream makers are used for small- and medium-scale production:

- vertical freezers with capacities of 4-20 litres per batch
- horizontal freezers with capacities of 8-35 litres per hour.

Both have freezing times of approximately 15 minutes.

The ice cream mixture is placed in the bowl and a rotor scrapes the frozen ice cream from the bowl wall and simultaneously incorporates air. Freezing is continued until all of the liquid is frozen at approximately –4°C to –7°C. This soft ice cream is then either sold directly or hardened in a freezer at –18°C. Fast freezing results in very small ice crystals, which are not detectable in the mouth and thus gives a smooth creamy consistency to the product.

There are important differences between the batch and horizontal types that affect the type of ice cream mix that is needed. A mix for a batch freezer requires a fat content of 6-8% and a sugar content of 12-13%, whereas the mix for a horizontal freezer should have a fat content of 9-10% and a sugar content of 14-15%. Batch freezers can only beat small amounts of air into the mixture as it freezes, to give an overrun after freezing of 50% or less. Horizontal freezers are more efficient at incorporating air and overruns can be up to 85%. As ice cream is usually sold by volume, the amount of air in the product has an important effect on profitability.

### Non-dairy Ingredients

Vegetable fats, including hydrogenated palm and coconut oils and salt-free margarine, are commonly used in ice cream in order to reduce costs. Sugars not only improve the flavour but also counterbalance fattiness and assist in providing a smooth texture. Dextrose or corn syrup modifies the melting point of ice in the ice cream to make it harder or softer. Stabilisers bind together the complex mixture of fats, sugars, air and ice crystals. Carboxymethylcellulose is widely used as a stabiliser and is blended with small quantities of gums to improve its action. Manufacturer's recommendations on quantities of stabilisers used should be closely followed. Both synthetic and natural flavours are used, often with a colour that matches the flavour (e.g. yellow with banana flavour, orange with mango flavour etc.).

### Formulating an Ice Cream Mix

It is important that small-scale producers understand how to develop new ice cream mixes to expand their range of products and compete effectively with other producers. 'Balancing' a mix involves maintaining the correct proportions between:

- fat and sugar that controls the 'fattiness' of the product
- water to solids that controls the texture.

Correct formulation of an ice cream mix requires knowledge of the composition of the ingredients, their cost and availability, and also depends on the type of freezer that is used. The typical fat and milk-solids-not-fat (MSNF, i.e. proteins, lactose, minerals, acids, enzymes, vitamins. Total milk solids = fat + MSNF) contents of dairy products used as ingredients in a mix are shown in Table 9.7 and typical formulations for different types of ice cream are shown in Table 9.8.

**Table 9.7 Typical composition (%) of common ice cream dairy ingredients**

| Product | Fat | MSNF | Sugar | Water |
|---|---|---|---|---|
| Full cream milk | 4 | 8.8 | | 87.2 |
| Liquid skimmed milk | | 9 | | 86 |
| Full cream milk powder | 27 | 70 | | 3 |
| Skimmed milk powder | | 97 | | 3 |
| Double cream | 48 | 4.5 | | 47.5 |
| Single cream | 18 | 7.2 | | 74.8 |
| Butter | 84 | | | 16 |
| Sweetened condensed milk | 9 | 22 | 44 | 25 |
| Evaporated milk | 9 | 22 | | 69 |

**Table 9.8 Different mixes for hard-frozen ice creams**

| Component | (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Type of ice cream | Standard | | Premium | | | | Super |
| Milk fat | 10.00 | 11.00 | 12.00 | 13.00 | 14.00 | 15.00 | 16.00 |
| MSNF | 11.00 | 11.00 | 10.50 | 10.50 | 10.00 | 10.00 | 9.50 |
| Sucrose | 10.00 | 10.00 | 12.00 | 14.00 | 14.00 | 15.00 | 15.00 |
| Corn syrup solids | 5.00 | 5.00 | 4.00 | 3.00 | 3.00 | — | — |
| Stabiliser | 0.35 | 0.35 | 0.30 | 0.30 | 0.25 | 0.20 | 0.15 |
| Emulsifier | 0.15 | 0.15 | 0.15 | 0.14 | 0.13 | 0.12 | 0.10 |
| Total solids | 36.50 | 37.50 | 38.95 | 40.94 | 41.38 | 40.32 | 40.75 |
| Water | 63.50 | 62.50 | 61.05 | 59.06 | 58.62 | 59.68 | 59.25 |
| Overrun (%) | 100–120[1] | 60–90 | 25–50 | | | | |

1 120% overrun is a legal maximum in some countries Source: Bonfoh, 2005.

## MILK CONFECTIONERY

In ACP countries that have significant Asian populations, there is a demand for a variety of dairy confectionery products such as *rasagolla, champakali, rasmalai, gulab jamun* etc. They are made from intermediary products such as *khova* – made by reducing milk whilst stirring constantly until it's almost solid and heat-acid coagulation (*chhana*) of milk. Both are granular, whitish creamy in colour, soft and spongy with a characteristic bland and fatty flavour. Fig. 9.11 shows stages in production of *chhana*.

*Rasagolla* is a round, creamy white, milk-based sweet made from *chhana* suspended in sugar syrup (Fig. 9.11). It has a smooth surface, distinct spongy and springy texture and the flavour of *chhana*.

| Stage in process | Notes |
|---|---|
| Cow's milk | Only fresh milk should be used |
| Heat | Boil for 10 minutes. Milk should be regularly stirred to avoid cream forming on the surface and prevent milk burning onto the pan |
| Cool | To about 80°C |
| Mix | Lemon juice or citric acid or curd is added slowly to the milk (20 g per 10 litres milk), with constant and slow stirring. Continue until coagulation is complete. Maintain the temperature at 80°C. It's important not to put in the lime juice/curd when the milk is boiling, and to wait till it cools slightly While the acid is being stirred in, turn on a very low flame to maintain the temperature, then switch off and allow to settle |
| Settle/drain | Allow to settle for 15 minutes. The coagulated milk is hung in a muslin cloth bag for about 2 hours to drain the whey. The coagulum is the finished product |
| Pack | In vegetable parchment paper or polythene bags |
| Store | The product has a shelf life of 2–3 days under refrigeration |

**Fig. 9.11 Production of *chhana***

| Stage in process | Notes |
|---|---|
| *Chhana* | Good quality fresh *chhana* |
| Mix | Add wheat flour (20 g per kg of *chhana*) gradually while kneading to smooth and pliable consistency |
| Shape | Take a small quantity of dough to make 2-cm diameter balls, slightly flatten them by pressing with fingers, place a cardamom seed in the centre of each ball, fold and shape into smooth balls. Keep the balls covered with a wet cloth |
| Mix | Prepare sugar syrup (1 litre of water to 1 kg sugar), heat to dissolve, filter and boil |
| Heat | Transfer balls to simmering sugar syrup and cook for 10–15 minutes on medium heat. On cooking the balls absorb syrup and swell |
| Cool | To room temperature |
| Pack/store | In glass jars |

**Fig. 9.12 Production of *rasagolla***

| Stage in process | Notes |
|---|---|
| Milk | Milk should be fresh, without any undesirable off-flavours or acid development. Buffalo milk with a minimum of 5% fat is preferred as it yields more *khova* with a better texture than cow's milk |
| Heat (high) | Boil whilst continuously stirring to avoid cream separation. Continue boiling until the milk starts to coagulate which is marked by an abrupt change in colour |
| Heat (low) | Once coagulation starts the temperature should be lowered to 85–88°C. Stir briskly and regularly scrape the milk solids from sides of the pan. The end point is marked by the solid mass leaving the sides and bottom of the pan |
| Cool | To room temperature |
| Press | Pat to form a compact mass |
| Pack | In vegetable parchment paper or polythene bags |
| Store | The product has a shelf-life of 2–3 days under refrigeration |

**Fig. 9.13 Production of *khova***

| Stage in process | Notes |
|---|---|
| *Khova* | Break fresh *khova* |
| Mix | Sieve flour (105 g) and baking powder (7.5 g) together before adding to *khova*. (900 g). Excess flour gives a leathery and soggy product while insufficient flour results in bursting and disintegration of the product during frying. Mix a little water, just enough to make a smooth mixture while kneading, and thoroughly mix to get a homogeneous mixture |
| Knead | To smooth and soft dough. Cover the dough with a wet cloth and put it aside |
| Mix | Prepare sugar syrup by dissolving 3 kg sugar in an equal quantityof water and boiling it for 5 minutes. If desired rose or cardamonessence can be added |
| Shape | Take small quantities of dough and shape them into balls of 2-cm diameter or oval forms. The balls should be smooth without any cracks on the surface |
| Fry | Heat *ghee* (clarified unsalted butter) in a shallow pan in a shallow pan until hot but not smoking. Fry the balls slowly on medium / low flame until they turn deep brown. It is important to avoid the formation of an uncooked hard core at the centre, which happens when the outside browns too fast |
| Transfer | Remove the fried balls from the *ghee* using a perforated ladle and immediately transfer them into sugar syrup. The fried balls swell in size and become soft as they absorb the syrup |
| Store | In syrup in closed glass jars or wide, deep stainless steel containers |

**Fig. 9.14 Production of *gulab jamun***

*Khova* is obtained by evaporating milk (Fig. 9.13). It is used in the preparation of many sweetmeats (*galub jamun, burfi*, milk cake, *doodh peda*, etc). Like *chhana*, it has a shelf life of 2–3 days under refrigerated conditions.

*Gulab jamun* is a milk-based sweet made from *khova*, refined flour and cane sugar. It is round or elliptical in shape with deep brown slightly crisp, outer surface and dull white, soft and porous inside (Fig. 9.14). It is stored floating in sugar syrup, the product has a distinct flavour of deep-fried milk solids, sugar syrup and added flavours.

## QUALITY AND TESTING

Good-quality raw milk has to be free of debris and sediment; free of off-flavours and abnormal colour and odour; low in bacterial count; free of chemicals (e.g., antibiotics, detergents); and of normal composition and acidity. The quality of raw milk is the primary factor determining the quality of milk products. Good-quality milk products can be produced only from good-quality raw milk.

The hygienic quality of milk is of crucial importance in producing milk and milk products that are safe and suitable for their intended uses. To achieve this quality, good hygiene practices should be applied throughout the dairy chain. Among the causes of small-scale dairy producers' difficulties in producing hygienic products are informal and unregulated marketing, handling and processing of dairy products; lack of financial incentives for quality improvement; and insufficient knowledge and skills in hygienic practices.

Milk testing and quality control should be carried out at all stages of the dairy chain. Milk can be tested for:

- quantity – measured in volume or weight;
- organoleptic characteristics – appearance, taste and smell;
- compositional characteristics – especially fat, solid and protein contents;
- physical and chemical characteristics;
- hygienic characteristics – hygienic conditions, cleanliness and quality;
- adulteration – with water, preservatives, added solids, etc;
- drug residues.

Examples of simple milk testing methods suitable for small-scale dairy producers and processors in developing countries include taste, smell, and visual observation (organoleptic tests); density meter or lactometer tests to measure the specific density of milk; clot-on-boiling testing to determine whether the milk is sour or abnormal; acidity testing to measure the lactic acid in milk; and the Gerber test to measure the amount of fat in the milk.

## HEALTH HAZARDS

Similar to all other food types, milk and milk products can cause food-borne illness. The quality of milk can be affected by such factors as pathogen contamination and growth, chemical additives, environmental pollution and nutrient degradation.

Microbiological hazards are a major food safety concern in the dairy sector because milk is an ideal medium for the growth of bacteria and other microbes. These can be introduced into the milk from the environment or from the dairy animals themselves. Milk can contain harmful micro-organisms such as *Salmonella, Escherichia coli* O157:H7, *Listeria monocytogenes, Staphylococcus aureus, Yersinia enterocolitica, Bacillus cereus, Clostridium botulinum, Mycobacterium bovis, Brucella abortus* and *Brucella melitensis.*

Chemical hazards can be unintentionally introduced into milk and milk products, making them unsafe and unsuitable for consumption. Milk can be contaminated when the milking animals consume feed and/or water that contain chemicals. Other causes of contamination may be inadequate control of equipment, the environment and milk storage facilities. Chemical hazards include detergents, teat disinfectants, dairy sanitizers, anti-parasitics, antibiotics, herbicides, pesticides and fungicides.

A zoonosis is an infectious disease that can be transmitted between vertebrate animals and humans. Zoonosis infections commonly associated with the consumption of milk and milk products are tuberculosis, brucellosis, leptospirosis, salmonellosis and listeriosis.

## RECEIVING, TRANSPORTATION AND STORAGE

### Introduction

Good receiving, transportation and storage practices help ensure that food reaches its destination in a safe condition without compromising quality. By providing an environment that reduces the risk of contamination and protects food from physical damage and temperature abuse, these practices control the safety and quality of your product until it is delivered to the customer. That's why it's important to have a documented preventive control program for receiving, transportation and storage. Your program should cover:

### Receiving and Storage

*General Guidelines*

- To control any hazards that could enter the plant, it's important to control incoming materials:
- Keep records of all shipments received at the plant.
- Create and maintain a list of approved suppliers for ingredients, packaging materials and non-food chemicals that are delivered to your plant.

- Before approving ingredient and packaging suppliers, consider auditing reports of their food safety program.
- Give your receiving staff a list of approved suppliers for non-food chemicals, ingredients and packaging.
- Make sure the area where you receive incoming materials is separated from your processing area to prevent contamination of food products.

Define specifications for incoming materials to make sure that they are food grade and free of any biological, chemical, or physical hazards. For example, cocoa powder can sometimes contain Salmonella, a known biological hazard. When receiving these materials, make sure they meet your specifications. This may involve one or more of the following:

- Analyse the material through a regular monitoring program (for example, grading raw milk and testing it for antibiotics).
- Ask your supplier for a "Certificate of Analysis" prior to or with each shipment and lot. A Certificate of Analysis demonstrates that your ingredients meet the appropriate specifications.
- Periodically sample and test incoming ingredients to verify they are meeting specifications.
- Visually inspect the material.

When storing materials, be sure to:

- identify the hazards associated with storing the materials you receive (such as inadequate temperature control, overhead pipes dripping condensation on your product, etc.)
- establish policies and procedures for eliminating or controlling those hazards
- keep storage areas clean and organized
- regularly monitor all storage areas to make sure requirements are being met

*Rejection of Materials and Exceptions*

Have a documented policy that describes when incoming material should be rejected and how to do it. It should also describe when to make exceptions and how to handle those situations. For example, if the delivery vehicle was dirty but the ingredients or packaging were sufficiently protected and intact, you can still receive the material if you:

- send a report to the supplier and the transporter documenting the conditions under which the materials were received
- document what you had to do to make sure it would not contaminate other materials or your product (for example, cleaning dust and debris off the outer wrap or the containers)

### Raw Milk

*Receiving*

All raw milk must be received in dedicated tank-trucks that meet the requirements under the regulations of the Milk Act. When raw cow or goat milk arrives at your plant, a certified Plant Milk and Cream Grader (PMCG) must grade it based on the regulatory requirements under the Milk Act. PMCGs should reject the milk if it does not meet the requirements.

If the tank-truck has been sealed, check the records to make sure the sealing program was followed. If any seals are missing and unaccounted for, the load may have been tampered with and should be rejected. If there is a logical explanation for the missing seal, the load may be accepted. In this case, be sure to document the reason why the seal was missing.

Make a record of each shipment received including information such as:

- volume
- temperature
- date and time of receipt
- person responsible for receiving
- where the product was stored
- any unusual activity you observed

### Storage

- If your plant cannot immediately process the incoming raw milk, make sure there are enough tanks or silos to store it.
- To minimize the growth of micro-organisms, empty, clean and sanitize the raw milk silos and storage tanks at least once every 48 hours.
- Have a written cleaning program for making sure the cleaning and sanitizing was done properly.
- Use storage tanks or silos that are easy to clean. This means:
  - they should be made of stainless steel
  - they should be designed so they can be cleaned either by hand (for smaller tanks) or using a CIP system (for larger tanks or silos)
- While the milk is in the storage tanks or silos, monitor the temperature and make sure it stays between 1°C and 4°C.

### Bulk Liquid Pasteurized Dairy Ingredients

*Receiving*

If you receive pasteurized dairy products in bulk tank-trucks, you will have to re-pasteurize them in your plant. This is because the tank-truck and the loading and unloading equipment (pipelines, hoses and pumps) are commonly shared with raw

milk and cannot be guaranteed to have been properly cleaned to prevent cross-contamination from raw milk.

If you receive pasteurized dairy products in bulk tank-trucks and you do not wish to re-pasteurize them, you must have specific vehicles, tanks, product transfer lines and transfer pumps dedicated to pasteurized product only at both the shipping and receiving facilities. These systems must be inspected and approved by your local government inspection agency.

- This practice can only be applied to certain products that would be subjected to a "kill step" during further processing such as drying or cooking.
- This practice is not acceptable for fluid milk and cream.

Have written policies and procedures to reduce any identified risks and make sure that pasteurized product does not become contaminated. These include:

- where the pasteurized product can be held before it is processed
- how long and at what temperature it can be held before it needs to be re-pasteurized
- how the product is protected from cross-contamination by raw product in the facility
- how the dedicated tank-truck is cleaned and sanitized

If the tank-truck has been sealed, check the records to make sure the sealing program was followed. If any seals are missing and unaccounted for, the load may have been tampered with. Reject the load.

Do not receive pasteurized dairy products in re-useable plastic totes, since they cannot be effectively cleaned. Collapsible totes with single-use plastic bag liners are acceptable.

When you receive pasteurized dairy products, document the temperature of the pasteurized dairy products (and the inside of the vehicle, if appropriate).

If products are delivered in a refrigerated truck, check the vehicle records to confirm the product was kept at between 1°C and 4°C throughout the entire journey.

### Storage

Store bulk pasteurized dairy products in dedicated tanks or silos. Never store them in a tank or silo that was previously used for raw milk. If you use tanks to store pre-pasteurized product that you are not going to re-pasteurize, empty and clean them no more than 24 hours after filling.

While the pasteurized product is in storage tanks or silos, monitor the temperature and make sure it stays between 1°C and 4°C.

If you receive pasteurized dairy products in containers (such as cartons, bags or totes), store these containers in a refrigerated area, monitor the temperature and make sure it stays between 1°C and 4°C.

Have a written procedure and keep records to make sure the refrigerated area is cleaned and sanitized regularly to minimize the risk of contamination from spills.

Rotate inventory so that oldest product is used first. Controlling the storage time and temperature of pasteurized dairy products helps to minimize the growth of micro-organisms.

**Packaged Ingredients**

*Receiving*

- When you receive ingredients, visually inspect them to make sure:
  - there are no signs of tampering such as broken seals on the packaging materials
  - the ingredients or packaging you have received are what you ordered
  - there is no opened packaging
  - there is no physical damage to the ingredients
  - there is no dust, debris or signs of pest activities on the ingredients
  - there is no sign of any water damage
  - there is no sign of any contamination from other materials that may be in the vehicle
  - the ingredients have been transported at the temperature recommended by the manufacturer. Use infrared thermometers to check the surface temperature, or open a package and use an immersion-type thermometer to check the internal temperature. If you open a package to check the temperature, throw the material away afterwards unless you can process it to get rid of any contamination that may have been introduced.
- From time to time, take samples of your ingredients and test them to make sure they meet your specifications.

*Storage*

- All ingredients should be stored:
  - according to the manufacturer's instructions
  - in a way that protects their integrity
  - in a way that minimizes the risk of contamination
  - Make sure all ingredients are clearly identified.

- Be aware of any ingredients that are allergens or contain allergens. Handle and store these ingredients in a way that does not contaminate other ingredients.
- In general, if an ingredient needs to be refrigerated, store it between 1°C and 4°C. Store frozen ingredients at -18°C or lower. Monitor and record these temperatures regularly.
- Each day, only bring out the ingredients needed for that day's processing. This reduces clutter in the processing and packaging areas, makes cleanup easier at the end of the day and prevents ingredients from getting wet or contaminated. Packaging material such as cardboard boxes may be a source of yeast and mould, so limiting the amount of ingredients you bring into the area helps to minimize the exposure to these micro-organisms.
- Any unused materials should be properly covered, labelled and returned to their appropriate storage conditions. Use up any partially used materials before opening a fresh package or container.
- Rotate ingredients in storage where appropriate so that the oldest product is used first to help make sure all products are used before their "best before" date. This usually means first in, first out (also known as FIFO).
- Protect ingredients stored at room temperature against contamination and conditions that could cause deterioration. This includes:
  - direct sun
  - excessive heating
  - moisture
  - external contaminants
  - rapid temperature changes that could affect their safety or quality
  - If you stack your ingredients, make sure:
  - there is not too much weight on any containers at the bottom on the stack
  - there is no danger the stacks could fall and damage the containers or expose ingredients to the environment
- Store materials in a way that allows you to clean the area effectively and control pests. For example, store items off the floor and approximately 45 cm (18 in.) away from the walls.
- Keep refrigerated storage areas at the proper humidity to prevent condensation, and keep them clean and sanitized to help prevent mould.
- Make sure condensate pipes empty into a drain to reduce contamination.
- Keep refrigeration units including their condensate collection trays and drain lines clean and maintained on a regular schedule to prevent the growth of mold, spoilage bacteria and pathogens.

- Treat condensate trays with sanitizer to prevent the growth of harmful bacteria that could cross-contaminate employees' clothing or skin or other areas of your plant and eventually food products.

**Packaging**

- Apply the same requirements you apply when receiving packaged ingredients.
- Storage
- All packaging materials should be stored:
  - in a way that protects their integrity
  - in a way that minimizes the risk of contamination
- Make sure all packaging materials are clearly identified.
- Make sure the packaging material is food grade. For example, get a certified letter of guarantee from the supplier.
- Protect packaging against damage, contamination and conditions that could cause deterioration. This includes:
  - direct sun
  - excessive heating
  - moisture
  - external contaminants
  - rapid temperature changes that could affect their safety or quality
- Store materials in a way that allows you to clean the area effectively and control pests. For example, store items off the floor and approximately 45 cm (18 in.) away from the walls.

**Non-food Chemicals**

- Apply the same requirements you apply when you are receiving packaged ingredients.
- Non-food chemicals include:
  - water-treatment chemicals
  - boiler-treatment chemicals
  - chemicals for cleaning and sanitizing
  - materials used for maintenance (such as oils, lubricants, Freon, oxygen and acetylene gases for welding, etc.)
  - other materials used for food contact surfaces

- Make sure all non-food chemicals you use are acceptable for use in a food plant. Acceptable products may be listed in the Reference Listing of Accepted Construction, Packaging Materials and Non Food Chemical Agents published by CFIA.
- If chemicals are not on the list, plant operators may seek assurance of their acceptability by obtaining either
  - a letter of non-objection from Health Canada, or
  - a letter of guarantee from the supplier of the chemical
  - For more information on the acceptability of chemicals, visit CFIA's website.
- Storage
- Store chemicals in a dry, well-ventilated area where there is no possibility that they will contaminate food or food contact surfaces.
- If chemicals are regularly used in food-handling areas, label them clearly and store them in a way that will not lead to contamination of food, food contact surfaces or packaging materials.
- Clearly label all bulk chemicals and any containers you use to dispense smaller portions of these chemicals. Do not use food product containers to dispense or dispose of chemicals.
- Only allow authorized and properly trained employees to handle chemicals.
- Make sure chemical spill kits are available to clean up spills.
- Take steps to contain spills that may occur to bulk chemical containers (such as installing a curb on the floor of the bulk chemical storage area).
- Make sure proper safety equipment is available, functioning and clean.

**Transport Vehicles**

The vehicles and containers used to transport food should be kept clean and in good condition. If you use the same vehicle or container for transporting different foods or for transporting non-food products, it must be effectively cleaned and disinfected (if necessary) between loads.

*Criteria for Receiving*

- When you receive ingredients or packaging materials, visually inspect the vehicle to confirm:
  - it is suitable for transporting food (it should be well built, maintained in good condition, easy to clean and enclosed to protect the load from dust, fumes and weather)

- it is clean:
- the operator can prove when it was last cleaned
- it doesn't have any odours
- there are no signs of rodent activity
- if other items are carried on the same vehicle, they are:
- not items that could potentially contaminate your ingredients or packaging materials
- properly separated from your ingredients or packaging materials (for example, by using physical dividers or plastic overwrapping)
- if the ingredients or finished product had to be kept at a specific temperature:
- the vehicle has kept them at that temperature
- the temperature was monitored and recorded throughout the journey

- Make a record of the condition of the vehicle for each delivery received.
- Sign shipment invoices and keep them on file as a record of receipt.

**Criteria for Shipping**

- Before products are loaded onto the vehicle, visually inspect the vehicle to make sure:
  - it is at the right temperature for the product being shipped
  - it can keep the product at the right temperature throughout the journey
  - the temperature can be checked during the journey
  - the vehicle is in good condition, clean, free of odours and free of any signs of rodent or insect activities
  - the product is effectively protected from contamination, including dust and fumes
  - cleaning records are available
  - no other items being shipped in the vehicle could contaminate your product
  - your product will be effectively separated from any other types of foods or non-food items in the vehicle during the journey
- Write down the results of your inspection.
- Make sure the finished product being loaded is in sound condition with no open, leaking or damaged containers. Load the vehicle in a way that prevents your product from becoming damaged or contaminated.

- For products requiring refrigeration, pre-cool the trailer, turn off the reefer immediately before loading (to prevent frost build-up), load quickly and then turn the reefer back on immediately after loading.

**Transport vehicles within plant**

This section covers equipment such as carts and forklifts used to move ingredients, packaging and finished product within your plant.

- Maintain this equipment carefully. Carts, motorized forklifts and hand trucks take a lot of abuse.
- Forklifts and hand trucks often have painted surfaces. Make sure the paint isn't flaking off, since this could contaminate the products.
- Wash transportation equipment frequently. Carts should be easily cleaned and designed to prevent water from collecting in them. Regularly clean and sanitize equipment wheels.
- Not all motorized forklifts can be used in all areas of the plant. Because propane may contaminate some stored food, use electric forklifts in food-processing areas.
- Create a monitoring program to make sure that any transport vehicles restricted to specific areas of the plant are used only in those areas. For example, a hand truck used to deliver ingredients into a raw batching area should not be used to deliver packaging materials into a finished product area.

**Product Returns**

Finished products may be returned to plant for several reasons. These include:

- expired shelf life
- customer complaints
- company product withdrawals
- recalls

These products may contaminate your plant environment, your equipment or other dairy products in plant. Finished products that have left your direct control must not be salvaged and may not be used for processing or rework.

Have a written policy that clearly defines how to handle product returns in your plant. It should include:

- clearly identifying product that has been returned
- creating a dedicated area for returns where they cannot contaminate other finished products or be mistaken for finished product to be shipped out
- monitoring the frequency of returns

- investigating unusually high numbers of returns
- documenting that returned products have been safely disposed

**Training**

Train the employees on receiving, transportation and storage programme. They should understand the dangers of improper procedures as well as what conditions can result in unsafe products or poor-quality products.

The training should cover:

- all the documents that make up your receiving, transportation and storage program (including records)
- how to access these documents
- how to fill out records
- what specifications your incoming materials should meet
- what conditions delivery and shipping vehicles should meet
- when to reject incoming materials and how to handle exceptions
- how to properly unload incoming materials and store them
- the proper storage conditions for ingredients, packaging and finished products
- the proper conditions for loading shipping vehicles and transporting finished products
- how to receive, store and handle all non-food chemicals
- how to handle returned products properly
- how to properly use and maintain transport vehicles within the plant such as carts, motorized tow motors, hand lift trucks, etc.

## STANDARDIZING MILK

**Purpose and Definition**

We know that liquid milk sold in the market is of different types with regard to its composition. Since the milk available to the processor may not necessarily be of the same composition as desired in the milk to be marketed, it is a very common practice to adjust the composition as per the requirement. Also, compositional modification (or adjustment) is necessary if the milk is to be converted into a certain product. A product must conform to the legal standards prescribed for it, or the quality standards set by the manufacturer. Product manufacture without appropriate compositional manipulation of milk may lead to poor quality product, or a product that does not meet legal requirements, or it may be an economical loss to the processor.

Standardization thus refers to the process by which the milk composition is

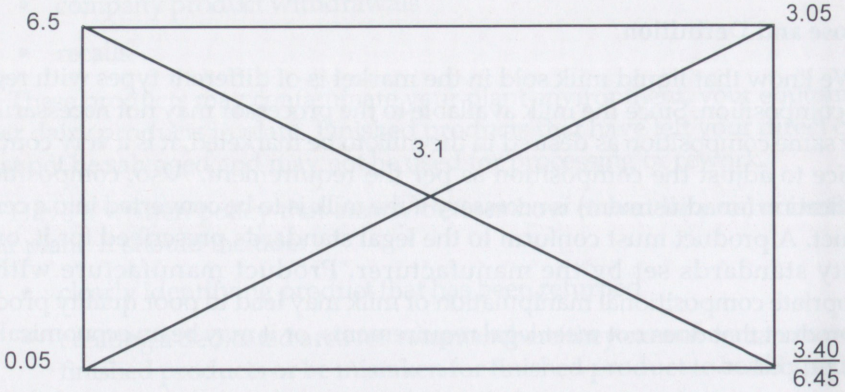
adjusted to the desired level. The most commonly considered compositional parameters are fat and SNF for market milk, although sometimes fat alone may be taken into account for standardization. For certain specific, product,-manufacturing applications even protein content may be adjusted. Accordingly, the process of standardization involves lowering or raising the level of a particular constituent(s) to the desired value specially fat.

**Standardization of Milk for Fat**

Often milk may be standardized to a certain value of a single component i.e. fat or SNF alone. This can be achieved by adding to the milk, a calculated quantity of a fat-rich product such as cream if the fat level is to be raised, or a low-fat or fat-free component e.g. skim milk, if the milk has excess fat. The fat content of milk can be reduced also by separating a calculated amount of cream of known fat percentage.

The calculation of the quantity of cream or skim milk to be added to milk, or cream or skim milk to be separated from it can be made by a simple method known as the Pearson's square method. It consists in drawing an arbitrary square (or, a rectangle), placing at the left concerns of the square, the values of fat content of the two products to be mixed and at the centre of the square, the desired fat percentage. Then, subtractions are made diagonally across the square, the smaller value being deducted from the larger one, and the differences are entered at the correspondingly opposite corners on the right- hand side. These two new values at the right corners are summed to obtain a third value. All the three values placed at the right represent the proportions or relative amounts of the given products to be mixed (top right figure for the amount of the product to the top left, bottom right figure for the product to the bottom left, and the sum for the final product). The following is an example of such a calculation:

500 kg of milk testing 6.5% fat to be standardized to 3.1% fat using skim milk containing 0.05% fat.



Thus mixing of 3.05 kg of 6.5% fat milk with 3.40 kg of the skim milk will yield 6.45 kg of milk containing the desired fat level i.e. 3.1%. Therefore, the quantity of skim milk required to be added to 500 kg whole milk will be

$(3.4 \times 500)/3.05=3.05$

Accordingly, 557.38 kg of 0.05% skim milk mixed with 500 kg of 6.5% fat whole milk will yield 1057.38 kg of milk having 3.1% fat.The single-component (fat-based) standardization is commonly used for cream meant for butter-making. It generally involves adjusting the fat percentage of a high-fat cream to the desired level by mixing it with the calculated quantity of skim milk (or, whole milk). Blending of the two components i.e. cream and skim milk or whole milk can be carried out by transferring the calculated quantities of the two(one after the other), to a tank (or, silo) with a provision for adequate mixing.

Continuous, on-line blending is much more desirable in a large-scale operation. This can be achieved on the cream separator itself by allowing sufficient cream to remix with the skim milk so that the mixture is a milk with the desired fat content; the balance cream flows through the cream line into the cream tank. This requires that the separator is fitted with a standardizing device.

**Standardization of Milk for Fat and SNF**

When milk is required to be standardized for both fat and SNF, the basis of calculation of the quantity of skim milk or cream to be added is the ratio of fat to SNF, and the total solids (TS) content. If the desired fat-to-SNF ratio is higher than the actual ratio in the available milk, then skim milk will be required to be added. On the other hand, when the desired ratio is lower, cream needs to be blended into the milk. It is, therefore, necessary that both the fat and SNF contents of the milk to be standardized, and those of cream or skim milk to be used are known. If the fat content of cream or skim milk (separated from a milk of known fat and SNF contents) is known, the SNF content can be estimated as under

i) $\text{SNF in cream, \%} = SNF_m \times ((100 - f_c)/(100-f_m))$ (Eq. 1)

ii) $\text{SNF in skim milk} = SNF_m \times ((100 - f_s)/(100-f_m))$ (Eq. 2)

where,

$SNF_m$ = SNF percentage in milk

$f_c$ = fat percentage in cream

$f_m$ = fat percentage in milk

$f_s$ = fat percentage in skim milk.

The amount of skim milk or cream required to be added to a given quantity of milk (so as to attain the desired levels of fat and SNF in it) can be worked out by using the following formulae:

i) $Q_c = Q_m \times ((R \times SNF_m)/ (f_c - (R \times SNF_c)) - F_m)$ (Eq. 3)

ii) $Q_s = Q_m \times ((f_m / R) - SNF_m) / (SNF_s - (f_s / R))$ (Eq. 6)

where,

$Q_m$ =Quantity of milk to be standardized

$Q_c$ = Quantity of cream required

$Q_s$ = Quantity of skim milk required

R = Fat/ SNF ratio desired.

$f_c$ = Fat percentage in cream

$f_m$ = Fat percentage in milk

$f_s$ = Fat percentage in skim milk

$SNF_m$ = SNF percentage in milk

$SNF_c$ = SNF percentage in cream

$SNF_s$ = SNF percentage in skim milk.

Alternatively, an algebraic method can be used taking 'x' quantity of cream or skim milk of known fat and SNF contents required to be added to the given quantity of milk with certain fat and SNF levels, and then solving (for x) an equation of the desired fat-SNF ratio:

$$R = \frac{f_c \text{ (or } f_s) \times \frac{x}{100} + f_m \left(\frac{Q_m}{100}\right)}{SNF_c \text{ (or } SNF_s) \times \frac{x}{100} + SNF_m \left(\frac{Q_m}{100}\right)}$$

where all values except that of x are known.

# 10

# Heat Treatment of Milk

**Topics**

*Introduction • Milk Composition • Reaction kinetics • Principles of heat transfer • Thermisation and tyndallisation • Sterilisation - safety and spoilage considerations • UHT processing • Post-sterilisation contamination • High-temperature processing (extended shelf life) • Heating Methods • Test Results • Equipment • Some heat transfer properties • Definitions and equations*

## INTRODUCTION

The aim of this chapter is to communicate the underlying principles for producing heat-treated milk which is safe and of high quality, based on many years of experience which has been gained from teaching, research and troubleshooting.

Although a small amount of milk is still sold as 'untreated' or raw, the two main heat treatments used for milk sold in the retail sector are pasteurisation and sterilisation. Treatments somewhere between these are also being used for extending shelf life. The main aims of heat treatment are to reduce the microbial population, both pathogenic and spoilage, in raw milk to inactivate enzymes and to minimise chemical reactions and physical changes. Some important ones are a decrease in pH, precipitation of calcium phosphate, denaturation of whey proteins and interaction with casein, lactose isomerisation, Maillard browning and modifications to the casein micelle. The overall effect is to alter the sensory characteristics, i.e. overall appearance, colour, flavour and texture and the nutritional value as well as making it safe and improving its keeping quality.

Most of the milk destined for processing into dairy products is also heat treated in some form, although some cheeses are made from raw milk. One industrial process is thermisation, which involves heating milk at temperatures between 57 and 68°C for about 15 s. Another is preheating or forewarming, applied to milks prior to evaporation and powder production.

Conditions can be as low as 72°C for low-heat powders and up to 90–95°C for 5–10 min for high-heat powders, although temperatures above 100°C for shorter times are also used. Patterns of consumption and preferences for milk vary from one country to another. For example, in Great Britain in 2003, 92.9% of heat-treated milk for drinking was pasteurised, 1.4% (in-container) sterilised and 5.7% ultra-high temperature (UHT) treated. In Australia, the corresponding figures for white milk are 91.9%, 0% and 8.1%, respectively. The balance is totally different in other European countries, and in some, such as France and Germany, UHT milk is the main milk product.

Going back about 50 years, only a limited number of products were available. These were mainly white milk, limited flavoured milks and some creams. Domestic refrigeration was not widespread and pasteurised milk stored in the larder would keep for only 24-48 h.

Sterilised milk with its characteristic flavour and slightly brown colour was quite popular in the UK and powdered, evaporated and sweetened condensed milks were available. UHT milk was in its infancy and fermented milks were for the future. Heat treatment was on a small to medium scale.

Jumping forward to the twenty-first century, one big change is the scale of operation, with a move towards highly automated large-scale, energy-saving continuous processes. In terms of product availability, we are now spoilt for choice. Milk is standardised for both protein and fat: semi-skimmed milk at 1.5-1.8 g fat 100 $g^{-1}$ is now popular, accounting for 57.3% of all liquid milk drunk in Great Britain in 2003, and skimmed milk at 0.1 g fat 100 g-1 provides a low-fat version. For those who prefer a richer fuller flavour, milk from Jersey and Guernsey cows and other rare breeds can be purchased; these no longer are said to contain 5 g fat 100 $g^{-1}$, but are now '95% fat-free'. There is a wide range of flavoured milks, and creams ranging from 12 to 55 g fat 100 $g^{-1}$, with textures ranging from thin coffee creams to very viscous spoonable dessert creams. Competing with cow's milk are milks from goat, sheep and buffalo, as well as specialty milks for cats and dogs.

The industry is rightly promoting the health benefits of components naturally present in milk, i.e. specific fat fractions, bioactive peptides, beneficial minerals, especially calcium and magnesium, or producing milks with a healthy image, for example fermented milks, lactose-reduced milk, melatonin milk and milks containing probiotic microorganisms, prebiotic compounds, plant sterols or fish oils. In many parts of the world, conditions are not conducive to producing fresh milk; hence reconstituted and recombined milk are produced. Some dairy companies are now also processing 'vegetable milks', based on extracts from soy, rice, sweet corn and other vegetables.

## MILK COMPOSITION

Although there are many sources of data for composition of milk and milk products, these report average values, giving no indication of the variability of raw milk due to breed, diet, climate and stage of lactation. This should not be forgotten

when processing milk or when using data from the literature. The complexity and changing composition of raw milk pose key challenges.

Milk is an emulsion, containing fat globules in the range 1-10 microns in diameter, dispersed in an aqueous phase. Above 45°C, all this fat will be in the liquid phase; below this temperature, it will start to crystallise. This is not an instantaneous process and during crystallisation, latent heat will be released. The proteins in milk are divided into two fractions: (a) the casein fraction and (b) the whey protein fraction.

The casein fraction is complex and exists in micelles with a size range of 30-300 nm. In the context of heat treatment, heat stability is very important and is influenced by several factors, particularly pH and ionic calcium.

The casein micelle is remarkably stable to heat, and good quality milk can withstand temperatures of 140°C for at least 10 min and often longer without coagulating. However, if the milk is not properly handled, its stability can deteriorate drastically. Some manifestations of poor heat stability are fouling or deposit formation on heat exchangers, sediment in milk and heat-induced thickening and coagulation. These problems tend to increase as the processing temperature increases, but are also dependent on raw milk quality.

In this context, a very important property is milk acidity, measured as pH or titratable acidity. Whereas pH is a direct measure of H+activity, titratable acidity is a measure of buffering capacity between its own pH and that of the colour change (from colourless to red) in phenolphthalein, which is about 8.3. The pH of milk may influence many other aspects related to quality, in particular, the colloidal stability of milk and other heat-induced reactions, such as Maillard browning and lactulose formation. Both microbial activity and microbial inactivation are also influenced by pH, as is enzyme activity. The pH of raw milk is usually between 6.6 and 6.7, but it can be outside this range - for example 6.40-6.89. Its exact value is influenced by its protein, mineral and acid contents. The pH of milk falls during heat treatment, but this is largely reversible on cooling. Researchers illustrated that pH could fall to below 6.0, when the temperature exceeds 100°C.

Another important determinant is ionic calcium, but its influence on heat stability is less well established, mainly because of difficulties in measuring it. Thus, the pH and ionic calcium in raw milk may be useful heat stability indicators, especially in sterilisation and UHT treatment of normal milk and in situations where milk is fortified with calcium or acidified. Milk is a bland fluid with a characteristic creamy flavour. Because of this blandness, it is very susceptible to off-flavours; for example, a rancid flavour may develop due to excessive agitation of raw milk. Raw milk from healthy animals has a very low microbial count, but it easily becomes contaminated with spoilage bacteria and perhaps some pathogenic microorganisms. These need to be inactivated and this is readily achieved by heat treatment. From the standpoint of the milk processor, raw milk composition and its microbial loading will vary from day to day.

## REACTION KINETICS

All thermal processes involve three distinct periods: a heating period, a holding period and a cooling period. In theory, all three periods may contribute to the reactions taking place, although in situations where heating and cooling are rapid, the holding period is the most significant. However, procedures are needed to evaluate each of these periods individually to determine the overall effect.

The two reaction kinetic parameters of interest are the rate of reaction or inactivation at a constant temperature (e.g. D and k values), and the effect of temperature change on reaction rate (z and E values).

For pasteurisation processes, the range of interest is 60–80°C, and for sterilisation, from 100 to over 150°C. Chemical reaction rates are less temperature-sensitive than microbial inactivation rates. Thus, using heat treatment at higher temperatures for shorter times will result in less chemical damage occurring for an equivalent level of microbial inactivation. In practice, deviations from first order reaction kinetics are often encountered, as are deviations from the log–linear relationships between processing time and temperature discussed recently.

## PRINCIPLES OF HEAT TRANSFER

In thermal processing, the aim is to maximise the rate of heat transfer ($Js^{-1}$ (W) or British thermal units (BTU) $h^{-1}$), i.e. to heat and then cool the product down as quickly as possible. This will improve the economics of the process and in many cases also lead to an improvement in product quality. Heating processes can be classified as direct or indirect. The most widely used is indirect heating, where the heat transfer fluid and the milk are separated by a barrier; for in-container sterilisation this will be the wall of the bottle and for continuous processes, the heat exchanger plate or tube wall. In direct processes, steam is the heating medium and the steam comes into direct contact with the milk. Indirect heating also implies that the two fluids will not come into direct contact. It is important to ensure that this is the case, and the integrity of the barrier is a very important safety consideration, especially in the regeneration section where the heating medium is the hot heat-treated milk.

The heating medium is usually saturated steam but hot water and hot air are sometimes used. At temperatures above 100°C, the steam and the hot water are above atmospheric pressure. For steam, there is a fixed relationship between its pressure and temperature, given by the steam tables. Thus, a steam pressure gauge will act indirectly as a second temperature-monitoring device. Discrepancies between temperature and pressure readings suggest that there may be some air in the steam or that the instruments are incorrect. Cooling is achieved using mains water, chilled water or glycol. Regeneration is used in continuous processes to further reduce energy utilisation. Heating can be by either batch or continuous processing.

## THERMISATION AND TYNDALLISATION

Thermisation is the mildest heat treatment given to milk. It is used to extend the keeping quality of raw milk when it is known that raw milk may be held chilled

for some time, prior to being further processed. The aim is to reduce the growth of psychrotrophic bacteria, which may release heat-resistant proteases and lipases into the milk. These enzymes will not be totally inactivated during pasteurisation and may give rise to off-flavours if the milk is used for cheese or milk powders. Conditions used for thermisation are 57–68°C for 15 s, followed by refrigeration. Thermised raw milk can be stored at a maximum of 8°C for up to 3 days. The milk should also be phosphatase-positive in order to distinguish it from pasteurised milk, which is phosphatase-negative. It is usually followed later by pasteurisation or a more severe heat treatment.

Another thermal process, which has been investigated, is tyndallisation; it involves successive heat treatments in order to inactivate spores. According to researcher, Tyndall in 1877 suggested that if a medium was heated at 100°C for 3 min on 3 successive days, first the vegetative cells would be killed and the spores would germinate, and then be killed on either the second or third days. In practice, such double heat treatments are rarely encountered, and the process is not successful in totally inactivating spores because of the unpredictability of the spore germination process. The same applies to double pasteurisation processes, which have not been found to be effective.

## STERILISATION - SAFETY AND SPOILAGE CONSIDERATIONS

Sterilisation of milk to enable it to be kept at room temperature for several months became a commercial proposition in 1894. Milk can either be sterilised in bottles or other sealed containers, or by continuous UHT processing followed by aseptic packaging. From a safety standpoint, the primary objective is the production of commercially sterile products with an extended shelf life. The main concern is inactivation of the most heatresistant pathogenic spore, namely *Clostridium botulinum*. Since milk is a low-acid food (pH > 4.5), the main criterion is to achieve 12 decimal reductions of *Cl. botulinum*. This occurs when a product is heated at 121°C for 3 min, at its slowest heating point. The microbial severity of an in-container sterilisation process is traditionally expressed in terms of its Fo value. This takes into account the contributions of the heating, holding and cooling periods to the total lethality and is expressed in terms of minutes at 121°C. It provides a useful means of comparing processes. The minimum Fo value for any low-acid food should be 3.

*Cl. botulinum* is rarely found in raw milk. More common sporeformers are *Bacillus* species of which some, such as *B. stearothermophilus* (now known as *Geobacillus stearothermophilus*) and B. sporothermodurans, form highly heatresistant spores, which are not destroyed by a process with an Fo of 3. These bacteria may cause spoilage, but they are not pathogenic. Thus, a minimum 'botulinum cook' will produce a product which is safe, but not necessarily sterile. For foods which may contain highly heat-resistant spores, a heat treatment achieving two or more decimal reductions is recommended, corresponding to an Fo value of 8. Target contamination rates should be less than 1 in every 10 000 containers.

Spore counts in raw milk have been rarely reported to exceed 103 cfu $mL^{-1}$, although researchers reported that they may reach 5000 cfu $mL^{-1}$. Spores are mainly derived from surfaces of teats in contact with bedding materials. The most common *Bacillus* spores isolated from teat surfaces are *B. licheniformis*, *B. subtilis* and *B. pumilis* with lower numbers of *B. cereus*, *B. firmus* and *B. circulans*. Most common in raw milk are *B. licheniformis*, *G. stearothermophilus* and *B. cereus*. Very heat-resistant spores, such as *G. stearothermophilus*, are usually only a small proportion of the total.

### In-container sterilisation

Foods have been sterilised in sealed containers, such as cans, for over 200 years. Milk was originally sterilised in glass bottles sealed with a crown cork, but more recently, plastic bottles have been used. Milk sterilisation really developed after 1930 with the advent of the crown cork, which helped with the mechanisation of the bottle-filling process, and the reuse of bottles. In general, the basic principles have remained the same.

The main aim is to inactivate heat-resistant spores, thereby producing a product which is 'commercially sterile', with an extended shelf life. Practical drawbacks of in-container sterilisation processes are that the product heats and cools relatively slowly, and that temperatures are limited by the internal pressure generated. However, many dairy products are still produced this way worldwide, including sterilised milk, evaporated milk and canned desserts such as custard and rice pudding.

Sterilised milk is still produced in many countries, and in essence, the manufacturing procedure is not too far removed from that used over 50 years ago. Milk is clarified using a centrifuge, e.g. a Bactofuge™, with claimed spore removal

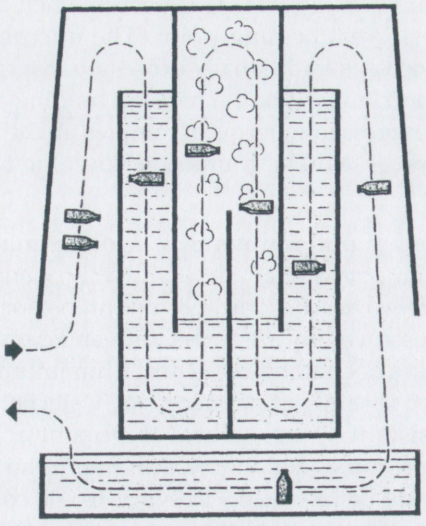


**Fig. 10.1 A continuous retort for sterilisation of glass or plastic bottles**

of greater than 99% (2 $\log_{10}$ reductions). It is heated using similar equipment to that used for pasteurisation. It is then homogenised at 63–82°C, for example at a single-stage pressure of about 20 MPa or double stage at about 17 and 3.5 MPa. It is then filled into glass bottles between 74 and 80°C under conditions which give minimal frothing, and sealed using a crown cork. Plastic bottles are sealed at a lower temperature of 54–55°C. Care should be taken to avoid conditions in balance tanks, which may be conducive to growth of thermophiles. Researchers cite sterilisation processing conditions of 110–116°C for 20–30 min, depending on the extent of cooked flavour and colour preferred by the consumer. Batch or continuous retorting processes may be used. Other processing details are outlined by the researchers; these include more detail on continuous retorts, such as hydrostatic or rotary valve sealed sterilisers, which are capable of higher temperatures and shorter times (132–140°C for 12 min), and the use of steam for glass bottles or steam/air mixtures for plastic bottles (Fig. 10.1).

Researcher recognised the need for ensuring that raw milk to be used for sterilisation was not heavily contaminated with bacterial spores. Today, this remains an important control variable. Sweet curdling was the chief bacterial fault, due to highly resistant spores of *B. subtilis* and *B. cereus*. Bacterial growth was found to produce other taints, such as carbolic, bad (e.g. oxidised) or cardboard taints. Researchers reported that the failure level of well-produced sterilised milks is of the order of 1 in 1000 units, although it may be as high as 5–10% in situations where there are large numbers of thermotolerant spores in the raw material or other contamination arising in the process.

A recognised test for ensuring adequate sterilisation is the turbidity test, developed by Aschaffenburg in 1950. This test measures whey protein denaturation and it is an indirect test (similar to phosphatase), as complete denaturation would indicate that the milk was adequately sterilised. Milk (20 mL) is mixed with ammonium sulphate (4 g), which causes casein and any associated denatured whey protein to precipitate. The mixture is filtered, producing a clear filtrate, which contains any undenatured whey protein present in the milk sample. The filtrate is then boiled, which causes any undenatured whey protein to be denatured, thereby producing a turbid solution, the amount of turbidity being proportional to the amount of undenatured whey protein in the milk. Some regulations state that sterilised milk should produce a negative turbidity result; i.e. it should be heated for such a time as to fully denature thewhey proteins. In principle, UHT heating of milk should result in some undenatured whey protein, but UHT processes with extensive heating and cooling profiles are more severe and will also give a negative turbidity result. Thus, the turbidity test will not always distinguishUHTmilk from sterilised milk. In fact, many UHT milk samples showa negative turbidity. Methods for distinguishing between sterilised and UHT milk have been discussed in detail by the researcher. However, this is made difficult by the wide range of times and temperatures which are permitted. This includes a combined process, which involves the production of milk under UHT conditions, e.g. 137°C for 4 s, and is filled into bottles which are then sealed and passed through a conventional retorting process. Although the retorting is much reduced, it is generally just sufficient to

ensure a negative turbidity result. In terms of determining the sterilisation effect, if this is to be treated as a single process, the critical point is to ensure that the milk does not become recontaminated in the intermediate filling process, especially with bacterial spores. This process was found to reduce the incidence of spoilage due to spore survivors. There is also plenty of opportunity for spore inactivation due to high temperatures being maintained for some considerable time.

Sterilised milk has a rich creamy appearance, perhaps helped by Maillard browning components, a distinct cooked flavour (rich, nutty, caramelised),which once acquired, makes other heat-treated products taste insipid. It is considerably browner than raw milk, the extent of browning depending on the severity of the heat treatment. Thus, Maillard browning contributes to product quality in terms of its colour and flavour, although not everybody will find sterilised milk to be as palatable as pasteurised milk.

Sterilisation causes more loss of nutrients than any other heat treatment. For example, losses of the water-soluble vitamins $B_1$ and $B_{12}$ have been reported to be 30-40% and 80-100%, respectively. Furthermore, it cannot be coagulated with rennet, unless calcium chloride is added.

To summarise, sterilised milk is still produced in quantity in some countries, with much of it now produced in retortable disposable plastic bottles with a metal foil cap, rather than in returnable glass bottles.

## UHT PROCESSING

### Introduction and Principles

UHT processing of milk combined with aseptic packaging was introduced to produce a shelf-stable product with minimal chemical damage compared with in-container sterilised milk. UHT milk may have a shelf life of up to 12 months, although in practice, it is usually consumed much earlier than this. In countries where it is a minor segment of the milk market, it is often used as a convenience product, and used when pasteurised milk is not available; whilst in countries where it is the major type of milk available, it is used regularly. In the former situation, UHT milk may need to be stable over a long period of time, while in the latter case, the desired shelf life may be ≤3 months.

UHT treatment is normally in the range 135–150°C in combination with appropriate holding times necessary to achieve 'commercial sterility'; i.e. microorganisms are unlikely to grow in the product under the normal conditions of storage. In practice, the products are checked for sterility by incubating at 55°C for 7 days or at 30°C for 15 days, and testing for bacterial growth.

Some useful bacteriological and chemical indices have been developed to describe the effects of a particular heating regime on the bacteria and chemical components of milk, respectively. The major ones are $B^*$ and Fo (bacterial) and $C^*$ (chemical). $B^*$ is a measure of the bacteriological effect of a heat treatment relevant to treatment at a reference temperature of 135°C. A process with $B^* = 1$ produces

a nine-decimal reduction of thermophilic spores assuming a z value of 10.5°C, and is equivalent to holding the product at 135°C for 10.1 s; this is the recommended minimal value of $B^*$ for a UHT process. This differs from the other bacterial index Fo, which is commonly used for in-container sterilisation, where the reference temperature is 121.1°C (250°F). While it is a good measure of the lethality of the heat treatment, it is more appropriate to heating at temperatures around 120°C than around 140°C. As indicated above, sterilisation processes should have an Fo ≤ 3 to ensure bacteriological safety. Although a direct correlation is not strictly correct, an Fo of 3 corresponds roughly to a $B^*$ of about 0.85.

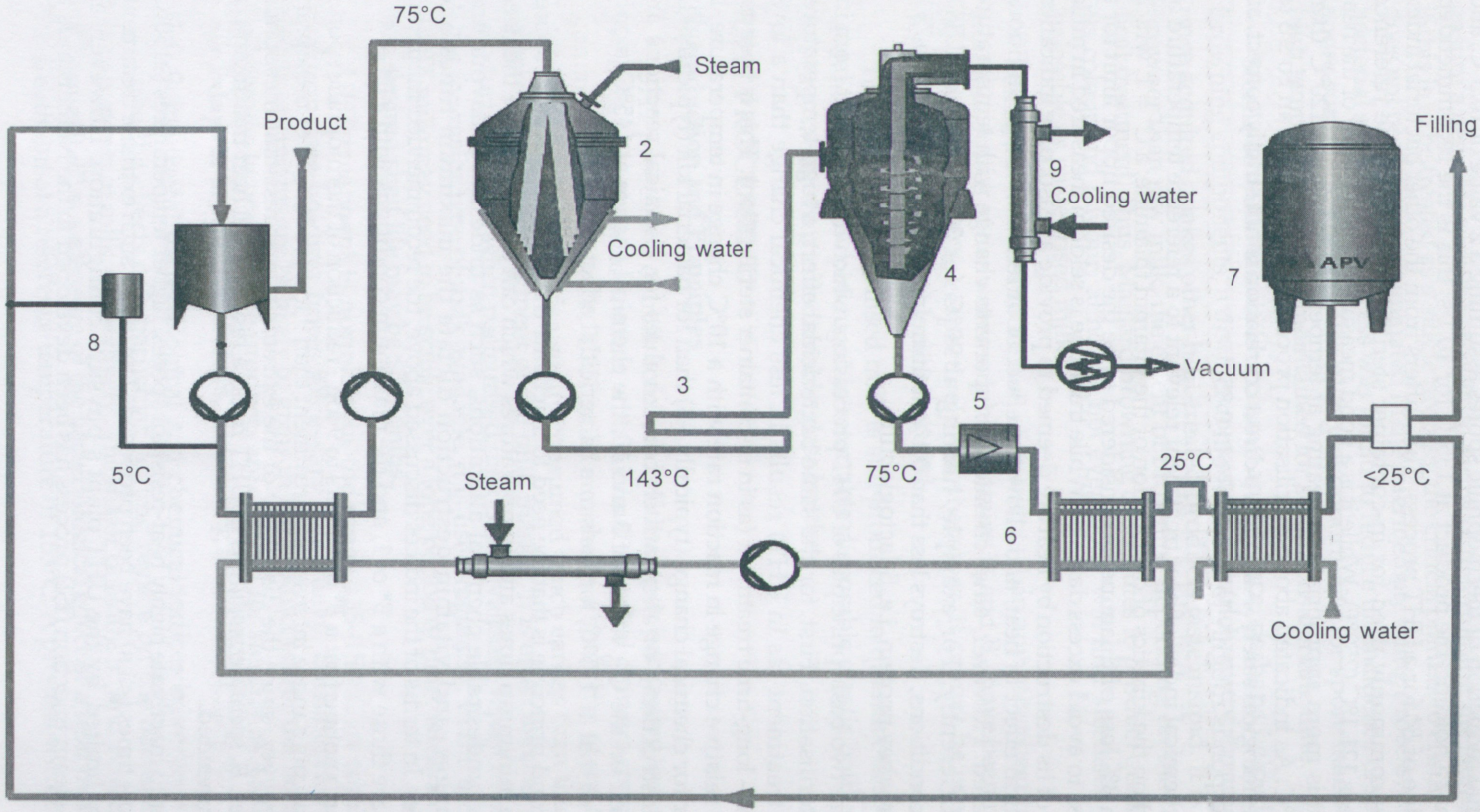
$C^*$ is a chemical index of heat treatment relevant to a reference temperature of 135°C based on the kinetics of destruction of the vitamin thiamine; a $C^* = 1$, which equates to a 3% loss of thiamine, is considered to be the desired upper limit for a UHT process to avoid excess damage. While thiamine is seldom measured in milk, the kinetics of its destruction by heat are deemed to provide a reasonable indication of the chemical effect of heat, and allow the effect of different heating conditions to be compared. However, other chemical components change with temperature and time differently. For example, heating at 90°C for 30 s, common UHT preheating conditions, destroys less than 0.1% of thiamine, but denatures up to 75% of the major whey protein, i.e. β-lactoglobulin.

There are two basic principles of UHT processing, which distinguish it from in-container sterilisation. First, for the same bactericidal effect, a high-temperature-short-time treatment (as in UHT) results in less chemical change than a low-temperature – long-time treatment (as in in-container sterilisation). This is because the $Q_{10}$, the relative change in reaction rate with a 10°C change in temperature, is much lower for chemical change (typically ~3) than for bacterial kill (typically~10 for spore destruction), or alternatively, the z values for chemical reactions are higher. Based on the $Q_{10}$ values of 3 and 10, the chemical change at 145°C is only about 2.7% of that at 115°C, for the same bactericidal effect.

The second principle is that the need to inactivate thermophilic bacterial spores dictates the minimum times and temperatures which can be used, while the need to minimise undesirable chemical alterations, such as undesirable flavour and colour changes, and vitamin destruction dictate the maximum times and temperatures. In terms of the indices discussed above, the recommended minimum conditions are those with a $B^*$ of 1, and the maximum conditions with a $C^*$ of 1.

It is worth noting that a $B^*$ of 1 refers to 9 $\log_{10}$ reduction of thermophilic spores, which represents a more intense heat treatment than 9 $\log_{10}$ reduction of mesophilic spores. However, since the discovery of the extremely heat-resistant mesophilic sporeformer, *B. sporothermodurans* in UHT milk, a higher level heat treatment has been recommended.

In order to inactivate highly heat-resistant spores, higher temperatures (≥150°C) for very short times (<1 s) have been proposed. The use of such extreme treatments is generally limited by the UHT plant's physical configuration. However, an innovative steam injection (ISI) process has been developed in the Netherlands to




1. Plate preheaters, 2. Steam infusion chamber, 3. Holding tube, 4. Flash vessel, 5. Aseptic homogeniser, 6. Plate coolers, 9. Condensor, 8. Sterilising loop and 7. Aseptic tank

**Fig. 10.2. The layout of a direct (infusion) UHT plant**

heat milk at 150–200°C for less than 0.1 s. It was shown to destroy heat-resistant spores.

### *Methods of UHT processing*

*Background*

UHT heating can be either 'direct' or 'indirect'. In direct heating, superheated steam is mixed with milk while, in indirect heating, a heat exchanger transfers heat across a partition between the milk and the heating medium, either steam or hot water. In THEs, the partition is the wall of the stainless steel tube, and in PHEs, it is the stainless steel plate.

The UHT process involves the following stages: preheating with heat regeneration, holding at preheat temperature, heating to sterilisation temperature, holding at sterilisation temperature, cooling and aseptic packaging (see Fig. 10.2). In addition, a homogenisation step is usually included either before or after the high-heat holding section. In commercial processing of UHT milk, preheating (to ~80–95°C) is usually achieved by using the hot processed milk to heat the incoming cold raw milk. This enables much of the heat used in the process to be regenerated and the cooling water requirement to be reduced. The preheated milk is often held for a short time (15 s to a few min) to denature the whey proteins, principally -lactoglobulin, to reduce their ability to foul, or deposit on, the hot surfaces of the high-temperature heating section. This processing approach, which is known as a 'protein stabilisation' step, is not usually employed in direct plants because they are much less likely to foul in the high-heat section than the indirect plants due to the reduced access of the milk to high-temperature surfaces.

The preheating and final cooling steps are always performed in indirect heat exchangers; however, the heating to sterilisation temperature after preheating can be either direct or indirect. This is the stage that characterises a plant as either 'direct' or 'indirect'. The cooling section immediately after the high-heat section also differs in the two systems: (a) direct plants use expansion cooling in a vacuum chamber and (b) indirect plants use THEs or PHEs.

All UHT processes involve aseptic packaging of the product into cartons, plastic bottles or laminated plastic cartons. This is an essential part of UHT processing as it ensures that the sterile product is not contaminated during packaging, thus enabling the product to be stored at room temperature for several months without spoilage by bacterial growth.

#### Direct Heating

There are two major types of direct heating UHT systems known as *steam injection* (steam into milk) and *steam infusion* (milk into steam). In the former, superheated steam is 'injected' into a stream of milk, while in the latter, milk is sprayed into or allowed to fall as a thin film or fine streams, through a chamber of superheated steam. A major feature of these two systems is the almost instantaneous rise in the temperature of the milk from preheat to sterilisation

temperature through the transfer of the latent heat of vaporisation of the steam to the milk. During this heating stage, steam is condensed, and the milk is diluted with water. The degree of dilution depends on the temperature rise of the milk, but for an increase in temperature of 60°C (e.g. from 80°C to 140°C), it is approximately 11%. After the milk passes through the high-heat-holding tube, the entrained water is removed in the vacuum chamber, which also rapidly cools the milk to approximately the same temperature as that of the preheated milk. To ensure neither dilution nor concentration of the milk occurs, the total solids of the incoming milk and the processed milk are monitored, and the temperature in the vacuum chamber adjusted if necessary. The steam used in direct UHTplants must be of high-quality, culinary grade. Poor-quality steam can lead to carry over of off-flavours into the milk.

**Indirect Heating**

In indirect heating using PTEs or THEs, the heating medium is either steam or superheated water. When water is used, it flows in the reverse direction to that of the milk. The reverse flow minimises the temperature differential between the two liquids and, in turn, minimises the amount of burn-on. Hot water is a significantly better heating medium than steam with respect to burn-on and flavour of the product as it enables a smaller temperature differential between the milk and the heating medium.

Heating from the preheat temperature to sterilisation temperatures and initial cooling of the sterilised milk is much slower in indirect systems than in direct systems. For example, reserachers reported less than 1 s for each heat transfer operation in direct heating compared with greater than 10 s with indirect methods. In practice, the heating times from preheat to final UHT temperature can be up to 100 s. Therefore, for a given temperature increase, indirect processes are more severe than direct processes. In other words, milk produced in an indirect plant is subjected to a greater heat load than milk processed in a direct plant, with equivalent bactericidal effectiveness.

The different heating processes and temperature–time profiles of direct and indirect systems give rise to several differences in the processing characteristics and parameters. Of particular note is the higher propensity of indirect plants to foul because of the large area of hot surfaces. In the first stages of the plant where the temperature reached is <100°C, the main reaction is denaturation of whey proteins, mainly β-lactoglobulin, with subsequent deposition of the denatured protein. In the higher-temperature (>100°C) sections of the plant, the major reaction is deposition of calcium phosphate, which has reduced solubility at high temperatures. Consequently, the deposit in the high-temperature sections is predominantly mineral, while the deposit in the lower-temperature section is predominantly protein. The build-up of fouling deposit causes a reduction in heat transfer and an increase in pressure. In commercial plants, the product temperature is maintained by increasing the temperature of the heating medium. This exacerbates the fouling because of the increased temperature differential between the heating medium and the product, and eventually, the plant has to be shut down for cleaning.

The degree of heat energy recovery is another difference between the two systems. In direct heating systems, less regeneration of heat is possible since the steam flashed-off in the vacuum chamber is condensed, and the useful heat is lost from the system. In indirect systems, all the heat in the hot sterile product at the sterilisation temperature (135–150°C) can be used in the regeneration section. Thus, heat regeneration in indirect systems is usually >90%, while it is only about 50% in direct systems.

### Aseptic Packaging

Following sterilisation and subsequent cooling, the sterile product is filled into a sterile container in an aseptic environment and hermetically sealed to ensure sterility is maintained throughout the handling and distribution processes. Two main aseptic packaging systems are used commercially. First, the type that uses pre-formed containers, and second, the type that forms, fill and seals the containers in the aseptic packaging system. For both systems, the containers can be either plastic or paperboard. In the first system, plastic bottles are pre-blown while in the second they are blown online. For paperboard cartons, in the first system, pre-cut and folded individual packages are assembled online, while for the second system, the cartons are formed, filled and sealed from a continuous roll of paperboard.

Whatever system is employed, the packaging material or container must be sterilised before being filled with the sterile product. The major sterilisation technique used is a combination of hydrogen peroxide and heat, which is a very effective sterilant for the surface of packaging material. A typical procedure is to treat the packaging material with 35% $H_2O_2$ at 70°C for 6 s followed by hot air treatment at 125°C to evaporate the residual $H_2O_2$. An alternative to $H_2O_2$ alone is a mixture of peracetic acid and $H_2O_2$ (4%). Gamma radiation is used to sterilise heat-sensitive packaging materials, such as plastics and laminates; an example is the flexible bags used in Intasept™ aseptic packaging machines.

It is important that a sterile environment is maintained during aseptic packaging to ensure the product remains sterile during transfer from the processing line to the sterile container. Following sterilisation of the filling machine with gaseous hydrogen peroxide, the air entering the filling machine is sterilised and filtered, and maintained at a positive pressure of about 0.05MPa in the filling chamber. Such precautions are necessary to prevent post-sterilisation contamination of the product with airborne bacteria and moulds.

### Factors affecting the quality of UHT milk

Raw milk quality is affected by: (a) growth of psychrotrophic bacteria and (b) heat-resistant spore-forming bacteria.

#### *Growth of Psychrotrophic Bacteria*

Good quality raw milk is essential for producing UHT milk with a long shelf life. In general, milk destined for UHT processing should be stored refrigerated

(<5°C) for no more than 48 h. Storage at higher temperatures and/or for longer times promotes the growth of psychrotrophic bacteria, which cause the production of lactic acid, reduction of the pH of the milk and also production of enzymes, notably proteases and lipases that can have considerable heat stability.

When the pH is reduced to ≤6.5, milk becomes unstable to heat. In UHT processing, such milk readily causes fouling of the heat exchangers, and the final product will show considerable sediment formation.

The UHT process destroys all vegetative bacteria and most sporeformers but does not inactivate some of the enzymes produced by psychrotrophic bacteria, such as *Pseudomonas* species, i.e. the most common bacterial contaminants of raw milk. Such enzymes are typically produced when the bacterial count exceeds ~$10^6$ cfu $mL^{-1}$. If milk with such bacterial counts is UHT processed, these enzymes, particularly proteinases and lipases, can remain active in the UHT milk. Since UHT milk is usually kept at room temperature and may be stored for several months, even traces of these enzymes can produce noticeable changes, and result in bitter flavour and gelation (due to proteinases) and rancid flavours (due to lipases).

### *Heat-resistant spore-forming bacteria*

Bacterial spores in rawmilk present the fundamental challenge forUHTprocessors.Without such organisms, there would be no need for the heating intensity of UHT to produce a shelf-stable product. The heat-resistant thermophiles, such as *G. stearothermophilus* and *B. licheniformis*, are the most commonly encountered. They can cause the 'flat sour' defect in UHT milk, which is characterised by acid production, but no gas production. However, these thermophiles do not grow in milk under 'normal' storage conditions (≤30°C), and have a growth optimum of ~55°C. They have been known to cause problems if the milk temperature reaches high levels during transportation.

Aconcern related to rawmilk quality is the occurrence of the bacterium *B. sporothermodurans*, which produces highly heat-resistant spores. This organism is mesophilic, which means that it can grow at room temperature. Fortunately, it does not appear to cause spoilage other than a slight discolouration of the milk, and seldom reaches counts of greater than 105 cfu $mL^{-1}$. However, it is extremely difficult to remove from contaminated equipment, and has caused the closure of some UHT plants. Because of its heat resistance, its spores may be present in UHT milk and be of little concern. However, the practice of reprocessing out-of-date UHT milk has been shown to enrich the UHT milk with this organism, and cause levels which are of concern.

Researchers investigated a case of 'obstinate contamination' of UHT milk from a company, and found two very heat-resistant mesophilic organisms. One was *B. sporothermodurans* and the other was identified as *Paenibacillus lactis*. This was the first time *Paenibacillus* had been isolated from UHT milk, although *Paenibacillus* spores have been previously reported to survive heating at 120°C. *Paenibacillus* spores have been isolated from silage and feed concentrates, which may be the origin of the organism in milk.

Other aspects that can affect the quality of UHT milk are given in the next three sections.

### Processing Times and Temperatures

The major effects of processing on the quality of UHT milk are due to the higher heat load in indirect systems and, to a lesser extent, the lower dissolved oxygen content in directly processed milk, caused by the concomitant extraction of air andwater in the vacuum cooling chamber.

The two major differences in product characteristics are in flavour and susceptibility to gelation during storage, i.e. age gelation. Indirectly processed milk has a more cooked flavour, and may develop more oxidised or stale flavours due to the higher dissolved oxygen level. However, directly processed milk is more prone to gelation. A major cause of age gelation is proteolysis catalysed by either the native milk plasmin or bacterial proteases resulting from growth of psychrotrophic bacteria, principally pseudomonads, in the milk before processing. Both plasmin and the bacterial enzymes have considerable heat stability; the higher heat load of indirect heating inactivates the proteases much more than does direct heating. While both plasmin and bacterial proteases cause gelation and also bitterness, their modes of action on the caseins are quite different. Bacterial proteases preferentially attack κ-casein in much the same way as chymosin in rennet, while plasmin preferentially attacks β-casein. Thus, the two proteases release different peptides, and this difference can be exploited to determine the cause of age gelation.

## POST-STERILISATION CONTAMINATION

A major consideration in the handling of milk after the high-temperature sterilisation is contamination.This may result from several sources, but two important ones are the seals in the homogeniser (if downstream) and the air supply to the aseptic packaging unit. Researcher showed that spores trapped under seals had enhanced heat stability, largely attributable to a very lowwater activity in their microenvironment, and could act as a reservoir of contaminating spores. Flat sour defects due to contamination by *G. stearothermophilus* can arise in this way. Frequent seal changes have been found to be an effective, although expensive, way of minimising such contamination.

Another microbial problem, which has caused problems in several companies in recent years, is the filamentous fungus, *Fusarium oxysporum*. This organism can cause an offflavour similar to blue-vein cheese in UHT milk within a few weeks, and it also produces gas. It is often detected when packages become swollen or 'blown'. It is a common fungus of plants and soils, and can enter UHT milk packages through contaminated air in the filling machine. Negative air pressures in aseptic filling areas may facilitate contamination of the packaging equipment if there is a source of the fungus nearby. Once the fungus has contaminated a filling machine, it is difficult to eliminate.

### Storage Temperature and Rime

UHT and in-container sterilised milks are subject to considerable chemical and physical changes during storage, although they are more noticeable in the latter. Each of the changes is dependent on both time and temperature of storage. For example, non-enzymatic browning develops during storage, and proceeds at a faster rate at higher temperatures. It is particularly noticeable above 30°C, and is accelerated in lactose-hydrolysed and sugar-sweetened products.

Age gelation, which is often the limiting factor for the shelf life of UHT milk, is also temperature and time dependent. However, the temperature dependence is only true up to about 30°C after which gelation is retarded. The exact reason for the retardation at higher temperatures is not known, but may be due to autolysis of the protease involved, excessive proteolysis at the caseins preventing the protein network get from forming or the formation of non-disulphide covalent cross links between the caseins preventing the gel formation.

Oxidation reactions occur, starting with oxidation of sulphydryl compounds produced during heating, then vitamin C and folic acid followed by fats. All these reactions are dependent on the level of dissolved oxygen. This is close to saturation in most products processed by indirect heating, but is reduced in products which have been subject to deaeration, and in directly treated products subjected to vacuum flash cooling. However, the levels of dissolved oxygen can increase if the sterile milk is stored for some time in an aseptic tank before packaging, if the packaging material is permeable to oxygen or if there is a headspace in the filled package. The headspace volume can vary considerably with the type of package. Researchers found that 1-L Tetra Brik packages to contain an average of ~8 mL, a 1-L Combiloc package ~34 mL and a 1-L plastic bottle ~58 mL. Directly processed milk packaged in the plastic bottles exhibited quite high dissolved oxygen levels (5.8-7.2 mg $L^{-1}$). The headspace volume can have a major influence on dissolved oxygen levels, and development of volatile carbonyl compounds caused by oxidation.

### Heat Stability, Eediment Formation and Fouling

A major concern withUHTprocessing is the heat stability of the milk. Milk having poor heat stability can give rise to fouling of heat exchangers and sediment formation, and it would be best to avoid UHT treating such milk. However, there is no test for quickly assessing the heat stability of milk to UHT processing conditions. The much researched heat coagulation test for UHT processing, which measures the time required to coagulate milk at 140°C, is not easy to perform, and it has not been established that it is useful for predicting susceptibility to sediment or fouling in UHT milk.

Most UHT milk contains a slight amount of sediment, which is not usually sufficient to be a problem. It has been found that sediment increases with severity of the treatment, and is present in greater quantities following direct processes. Researcher found that little sediment was formed in UHT cow's milk if the pH was

kept above 6.62; below this value, sedimentation increased rapidly. In contrast, sedimentation was severe in goat's milk when the pH was below 6.9. Similar trends were observed for concentrated skimmed milk, which were found to be stable above pH 6.55, but below this value, severe sedimentation occurred. However, on some occasions, a more voluminous sediment appears, and it is more of a problem in goat's than in cow's milk.

Researchers suggested that ionic calcium may play a role in sediment formation in UHT goat's milk. Up until recently, the only detailed study on ionic calcium in cow's milk at its natural pH was that of White & Davies. A good correlation was found between ethanol stability and ionic calcium for 132 individual cows. Ionic calcium was measured on ultrafiltration permeates derived from each individual milk, using a chemical method. Thus, there is considerable evidence that ionic calcium varies considerably both in milk from individual cows and in bulk milk, and that this may influence heat stability. However, despite this, its measurement is not routinely practiced as a quality assurance procedure.

One alternative test is the ethanol stability test. By adding equal volumes of different strength alcohol solutions to milk, one can establish the concentration which just fails to cause the milk to coagulate - this is termed the ethanol stability. Pioneering work on understanding factors affecting ethanol stability, and the mechanisms was conducted by researchers starting in the 1980s.Researcher recommended that milk should be stable in 74% ethanol to be suitable for UHT processing.

One key question is whether there is a correlation between ethanol stability and stability to UHT processing conditions. The authors' experience with pilot plant and laboratory experiments on cow's and goat's milk suggests that reducing ionic calcium is beneficial in terms of reducing fouling of heat exchangers and sediment formation. Also, reducing ionic calcium was found to increase ethanol stability.

Therefore, in situations where sediment formation or fouling is a problem, the following suggestions are offered: routinely monitor pH, ethanol stability and, if possible, ionic calcium in raw milk to establish their contribution to fouling and sediment-related problems. Over time, this should provide data to be able to assess, understand and eventually reduce the problem.

As intimated by the researchers, raw milk with an ethanol stability below 74% is likely to be problematic. There are two main reasons why ethanol stability may be low. The first and most likely is a high microbial count, and the second is due to a salt imbalance. The former situation is likely to arise with milk of poor hygienic quality or poor refrigeration. As raw milk quality deteriorates, i.e. as its bacterial count increases, its pH will fall, which in turn will increase ionic calcium and reduce ethanol stability.

However, as researcher cautions, milk with a low ethanol stability may still not be of poor microbial quality; it may just have a salt imbalance. In this context, salt imbalance refers to a combination of circumstances that leads to the micelle being made more susceptible to coagulation. For example, any factors which reduce the

negative charge, such as concentrations of $H^+$, $Ca^{++}$, $Mg^{++}$, $Na^+$ and $K^+$ ions, aswell as the proportions of different casein fractions in the micelle. One example is goat's milk, which has a high concentration of ionic calcium compared to cow's milk. Similar detrimental changes may occur to milk when calcium supplements are added, or when the pH is reduced by whatever means. Where permitted, stabilisers, such as disodium hydrogen phosphate and trisodium citrate, may help improve stability; their effect is to reduce ionic calcium, increase pH slightly and increase ethanol stability. Levels used are between 0.05 and 0.2 g 100 $g^{-1}$, but practical experience should dictate. One possible drawback is more browning, brought about by the increase in pH. This is not likely to show immediately after UHT processing, but may manifest itself during storage, especially when the product is stored at above 30°C.

Overall, it is recommended that ionic calcium, pH and ethanol stability are measured to develop a better understanding of how this relates to UHT plant performance and indicators of fouling and sediment.

It has also been suggested that factors that give rise to sediment formation are also responsible for fouling. Much of the research on the mechanism of fouling has focused on the role of β-lactoglobulin denaturation, whereas sediment research has revolved around casein micelle aggregation, in terms of pH and more recently ionic calcium. Of course, denatured β-lactoglobulin is involved through its aggregation with the casein micelle on heating. The mechanism of fouling in UHT plants is usually explained in terms of two types of fouling, A and B. Type A deposit is soft and voluminous and forms between ~75 and ~110°C in the preheat section; while type B deposit is hard and granular, and forms in the high-temperature sections of the plant. The two deposits differ considerably in composition, with type A being predominantly protein and type B being predominantly mineral, largely calcium phosphate. This mechanism, therefore, focuses on whey protein denaturation and mineral deposition, but does not consider the role of casein. A comprehensive mechanism of fouling and sediment formation involving whey protein denaturation, casein destabilisation and mineral insolubilisation is, therefore, still required.

## HIGH-TEMPERATURE PROCESSING (EXTENDED SHELF LIFE)

There is a requirement to further increase the shelf life of pasteurised products, both for the convenience of the consumer and to provide additional protection against temperature abuse. However, it is important to avoid the onset of cooked flavour, which results from severe heating conditions. High-temperature pasteurisation has been introduced to meet this requirement. It is a continuous heat treatment between HTST pasteurisation and UHT sterilisation, and is used to produce what has become known as extended shelf life (ESL) milk. It is also known as 'ultrapasteurisation', although this term has a different meaning in some countries. ESL milk can also be produced using non-thermal technologies, such as microfiltration and bactofugation, but its production by heating processes only is discussed here.

There is no single definition for ESL milk; however, the EU legislation states that pasteurised milk, which shows a negative reaction to the peroxidase test, must be labelled 'high temperature pasteurised milk'. In a recent review on ESL milk, Researcher used the following definition: *ESL products are products that have been* treated in a manner to reduce the microbial count beyond normal pasteurisation, packaged under extreme hygienic conditions, and which have a defined prolonged shelf life under *refrigeration conditions.*

A range of different temperature-time combinations has been suggested for producing ESL milk. HTST pasteurisation (72°C for 15 s) does not destroy sporeformers or thermoduric non-spore-forming bacteria, such as coryneforms, micrococci and thermoduric streptococci. As discussed above, increasing the temperature of pasteurisation from 72°C to~90°C destroys some of the thermoduric non-spore-forming bacteria, but has a detrimental effect on shelf life. Thus, this temperature range is unfavourable for ESL processing. Therefore, an approach is to use temperatures >100°C for very short times. Researchers showed that milk heated to 115°C for 2 s had a much better keeping quality than milks heated at both 72°C for 15 s and 90°C for 15 s. Researchers reported that treatment of milk at temperatures ≤117.5°C resulted in high total counts (>$10^6$ cfu $mL^{-1}$) after 13 days, whereas milks treated at temperatures ≥120°C showed counts of <$10^2$ cfu $mL^{-1}$ after storage at 7°C for >40 days. It appears that heating at e"120°C is required to inactivate psychrotrophic spore-forming organisms, such as *B. cereus* and *B. circulans.* The upper temperature limit, which Blake *et al.* (1995) concluded was 134°C, is governed by the heat-induced chemical changes, and can cause flavour impairment. Thus, the most common conditions for ESL heat treatment are in the 120–130°C range for a short time (<1 to ~4 s).

Another approach to produce ESL milk is to use small amounts of a bacteriocin. The addition of small amounts of nisin (40 IU $mL^{-1}$) was also effective in reducing microbial growth following heat treatment at 72°C for 5 s, and even more effective at 90°C for 15 s. It was particularly effective at inhibiting *Lactobacillus* spp. at both temperatures. Results for milk heat treated at 117°C for 2 s with 150 IU $mL^{-1}$ nisin were even more spectacular. Such milks have been successfully stored for over 150 days at 30°C with only very low levels of spoilage. Local regulations would need to be checked to establish whether nisin is a permitted additive in milk and milk-based beverages.

A key aspect of the heating conditions to produce ESL milk with a flavour similar to that of pasteurised milks is short heating, holding and cooling times. This is most easily achieved by direct steam heating, either steam injection or steam infusion, for example in the APV Pure-LacTM system.

For ESL milk, the lowest advisable packaging level is ultraclean, although aseptic packaging is recommended by some authors. There is no doubt that the longest shelf lives are obtained with aseptic packaging, but this is achieved at a cost, i.e. the cost of the aseptic packaging equipment installation and maintenance. For this reason, ESL milk is usually packaged in ultraclean rather than aseptic systems.

## HEATING METHODS

The move is on for ultra-high temperature (UHT) pasteurization, which will extend the shelf life of milk and other dairy products. UHT pasteurization is being used because consumers are buying more dairy products in bulk, dairy operations are becoming larger, and population expansion is increasingly causing consumers to be located in more remote locations throughout the world.

Traditionally, milk is pasteurized at 180- 185°F (about 85°C) and refrigerated. Other dairy products are commercially sterilized by subjecting product to temperatures in excess of 100°C, and then packaging it in airtight containers. The basis of UHT is sterilization of the product before packaging, then packaging it in pre-sterilized containers in a sterile atmosphere. Processing dairy products in this manner, using temperatures exceeding 135°C, permits a reduction in the required hold time to 2-to-5 seconds, and yields a shelf stable product that can be stored for extended period of time at ambient temperature.

Some examples of food products processed under UHT conditions are:

- Liquids such as milk, juices, yogurts, cream, and salad dressings.
- Foods with small particulates like baby food, soups, sauces, and stews.
- Soy based products in order to inactivate bacteria and reduce off flavors.

The traditional problems or difficulties in using a UHT process have been:

- **Sterility**: The complexity of the equipment requires more highly skilled operators to maintain sterility through out the aseptic process.
- **Particulate Size**: With larger particulates comes the danger of overcooking of the product surface.
- **Product Quality:** Heat stable lipases or proteases can lead to flavour deterioration. In a number of cases flavour deterioration has caused a more pronounced cooked flavour for UHT milk as an example.

### Current UHT Cooking Methods

*Indirect Heating*

With this method the heating medium and product are not in direct contact with each other. As mentioned earlier, the types of heat exchangers are plate and frame, tubular, and scraped surface. The advantage of using these types of exchangers is that you do not need culinary steam since the two media are kept separate. However, each type of exchanger has its own disadvantages.

- **Plate and Frame Exchangers**: While they are typically easy to inspect and take up less floor space than other types of indirect exchangers, they are limited by gasket temperatures and pressures. In most cases the EPDM gasket is limited to a maximum 160°C. The plates, while easy to take apart, can over time become fatigued as they flex from the constant high

temperature steam and lower temperature product passing over the contact surface. Liquid velocities are usually lower for a plate and frame exchanger arrangement and as such can lead to uneven heating and potential burn-on and browning.

- **Tubular Exchangers**: While they have fewer seals and therefore do not suffer as much from gasket limitations or plate fatigue, they typically take up more floor space and are not easy to inspect. Heating may be more uniform, however browning or burn-on is possible because of the large surface area required to achieve the desired set point.
- **Scraped Surface Exchangers**: This type of exchanger forces product through a jacketed tube in which a set of rotating blades is constantly moving product from the outer walls toward the center. The product is more evenly heated and there is less opportunity for product browning or burn-on. Scraped surface exchangers are also more suitable for highly viscous products and products containing particulates. A general negative has always been the time and cost required for inspection and maintenance of this equipment.

A final point concerning indirect heat exchangers is that while they do a good job of heating product at a fixed liquid flow rate, they suffer when the liquid side flow rate varies. The potential for burning increases as the liquid flow rate decreases.

**Steam Infusion**

The general concept is to take the liquid product stream and have it pumped at a higher pressure through a distribution nozzle into a chamber filled with slightly lower pressure, culinary quality steam. This system is characterized by cascading a small volume of product through a large steam chamber. The product then collects at the bottom of the chamber and is fed forward via a timing pump.

Product temperature is generally controlled by pressure. Additional holding time is accomplished through the use of hold tubes, plate and frame exchangers or tubular exchangers. This is followed by flash cooling in a vacuum chamber where all added moisture is removed as needed. Variations of this method involve 1) preheating the product to a desired set point before the addition of direct contact steam, or 2) using the steam infusion method first followed by flash steam removal and perhaps reheating to a uniform set point.

All of these steam infusion methods accomplish the same thing:

- Instantaneous heating and rapid cooling.
- Lack of overheating or burn-on.
- Heating of low and high viscosity products.
- Use of variable product flow rates.

The negatives of steam infusion are:

- **Size**: The infusion chambers take up a sizeable amount of useable production floor space.
- **Sanitation**: These systems are not easilycleaned.
- **Capital Outlay**: Units require high, initial capital investment.
- **Operations**: A fairly skilled work force is required to monitor pressures, feed pump flow rates, etc.
- **Need for a Timing Pump**: Added equipment and operating cost.

### Direct Steam Injection

*More than a Viable Alternative*

Like steam infusion, Direct Steam Injection uses culinary steam to quickly heat a food product to the desired UHT temperature. Direct Steam Injection blends the liquid stream with the higher pressure steam using multiple orifice injectors, static mixers or venturi type injectors. The key is to get the product to quickly absorb all the steam energy and elevate its temperature as quickly as possible. Unlike steam infusion it does not require a large chamber filled with steam with a higher pressure liquid cascading through it to achieve the quick heat transfer of steam and liquid.

The immediate advantage of this type of mixing is that the mixing chamber isn't much bigger than the liquid transfer piping. The overall assembly has a minimal foot print and the mixing chamber itself is especially small and easy to clean or inspect. Often the chamber requires no more than opening three quick release clamps to remove the device from the process line.

Because the steam has intimate contact with the product, the Direct Steam Injection method maximizes heat transfer. Another important feature is that the same system can, for the most part, process a wide variety of slurries to a desired set point. Some systems, depending on their designs, can even process particulates with little or no product degradation.

Another advantage is that Direct Steam Injection systems are available in a range of sizes capable of handling product capacities from 240-liters/hour to +20,000-liters/hour. Direct Steam Injection systems also require low capital investment, potentially half as much as any other method being considered.

The main disadvantages to using Direct Steam Injection or even steam infusion are:

- Steam injection like steam infusion requires the use of culinary steam.
- **Dilution**: Added moisture must be removed using an atmospheric or vacuum flash chamber. Depending on product specifications, this can be minimized or eliminated by preheating upstream of the steam injector.

- **Sanitation**: Current 3A standards call for COP on steam injector systems so a backup unit or removable spool piece should be considered.

## TEST RESULTS

One manufacturer of Direct Steam Injection systems and an early pioneer in Sanitary Direct Steam Injection Heating, Pick Heaters, regularly tests a variety of food products to confirm cook temperature can be achieved and maintained while maintaining product integrity.

Recently a series of tests were conducted on whole milk with 4.5% butterfat to determine whether UHT temperatures could be achieved without browning and off flavors. Using 140-psig steam and liquid pressures of 100-psig, their Direct Steam Injection system was easily capable of a 40ÚC temperature rise when the product was preheated to 95ÚC. The product retained good full-bodied mouth feel, no browning and no strong burnt smell typically found in a UHT process. And while there was a little filming on the injector, it was quickly cleaned using traditional CIP methods.

### Conclusion

Heating is the major processing treatment applied to milk. In most countries, all market milk and most, if not all, milk used for manufacture undergoes some formof heat treatment. The types of heating vary from the mildest treatment, thermisation, through pasteurisation, high-temperature heating or ESL treatment, to UHT and in-container sterilisation, the most severe treatment. All processes reduce bacterial spoilage, and all but thermisation destroy most pathogenic organisms. The technologies used for heating are very mature and have served and continue to serve the consumer and the dairy industry very well. Considerable research has been carried out on the effects of the different methods and severity of heating on the bacterial flora and the chemical constituents of milk, and these are now reasonably well understood. This understanding has enabled the dairy industry to meet challenges related to heating. For example, there has been increasing consumer demand for minimal processing and natural flavours and, hence, UHT milks with very little cooked flavours. However, the discovery of very heat-resistant bacterial spores in UHT milk some years ago suggested a need for more intense UHT treatment to ensure sterility. These apparently conflicting demands were met by development of technologies capable of very rapid direct heating to high temperatures followed by rapid vacuum cooling. This development was possible through an understanding of the different kinetics of bacterial destruction and chemical reactions. The most recent development has been the introduction of ESL milk to satisfy the demand for a longer lasting market milk with a taste similar to that of pasteurised milk. Further developments in this technology can be expected, possibly in conjunction with some forms of non-thermal processing.

The smaller direct contact steam injection system offers a number of important advantages over the other UHT methods being used today. Direct Steam Injection

is more than a viable alternative for UHT processing, and should be given serious consideration.

## EQUIPMENT

Liquids can be heated and cooled in a batch process, in a heat exchanger, or in a packaged form. Moreover, direct mixing with steam can be combined with heat exchange. Highly viscous liquids can be heat-treated in a scraped-surface heat exchanger. Originally, batch processing was in general use for pasteurizing beverage milk. It is the so-called *holder pasteurization*, e.g., 30 min at 63°C. The method is still in use in the manufacture of starters, whipping cream, and other small-scale products. Usually, the vats involved are fitted with an agitator; steam or hot water circulates through a double jacket, followed by cold water. Among the advantages of holder pasteurization are the simplicity, flexibility, and satisfactory temperature control (little fluctuation in temperature occurs unless highly viscous liquids are treated). A drawback is that the warming and cooling times are long (excessive for large containers). Furthermore, regeneration of heat is not well possible and connection to continuous processes is awkward.

Currently, flow-through heaters or heat exchangers are commonly used. Hot water or condensing steam constitutes the heating medium. Sometimes, vacuum steam heating is applied to minimize the difference in temperature with the liquid to be heated.

In *plate heat exchangers*, a large heating surface is assembled in a confined space and on a small floor area. The heating agent and the incoming liquid are present in thin layers and are separated by a thin wall, i.e., a plate. Because of the large heating surface per unit volume of liquid that is to be heated, the difference between the temperature of the heating agent and the temperature of the liquid to be heated can be small, e.g., 2°C when milk is heated from 65°C to 75°C. This may be an advantage for some heat-sensitive products, where fouling of the heat exchanger is greater for a higher wall temperature. Furthermore, warming and cooling proceed rapidly in plate heat exchangers.

Another advantage is that the energy consumption (for heating and cooling) can be relatively small because heat can be regenerated. When the milk enters the heat exchanger, it is warmed by milk that has already been heated and this, in turn, is at the same time cooled by the milk coming in. The latter is subsequently heated further by hot water (or steam). It may then flow through a holder section to achieve a sufficient heating time. After being cooled by the incoming milk, it is cooled further by means of cold water (or another cooling agent). Note that the liquids being heated and cooled are always in counter-flow and that the temperature difference between the two remains constant.

A plate heat exchanger is made up of various sections connected in series, including a regeneration section, a heating section, a holding section (may also be a tube), and cooling sections. Each section consists of a great number of plates, being partly connected in parallel and partly in series. In this way, the liquid is properly

distributed among the plates and arrives at a speed that is high enough to reduce fouling. The plates are shaped in such a way as to greatly enhance turbulence in the liquid. This enhances heat transfer and diminishes fouling. Plate heat exchangers have some disadvantages. The main one is that leakage can occur, for instance, due to an imperfect or worn-out rubber gasket between two plates. If as yet unpasteurized milk in the regeneration section leaks into the milk that has passed the holder section, contamination with undesirable bacteria may occur. To prevent this, the pressure on the milk after the holder must be higher than that on the milk before the holder; this requires an additional pump.

Another problem is that the pressure on the milk cannot be very high. There is only a very small distance between plates and this precludes the heat treatment of highly viscous liquids because a high pressure would be needed to overcome the high flow resistance. It is also difficult to heat milk to well above 100°C because it needs an overpressure to prevent boiling. Finally, liquids containing small particles, say > 50 μm, and liquids that cause heavy fouling also give problems.

In the remaining part of this subsection we will primarily discuss *sterilization*. When a flow-through process is applied, some additional measures must be taken. In the first place, the equipment should not only be clean prior to operation but also sterile. This concerns the heat exchanger and all downstream equipment like pipelines, valves, balance tanks, packaging machine, etc. The equipment must therefore be sterilized; this is generally achieved by operating it with water, which may take about half an hour. Moreover, the plant must be designed in such a way that recontamination can be rigorously prevented. Finally, the packaging material must be sterilized.

*Tubular heat exchangers* generally have a smaller heating surface per unit volume of liquid to be heated than plate heat exchangers. Accordingly, the difference in temperature between the heating agent and the incoming liquid will generally be greater.

To restrict fouling and enhance heat transfer, high flow rates are used, which necessitates high pressures. But this causes no problems because tubes are much stronger than plates; some tubular heat exchangers even have no sealing gaskets but have (spirally bent) concentric tubes. Tubular heat exchangers can readily be applied to obtain very high temperatures (e.g., 150°C). Accordingly, they are excellently fit for indirect UHT treatment. Like a plate heat exchanger, a tubular heat exchanger can be built of regeneration, heating, holding, and cooling sections. In modern heat exchangers, the milk may be in counter-flow with water throughout the apparatus. The water is kept circulating and is heated by means of indirect steam heating immediately before it heats the milk to the maximum temperature desired. In such cases, the flow rates on either side of a separating wall need not be the same, implying that the temperature difference is not constant. Often, up to about 90% heat regeneration is achieved. This method of working has advantages with respect to temperature control, rapid and even heat transfer, and saving of energy.

In *UHT treatment with direct heating* there is no wall between the heating agent and the liquid to be heated but the heating agent (steam) is injected into the liquid or the other way around. Thereby an almost instantaneous heating to the desired temperature (e.g., from 80°C to 145°C in 0.1 s) occurs, provided that the steam can immediately condense. For this to happen, finely dispersed steam and a significant back pressure in the liquid are needed. The incoming liquid becomes diluted with the condensing steam. Of course, the steam has to be of high purity. After maintaining the liquid at the desired temperature for a few seconds, it is discharged in a vessel at reduced pressure. Here almost instantaneous evaporation of water occurs, causing very rapid cooling. The amount of water that evaporates should equal the amount of steam that had been absorbed.

Steam injection heating causes disruption of fat globules and some coagulation of protein. Homogenization (aseptic) at high pressure redisperses the coagulum. Without homogenization, the product gives an inhomogeneous astringent or nonsmooth sensation in the mouth, and a sediment tends to form on storage. The heating section (up to, e.g., 80°C) that precedes the steam injection, and the cooling section that is connected after the 'flash cooling' (starting from, e.g., 80°C), may be plate heat exchangers. Therefore, holding times are often extended or the warming that precedes the steam injection is made slower and extended to a higher temperature. Also, in indirect UHT treatment, long warming times are sometimes applied to obtain a fuller inactivation of plasmin. In this way, the difference between traditionally sterilized and UHT milk is diminished.

The so-called vacreator is quite similar to a direct UHT heater but in this apparatus the liquid is heated to pasteurization temperatures. The essential detail is evaporative cooling in vacuum, aimed at the removal of volatile flavor compounds. Such apparatus is occasionally used for pasteurizing cream for butter manufacture. Disadvantages include the considerable damage (coalescence and disruption) to fat globules and the limited heat regeneration. A variant of steam injection is infusion. In this method the milk is sprayed into a steam flow.

### Autoclaving

Heating a liquid in a hermetically sealed container (rarely larger than a liter) has the distinct advantage that recontamination of the heated liquid by microorganisms can be readily prevented. That is the reason why this method of working is primarily applied in sterilization. However, it suffers from serious disadvantages. The long warming and cooling times and the large temperature differences inside a can or bottle result in undesirable changes like browning and sterilized milk flavor. Agitating or revolving the containers during the heat treatment may restrict these changes because it considerably enhances heat transfer and evenness of temperature. However, its technical operation is difficult because the heating by steam must occur under pressure, at least when sterilization is applied. Heating to 120°C corresponds to a gauge pressure of 1. The easiest method of heating is batchwise in a steam closet or autoclave.

But that implies low capacity, and high energy costs (regeneration is impossible), and is very laborious. Because of this, continuous sterilizers are commonly applied. For bottles, it mostly concerns a hydrostatic sterilizer in which the bottles pass twice through a water seal, about 10 m high because of the 1-bar gauge pressure. In some machines, the bottles undergo a rocking motion, whereby heat transfer is speeded up. For cans, the so-called 'cooker and cooler' with rotary air locks is usually used. Continuous sterilizers allow considerable heat regeneration.

## SOME HEAT TRANSFER PROPERTIES

**Density** ($\rho$, **kg m$^{-3}$**): mass/volume; for solids, must distinguish between bulk density and solid density; for foams, overrun is used. Natural convection is due to density differences.

**Specific gravity**: mass/mass; dimensionless quantity; the termoriginal gravity is often used in brewing examples.

**Viscosity** ($\mu$, **Pa s or Nsm$^{-2}$**): a measure of the internal friction within the fluid; the ratio of shear stress/shear rate; distinguish between Newtonian and non-Newtonian fluids.

**Specific heat (c, J kg$^{-1}$ K$^{-1}$)**: the amount of energy required to raise unit mass by unit temperature rise; water has a high value compared to other food components.

**Latent heat ($h_{fg}$, kJ kg$^{-1}$)**: the amount of energy required to convert unit mass of material from solid to liquid or liquid to gas at a constant temperature; water has a high value.

**Specific enthalpy (H, kJ kg$^{-1}$)**: enthalpy = $U + PV$, and specific enthalpy is the enthalpy/mass; at constant pressure, enthalpy changes are equivalent to heat changes.

**Thermal conductivity (k,Wm$^{-1}$ K$^{-1}$)**: this is a measure of the rate of heat transfer through a solid; foods are poor conductors of heat.

**Thermal diffusivity** ($\alpha$, **m$^2$ s$^{-1}$**): this measures how quickly a material changes in temperature, when energy is added or removed; it is evaluated from thermal conductivity/density × specific heat – ($k/\rho c$)

**Heat film coefficient (h, W m$^{-2}$ K$^{-1}$)**: a measure of heat transfer by convection, within a fluid; compare - gases (low), liquids (intermediate) and condensing vapours (high).

**Overall heat transfer coefficient (OHTC) (U, Wm$^{-2}$ K$^{-1}$)**: a measure of the overall heat transfer performance of a heat exchanger,which accounts for all the individual resistances to heat transfer; high OHTC values are desirable, and OHTC is reduced by fouling.

## DEFINITIONS AND EQUATIONS

Temperature conversion

$°C = (°F-32) \times 0.56$

$K = °C + 273.15$

Temperature difference

$°C = °F \times 0.56$

$1°C = 1K$

Energy units

4.18 J = 1 calorie

1 BTU = 1.055 kJ

1 therm = 105 BTU

1 kWh (unit) = 3.6 × 106 J or 3.6 MJ

**Heat exchanger design:** rate of heat transfer $Q = UA\Delta\theta_m$

$Q$ = duty ($Js^{-1}$);

$U$ = overall heat transfer coefficient ($Wm^{-2}\ K^{-1}$);

$A$ = surface area ($m^2$),

$\Delta\theta_m$ = log mean temperature difference (K).

# 11

# Centrifugation - Separating Cream from Milk

**Topics**

*Principles of Centrifugation • Clarification • Separation • Dairy Centrifuges and Separators • Standardization • Standardization Problems • Pearson square approach to above problem*

Centrifugal separation is a process used quite often in the dairy industry. Some uses include:

- clarification (removal of solid impurities from milk prior to pasteurization)
- skimming (separation of cream from skim milk)
- standardizing
- whey separation (separation of whey cream (fat) from whey)
- bactofuge treatment (separation of bacteria from milk)
- quark separation (separation of quarg curd from whey)
- butter oil purification (separation of serum phase from anhydrous milk fat)

## PRINCIPLES OF CENTRIFUGATION

Centrifugation is based on Stoke's Law. The particle sedimentation velocity increases with:

- increasing diameter
- increasing difference in density between the two phases
- decreasing viscosity of the continuous phase.

If raw milk were allowed to stand, the fat globules would begin to rise to the surface in a phenomena called creaming. Raw milk in a rotating container also has centrifugal forces acting on it. This allows rapid separation of milk fat from the skim milk portion and removal of solid impurities from the milk.

A separator is a centrifugal device that separates milk into cream and skimmed milk. Separation was commonly performed on farms in the past. Most farmers milked a few cows, usually by hand, and separated milk. Some of the skimmed milk was consumed while the rest was used to feed calves and pigs. Enough cream was saved to make butter, and the excess was sold.

Today, milk is separated in industrial dairies. Sufficient cream is returned to the skimmed milk before sale.

Manual rotation of the separator handle turns a worm gear mechanism which causes the separator bowl to spin at thousands of revolutions per minute.

When spun, the heavier milk is pulled outward against the walls of the separator and the cream, which is lighter, collects in the middle. The cream and milk then flow out of separate spouts. Some floor model separators were built with a swinging platform attached to the stand. The bucket for collecting the cream was put on the platform, and a much larger bucket was set on the floor to collect the milk. Some floor model separators had two swinging platforms. Smaller versions of separators were called table-top models, for small dairies with only a few cows or goats.

Gustaf de Laval's construction made it possible to start the largest separator factory in the world, Alfa Laval AB. The milk separator represented a significant industrial breakthrough in Sweden. Within the first decade of the 1900s, there were over twenty separator manufacturers in Stockholm. Separators in modified form are also used on ships to purify oil, which may have been their original use, because in its original form de Laval proposed the separator for use in his steam turbine. De Laval's turbine used mechanically lubricated journal bearings which weren't insulated from the inside of the turbine. When the steam condensed into water it contaminated the oil. To purify the oil a centrifugal separator was used, which was later adapted to the dairy industry.

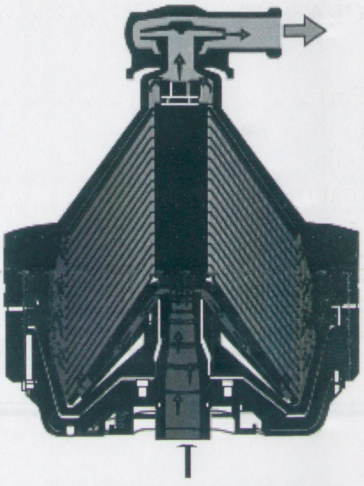


**Fig. 11.1. In a centrifugal clarifier bowl, the milk enters the disc stack at the periphery and flows inwards through the channels.**

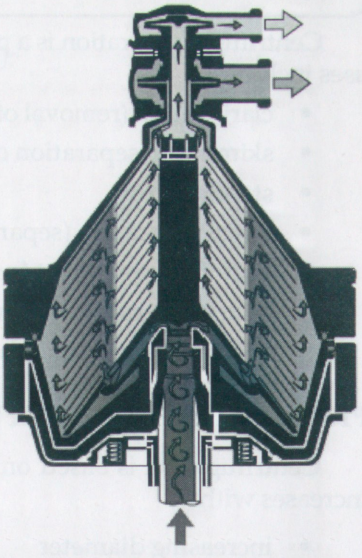
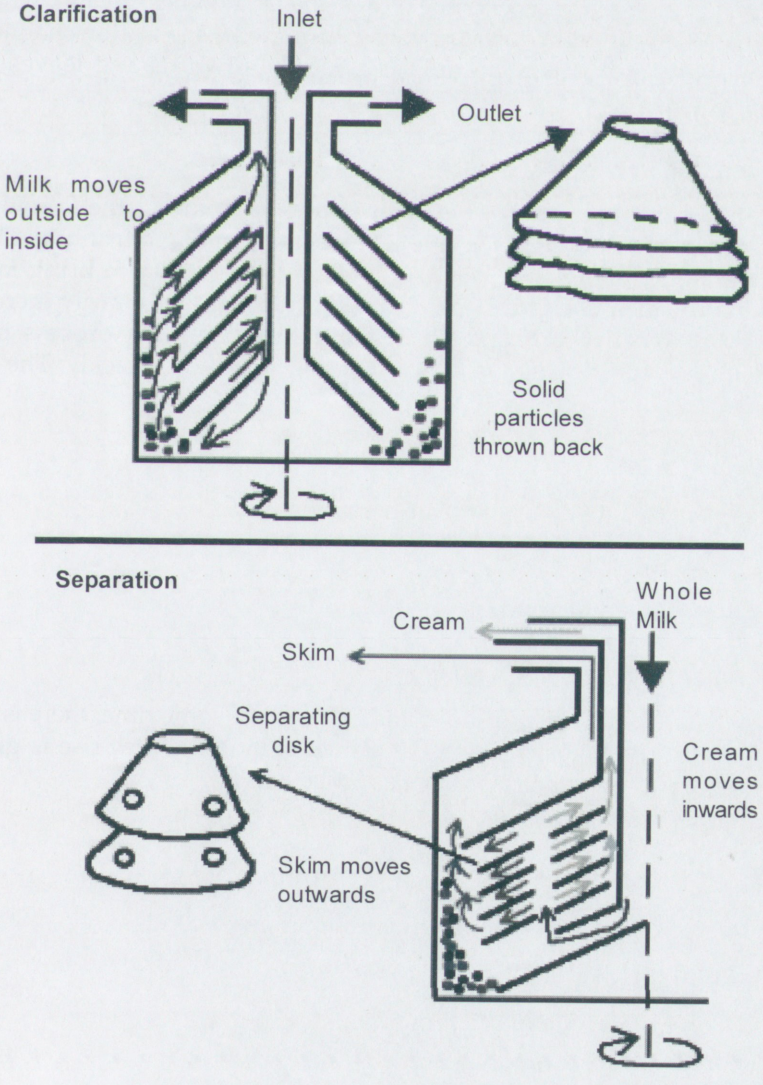


**Fig. 11.2. In a centrifugal separator bowl, the milk enters the disc stack through the distribution holes.**

## CLARIFICATION

Separation and clarification can be done at the same time in one centrifuge. Particles, which are more dense than the continuous milk phase, are thrown back to the perimeter. The solids that collect in the centrifuge consist of dirt, epithelial cells, leucocytes, corpuscles, bacteria sediment and sludge. The amount of solids that collect will vary, however, it must be removed from the centrifuge.

More modern centrifuges are self-cleaning allowing a continuous separation/ clarification process. This type of centrifuge consists of a specially constructed bowl with peripheral discharge slots. These slots are kept closed under pressure. With a momentary release of pressure, for about 0.15 s, the contents of sediment space



are evacuated. This can mean anywhere from 8 to 25 L are ejected at intervals of 60 min. For one dairy, self-cleaning translated to a loss of 50 L/hr of milk.

The following image is a schematic of both a clarifier and a separator.

## SEPARATION

Centrifuges can be used to separate the cream from the skim milk. The centrifuge consists of up to 120 discs stacked together at a 45 to 60 degree angle and separated by a 0.4 to 2.0 mm gap or separation channel. Milk is introduced at the outer edge of the disc stack. The stack of discs has vertically aligned distribution holes into which the milk is introduced.

Under the influence of centrifugal force the fat globules (cream), which are less dense than the skim milk, move inwards through the separation channels toward the axis of rotation. The skim milk will move outwards and leaves through a separate outlet.

### Creaming Separation

If milk is left to stand for between 30 and 40 minutes, the fat will naturally separate, according to the International Livestock Research Institute. This process is known as creaming and uses the principles of gravity to bring fat globules together and cause them to rise. Manufacturers can temporarily increase milk's temperature to between 20 and 50° Celsius to help speed the process, because the higher temperatures cause the fat to come together more quickly. The fat portion can then be skimmed off the top.

#### *Separation Processes*

There are two methods of cream separation viz.,

- Gravity Method
- Centrifugal Method

#### *Cream separation by gravity method*

When milk is allowed to stand undisturbed for some time, there is a tendency of fat to rise. The velocity or rate at which the fat globules rise is given by the following equation, which is known as Stoke's Law:

$$V = (2/9) * Gr2 * (ds - df) / N$$

where,

V = rate of rise of fat globule in centimeter per seconds

r = radius of fat globule

G = Force of gravity (981 dynes)

N = Viscosity of skim milk

ds = density of skim milk

df = density of fat globule

### Gravity Separation

If the idea of waiting 30 to 40 minutes to separate milk fat sounds too long, a faster option exists: centrifugal separation. This milk separation method requires a centrifuge -- a machine that spins at a very fast rate. When milk is placed into a centrifuge, the force of gravity is about 5,000 to 10,000 times greater than the Earth's gravitational pull, according to the International Livestock Research Institute. Milk centrifuge separators have different channels where the fat and skim milk portions are separated and collected. Another benefit of the centrifuge method is that particles such as bacteria also can be separated.

### Nutrient Composition

The results of milk fat separation for skim milk production can reduce fat and calories without removing calcium, a mineral vital for healthy bones. For example, whole milk contains 3.34 grams of fat, while skim milk has 0.18 grams of fat, according to Encyclopaedia Britannica. Milk packagers can set the desired fat content when using the centrifuge process. Skim milk must be less than 0.5 percent milk fat, but most skim milk contains less than that. Skim milk also has about 26 fewer calories than whole milk.

## DAIRY CENTRIFUGES AND SEPARATORS

The main purpose of this process is to remove impurities from milk. If process conditions are favorable, there will also be an efficient reduction in the amount of leucocytes and bacteria.

### Milk Clarification

The main purpose of this process is to remove impurities from milk. If process conditions are favorable, there will also be an efficient reduction in the amount of leucocytes and bacteria. All centrifuges perform as clarifiers, but normally only machines with a high hydraulic capacity are used for this application. The clarifier can operate either with cold milk below 46°F (8°C) or hot milk, 122 – 140°F (50-60°C).

### Hot Milk Separation

The largest application for centrifuges in dairies is hot milk separation. The objective is to separate the globular milk fat from the serum, the skim milk. The separation process is normally incorporated into the pasteurization line and also combined with a direct, in line fat standardization system for both milk and cream. Separation normally takes place at 122-140°F (50-60°C). The fat content of the cream discharged from the separator can be regulated to a level between 20 and 70%.

### Whey Clarification

To maintain optimum fate separation conditions during a long period of time, the fines should be removed from the whey before separation. The most efficient way to reduce the fines is to install a centrifugal hermetic clarifier ahead of the whey separator. The interval between sediment discharges from the fat separator can then be as long as that for the milk. Flow rate, fines content and skimming efficiency are important parameters for the choice of clarifier.

### Cold Milk Separation

Cold milk separation at 39-41°F (4-5°C) is practiced when heating the milk is undesirable. The viscosity and characteristics of the cream allow temperatures preclude any other form except hermetic separation. Owing to less favorable processing conditions, the skimming efficiency of a cold milk separator is lower than that of the corresponding hot milk type. The fat content in the skim milk can vary between 0.1 and 0.2% and the maximum fat content in the cream at 39°F (4°C) is normally 45%.

### Whey Separation

Whey contains small amounts of fat which vary between 0.15 and 0.40% and have to be removed before further processing. The whey characteristics differ from those of milk in such a manner that the conditions of whey separation are more favorable. If the whey is pre-clarified, the skimming efficiency can be equal to that achieved in milk separation. Hermetic whey separators can also produce cream with a fat content higher than 40% and at temperatures below 95°F (35°C).

## STANDARDIZATION

The streams of skim and cream after separation must be recombined to a specified fat content. This can be done by adjusting the throttling valve of the cream outlet; if the valve is completely closed, all milk will be discharged through the skim milk outlet. As the valve is progressively opened, larger amounts of cream with diminishing fat contents are discharged from the cream outlet. With direct standardization the cream and skim are automatically remixed at the separator to provide the desired fat content (see the diagram to the right to explain the automatic standardization process) .

## STANDARDIZATION PROBLEMS

1. If a dairy has 160 kg of 40% cream and wishes to standardize it to 32% cream, how much skimmilk must be added?

### Mass Balance Approach

let x = kg skimmilk

y = kg of 32% cream

Mass Balance (mass into the process = mass out of the process) $160 + x = y$

Component Balance for Fat (fat into the process = fat out of the process)

$.40\ (160) = .32\ (y)$ which says 40% of 160 kg comes in and 32% of y goes out

$.40\ (160) = .32(160 + x)$ substituting our equation for y

$.32x = 64 - 51.2$

$x = 40$ kg skimmilk

2. How much cream testing 35% fat must be added to 500 kg of milk testing 4% fat to obtain cream testing 10% fat?

**Mass Balance Approach**

let x = kg 35% cream

y = kg of 10% cream

Mass Balance $x + 500 = y$ mass in = mass out

**Component Balance for Fat**

$x\ (.35) + 500\ (.04) = y\ (.10)$ again, fat in = fat out

$x\ (.35) + 20 = x(.10) + 50$

$.25\ x = 30$

$x = 120$ kg of 35% cream

## PEARSON SQUARE APPROACH TO ABOVE PROBLEM

A shortcut for 2-component mass balances

Place desired percentage in the centre of a rectangle

Place percentage composition of two available streams in left corners of rectangle

Cross subtract lower from higher numbers for right corners of triangle (higher number - lower number)

Use right corners as ratios of two streams

# 12

# Homogenization

**Topics**

*Introduction • Objectives of Homogenization • History • Definition • Merits of Homogenization • Demerits • Principles of Homogenization • Homogenizer • Process • Types of Homogenizer • Details of Homogenization • Theories of Homogenization • Factors Affecting Homogenization • Stage of Homogenization • Efficiency of Homogenization • Operation of the Homogenizer • Maintenance of Homogenizers • Advantages and Disadvantages of Homogenized Milk • Viscolised Milk • Viscolised Milk • High Pressure Homogenization Technology • Checking the Efficiency of Homogenization • Colloids Applications • Zeta Potential • Creaming*

## INTRODUCTION

Homogenization implies mechanical treatment to break fat globules into smaller size of 2 μm or less and uniformly disperse them in milk. Homogenization in the dairy industry is used principally to prevent or delay the formation of a cream layer in full cream milk by reducing the diameter of the fat globules. After homogenization, size of fat globules becomes less than 2μm. The average size of milk fat globule in milk is 2-12 μm. The number of fat globules is 3-4 billion in a milliliter of milk.

In the past, pasteurized milk usually was not homogenized, although the flavor of the milk becomes fuller by homogenization. A certain amount of cream was permitted to form to show the consumer clearly the full cream character of milk. Sterilized milk, evaporated or condensed milk and cream are generally homogenized.

Homogenization or homogenisation is any of several processes used to make a mixture of two mutually non-soluble liquids the same throughout. This is achieved by turning one of the liquids into a state consisting of extremely small particles distributed uniformly throughout the other liquid. A typical example is

the homogenization of milk, where the milk fat globules are reduced in size and dispersed uniformly through the rest of the milk.

Homogenization is the process of breaking down the fat globules in milk so that they stay integrated rather than separating as cream. When you see milk or yogurt labeled as "cream on top," it means that milk has not been homogenized. Homogenization is a purely physical process; nothing is added to the milk. Some scientists argue that milk is more digestible when homogenized and others argue that the milk fat globules are too rapidly absorbed when homogenized.

## OBJECTIVES OF HOMOGENIZATION

Homogenization results in milk or milk products in which the fat globules are reduced in size tosuch an extent that no visible cream separation occurs in the milk. This process basically results in milk of uniform composition or consistency and palatability without removing or adding any constituents. Homogenization increases the whiteness of milk, because the greater number of fat globules scatters light more effectively. Homogenized milk is less susceptible to oxidized flavor, and the softer curd formed by it when entering the stomach aids digestion.

## HISTORY

Homogenization was introduced by Buttenberg (1903) of Germany, and later developed in 1904 in London and the milk produced by this process was known as 'Gaulin milk'.

## DEFINITION

Homogenization can be defined as the process in which fat globules in milk are broken down to a size small enough to prevent the formation of a cream layer. Homogenizer is a machine, which disintegrates the fat globules of milk.

According to the United States Public Health Services (USPHS), 'homogenized milk is one that has been treated in such a manner as to ensure the break-up of the globules to such an extent that after 48 hours of quiescent storage, no visible cream separation occurs in milk and the fat percentage of the milk in the upper 10% portion, i.e., in the top 100 ml of milk in a quart bottle or of proportionate volumes in containers of other sizes, does not differ by > 10% of itself from the fat percentage of the remaining milk, as determined after thorough mixing'.

The number of fat globules in homogenized milk is about 10,000 times greater than those in unhomogenized milk. The size of fat globule is reduced to < 1 micron, while normal fat globule size averages 2-12μm in milk. The number of fat globules will be increased, but total volume of fat globules will remain almost same. The surface area of newly formed smaller fast globules is increased by 4-6 folds.

The majority of milk sold in the US is homogenized. The process is so prevalent and expected by consumers, that the federal classification of milk is "homogenized." The law does not require us to label our milk "homogenized". Except for Organic

Valley Grassmilk, Grassmilk Yogurt and Heavy Whipping Cream, all other Organic Valley fluid milk products are homogenized.

## MERITS OF HOMOGENIZATION

- No formation of cream layer/plug
- Fat will not churn
- Thick body and rich appearance
- Produces soft curd, easily digestible
- Less susceptibility to oxidation

## DEMERITS

- Increased cost of production
- Fat from returned homogenized milk is difficult to salvage.
- Sediment is greater
- May produce rancidity if temperature is not kept adequately high.

## PRINCIPLES OF HOMOGENIZATION

In raw milk, the diameter of the fat particles varies from 2 to 12μ, while a diameter of about 2μ or less is required to keep the fat from rising in stored condition. The milk is forced at high pressure through a narrow slit (spring loaded valve), which is only slightly larger than the diameter of the globules. The velocity of milk in the narrow slit can be 100 - 200 m/s. This can cause high shearing stresses, cavitations and micro-turbulence. The globules becomes deformed, wavy and then breakup.

## HOMOGENIZER

The homogenizer consists of a high pressure, reciprocating pump driven by a powerful motor, and a back pressure device i.e. homogenizer head. It is equipped with a set of valves and valve pressure screws that enable the exposure of liquid products to very high pressures. To withstand the high pressure and velocity and to prevent the wearing of the head, a special metal alloy 'stellite', which is noted for its hardness, is used for making the homogenizer valve. The power source is an electric motor built into the unit. The motor drives the crank and piston assembly either by a pulley or by a set of gears, both of which greatly reduce rpm to provide a suitable speed for the pistons. The gears, cranks, and drive shafts run in an oil bath. The pistons (commonly three sometimes five or seven in number) are usually straight rods giving a small displacement. The pistons extend from the crank shaft in the crank housing, into the pressure chamber in the homogenizer head. Each piston passes through a packing gland especially designed both to prevent product leakage, despite high operating pressures and to facilitate sanitation. The parts of homogenizer head are precisely ground and made to fit together in correct position

in order to avoid any leak. The wear and tear of homogenizer head is also frequently checked because if there is any shell gap the fat globules may escape through it and lower the efficiency of homogenization.

## PROCESS

The high velocity of milk confers high kinetic energy. The energy is dissipated into heat and since the passage time through the slit is small (< 0.1 m/s), the average energy density is very high. Such high energy densities lead to very intense turbulence (Reynolds number > 40,000). The pressure fluctuations are not desirable.

When the flow velocity in the valve slit is at its maximum, local pressure is less than zero. A negative pressure may cause cavitations (i.e. the formation and sudden collapse of vapour bubbles). The collapsing process creates huge shock waves, which may disrupt particles. The degree to which this happens in homogenizers varies. In most cases, globule disruption primarily is caused by turbulent eddies. The small globules do not rise to the top of milk but remains suspended in the milk or rise very slowly. Immediately after the globules are broken down, they show a tendency to cluster and rise to the top of the milk. Two-stage homogenization prevents this. The second stage breaks up any clusters, thus ensuring better dispersion on the fat throughout the milk.

## TYPES OF HOMOGENIZER

### High Pressure Homogenizer

This type of homogenizer consists of single acting triplex pump with each cylinder having suction and discharges valves. The discharge valve of each pump empties into a common discharge pipe, in which a special valve is placed. The pressure ranges between 35-350 bar (500-5000 psi) depending on the type of construction of the valve.

### Low pressure-rotary type homogenizers

Usual operating pressures are below 35 bar (500 psi). The construction is so designed that milk is subjected to grinding and shearing action.

### Sonic vibrators

The milk is subjected to high frequency vibration in a device called sonic vibrator or oscillator. The machine consists of a flat disc actuated by an electric magnet located over an anvil containing a hole, through which milk enters. The milk passes through the space between the disk and an anvil, and the vibrating action of the disk against the film of milk hammers the fluid at high frequency.

## DETAILS OF HOMOGENIZATION

Homogenizers can be single-stage, double-stage or even multi-stage type. Single-stage homogenizers are equipped with only one homogenizing valve. A

homogenizing valve usually consists of a valve, valve seat and an impact ring. These are held within a valve body. On the other hand, double-stage homogenizers are equipped with an additional homogenizing valve.

Commercial milk homogenizers are high pressure reciprocating pumps, each having a sanitary head upon which is mounted a homogenizing valve or valves. Homogenizers have 3, 5 or 7 pistons driven by an eccentric shaft through connecting rods and cross heads. The pump head contains a section manifold with passages connecting to the individual cylinders. Each cylinder has suction and a discharge valves, either poppet or ball type. The valves are spring loaded in some models.

The homogenizing valve accomplishes its intended function by restructuring the product flow area which results in the pressure commonly known as "the homogenizing pressure." The degree of homogenizing effect can be controlled by regulating this pressure, or corrected by the restriction to flow. A pressure gauge is used to indicate the pressure. Generally, double-stage homogenizers are used and pressure of 140 bar (2000 psi) and 35 bar (500 psi) in the first and second stages respectively are maintained.

## THEORIES OF HOMOGENIZATION

The principle underlying the process of homogenization is to subject the fat globule to enough severe conditions, which disrupts it into smaller globules. The newly formed fat globules are maintained in dispersion for sufficient time to allow milk fat globule membrane (MFGM) to be formed at the fat-serum interface. The following theories have been proposed to be responsible for the entire phenomenon.

**Shearing or Grinding**: As milk is passed at high pressure (velocity ~ 200-300 m $s^{-1}$) through the homogenizer valve (~ 100 mm gap), fat globules undergo shearing action. The shear between fat globules and the surface of the homogenizer wall coupled with wire drawing effect results in elongation of the fat globules which progressively becomes unstable. These phenomenon result in subdivision of the fat globules. Furthermore, the difference in velocity of the faster moving serum phase at the centre of the liquid stream as compared to the liquid near the edge of the stream causes the fat globules to grind against each other. The turbulence created by the difference in velocity and eddy currents of the liquid add to the shear effects and thus enhance the process of disruption of the fat globules.

**Exploding**: This theory suggests that during homogenization, there is build up of tremendous pressure. When this pressure is suddenly released, the internal pressures within the fat globules pull the globule apart with exploding effect. This results in disintegration or subdivision of fat globules into smaller globules.

**Splashing/Shattering**: As the high homogenizing pressure is attained in the homogenizer, the homogenizing valve releases the highly compressed milk at very high velocity. The liquid suddenly strikes a retaining wall/ perpendicular surface.This causes splashing or shattering effect on the fat globules resulting in break down of globules into smaller sizes.

**Acceleration and Deceleration**: This theory relates sudden change in velocity of milk as it passes through homogenizer to the homogenization effect. When milk enters the homogenizer valve, velocity of milk changes from almost static to very high velocity. As it comes out of the valve, there is sudden deceleration at a rate at which it was accelerated. This sudden change in velocity results in shattering effect leading to division of fat globules.

**Cavitation**: It is postulated that as the milk passes through the homogenization valve, the initial homogenization pressure decreases sharply due to sudden increase in the velocity of milk. Depending on the back pressure that exists outside the homogenizer valve, the pressure can drop to as low as the saturated vapour pressure of liquid. This leads to formation of vapour bubbles due to cavitation. Cavitation generates shock waves, which could be in excess of 1600 kg/cm2 in intensity. Due to overlapping of these shock waves, disintegration of the fat globules may occur.

### Shattering and impact

The fat globules are shattered by impinging them on the retaining value or impact ring.

### Explosion

With the release of high pressure, the fat globules explode.

### Shearing and grinding

The fat globules are subjected to unequal forces as milk flows at different velocities in a fluid stream. As a result of the shearing action between globules, it deforms the fat globules beyond its yield point.

### Cavitation

The vapor bubbles or cavities are formed within the medium and as they collapse subsequently, energy in the form of shock waves is released breaking up of fat globules.

### Attenuation

The fat globule disruption is attributed to violent changes in the velocities of milk as it passes through the unit rendering a fragmentation slight effect on the mean particle size.

## FACTORS AFFECTING HOMOGENIZATION

### Homogenization Pressure

In a single-stage homogenizer, usually 140-175 bar (2000-2500 psi) pressure is sufficient for milk having up to 6.0% fat. Higher pressure may increase the tendency of the milk to curdle when cooked, due to the increased destabilizing effect on milk

proteins. For milk on milk products with > 6 % milk fat, two-stage homogenization is needed to prevent fat clustering pressure of 140 bar (2000 psi) and 35 bar (500 psi) in the first and second stage respectively are applied in two-stage homogenization.

## STAGE OF HOMOGENIZATION

For milk with more than 6% fat, two stage homogenization is better. If the broken up fat globules have a tendency to agglomerate after the first homogenizing stage (150-200 bar), they can be re-dispersed employing 20-40 bar in the second stage.

### Temperature of homogenization

The enzyme lipase should be inactivated prior to homogenization by pre-heating milk to a temperature of 60°C or above. At this temperature fat is already in molten condition. In routine practice, the milk is heated to 65-70°C (149-158°F) for homogenization.

### Fat content

Homogenization becomes less effective with increasing fat content. When the fat content is high, raising the temperature improves homogenization efficiency. When the fat content is high, the newly created total fat globule surface is so large that the material (plasma protein) becomes insufficient to form new membranes on fat.

## EFFICIENCY OF HOMOGENIZATION

### Degree of Homogenization

The degree of homogenization is defined as the ratio of the volume of fat with fat globules diameter of < 0.7 μ to the total fat content of milk or cream. The value of 0.7 μ was based on the fact that fat globules with smaller diameter do not form clumps because they break up again due to Brownian movements.

### Farrall Index

It is a widely accepted microscopic method for determining the homogenization efficiency. This index may be defined as the number of fat globules having < 2 μ in diameter. The efficiency of homogenization is apparently based on the number of fat globules larger than 2 μ (if any), as measured under specified conditions.

## OPERATION OF THE HOMOGENIZER

**Before starting the homogenizer, the following points should be checked**

- Water is turned on to lubricate and cool the pistons.
- Pressure controls are checked to see that they are in idling position.

- Check availability of the product to the machine.
- Check during starting of the machine whether the oil pressure records > 1 $kg/cm^2$; otherwise the starter will trip.

**Starting of homogenizer machine**

- The motor is started.
- The homogenizer is run on water for about 5 min., then it is stopped and the water drained off by slackening the inlet union, which is tightened subsequently.
- The machine is checked for any leaks.
- The homogenizer is provided with milk supply by adjusting the 3-way valve accordingly.
- As soon as the machine starts pumping at full capacity, the pressure adjusting handle of the second stage valve is adjusted to the desired pressure, followed by adjusting the first stage pressure. This can be observed in the single pressure gauge provided.
- The product discharge from the machine is diverted back till the desired homogenizing pressure is obtained.
- When normal operation is attained, the bypass valve is turned to direct the product flow into the processing system.

Note: In some homogenizers, there might be provision to release the air from the machine.

**Closing down the Homogenizing Operation**

- At the end of the run, the product flow is diverted.
- As soon as the product to be homogenized is emptying out, the water is filled into the hopper for flushing.
- The first stage valve pressure and subsequently the second stage valve pressure are released in that sequence.
- The homogenizer can now be cleaned by switching over to the cleaning sequence.
- After the cleaning is over, the homogenizer is stopped.

**Precautionary Measures**

- The homogenizer should never be run dry. Adequate feed must be maintained at all times to prevent starving of the machine.
- Before the homogenizer is put to use again, the strainer from the inlet chamber should be removed, cleaned and refitted.

- The homogenizer should always be started with both homogenizing valve handles in released position.
- The pressure should be built up gradually.
- While the homogenizer is running, the 3-way valve on the product delivery line should never be closed; otherwise it may result in severe damage to the machine.

## MAINTENANCE OF HOMOGENIZERS

- The oil level should be inspected daily for maintaining desired level.
- Periodic check-up of the lubricating system must be done. Change the lubricating oil before 500 operating hours or 6 months or sooner, when it becomes emulsified.
- Inspect oil seals for preventing any chance of mixing of water with oil.
- Leakage can be prevented by tightening the nuts or couplings, inserting or replacing gaskets or replacing the pistons.
- The homogenizer gauges should be checked periodically for accuracy against a standard gauge.
- The homogenizer valves must be regularly inspected and if required, they should be lapped with a fine abrasive for perfect sealing.
- While dismantling the valves, care must be taken to avoid scratches. They must be kept on a rubber mat.

## ADVANTAGES AND DISADVANTAGES OF HOMOGENIZED MILK

### Advantages

- Prevents removal of fat/cream from milk
- Homogenized milk results in softer curd and therefore easily digested by infants
- Churning of fat does not occur during bulk transportation
- Fat is uniformly distributed and therefore gives uniform consistency
- Homogenized milk is comparatively resistant to development of oxidized flavour defect

### Disadvantages

- Homogenization offers possibility of incorporation of foreign fat into milk
- Homogenized milk is prone to development of 'sunlight' or 'activated' flavour defect

- Homogenized milk if returned unsold from the market is difficult to salvage as centrifugal separation of fat is not possible

## VISCOLISED MILK

Viscolised milk refers to a product, which has unusually deep cream/fat layer resulting from admixing of homogenized cream, skim milk and/or whole milk. The homogenized fat forms very loose clumps with the unhomogenized fat globules and rise to the surface giving an appearance of deep cream layer. The unfair traders who first separate the cream, homogenize it and then remix with the skim milk sometimes practice it. This gives the remixed milk a very rich and creamy surface appearance and thus deceives the consumers.

## DESIGN AND OPERATION OF HOMOGENIZERS

There are several types of homogenizer valves and therefore designs of homogenizers vary depending on the manufacturers. However, many homogenizers used in the dairy industry have been developed based on the principles introduced by Gaulin. Homogenizers essentially consist of two components - a piston pump to generate high pressure and a homogenizing valve. The homogenizer pump is generally a positive displacement pump with at least three and sometimes five or seven pistons, which operate consecutively to generate steady pressure. Single piston pumps generate pulsating output with fluctuating pressure thereby resulting in poor homogenization. The pump block is generally made of stainless steel but the piston seal rings are of a soft composite material.

Homogenizer valves, used for milk may be either a 'poppet type' or 'ball type'. A poppet design has relatively large contact surfaces and provides close fitting seal. If maintained properly, 'poppet' valves give better performance with low viscosity liquids like milk. 'Ball' valves can exert greater pressure on the much small seal area and are therefore, suitable for high viscosity liquids or suspensions with smaller particles.

Milk from high pressure manifold enters into the centre of the valve seat. The internal diameter of the valve seat is smaller than the manifold. As it passes into the narrow gap between the fixed and the adjustable faces of this valve, milk velocity gets accelerated. The gap is maintained against the feed pressure by a counter force exerted by an adjustable heavy duty spring. Shear effects result from the high velocity gradients between the liquid and the surface of the homogenizing valve. Turbulence also results from the high velocity of the liquid in the valve,causing eddy currents within the flow. Liquid which passes across the valve at about 200-300 m $s^{-1}$ suddenly drops in pressure to below saturation vapour pressure.

This permits microscopic bubbles to form for a few microseconds before collapsing.The high velocity jet of milk then impinges on a perpendicular impact ring. These effects contribute to the disruption of the fat globules. Homogenizer valves are made of very tough corrosion resisting alloys such as stellite. Better

resistance to corrosion can be achieved by using tungsten carbide and ceramic valves, which are used by many manufacturers in modern homogenizers.

As the fat globules are subdivided into smaller globules, there is increased surface area of the newly homogenized fat globules. The original milk fat globule membrane (MFGM) material is not sufficient to cover this. Proteins, particularly casein micelles migrate from serum phase to form new membrane material with the existing MFGM. This may result in sharing of the casein micelles and therefore some aggregation of fat globules could take place thus defeating the purpose of homogenization. A second stage homogenization therefore, becomes essential at reduced pressure (almost 20% of the first stage pressure (175 kg/cm$^2$) or upto 35 kg/cm$^2$). This enables aggregated fat globules to be disrupted for formation of stable emulsion of finely dispersed fat globules.

## HIGH PRESSURE HOMOGENIZATION TECHNOLOGY

With significant improvement in understanding of machine design, material strength and fluid mechanical knowledge, homogenizers with higher pressure capabilities have been developed. Such high pressure homogenizers could be operating based on two principles: (i) Conventional valve type homogenizer operating at a far higher pressure; (ii) Micro-fluidization based on the principle of collisions between high speed liquid jets.

### Conventional Valve Type High Pressure Homogenizers (HPH)

Such high pressure homogenizers work on the principle of conventional ball-and-seat type homogenizer valve. Highly abrasive resistant and durable components of high pressure homogenizers are made from best quality stainless steel, high alloy compositions and new ceramic materials. This allows these systems to operate at pressures upto 2550 kg/cm$^2$ or more. Besides the regular use in emulsion formation, these high pressure homogenizers find applications in inactivation of enzymes and bacteriophages and also in destruction of micro-organisms. Destruction of bacterial cells by HPH is due to several physical phenomenon viz., pressure drop, cavitation, shearing,turbulance and collision. These systems can be therefore, used as a combined process for pasteurization and homogenization.

### Microfluidization Technology

The microfluidizer operates under a different principle as in this case the liquid being processed is divided into micro streams that are so projected that these collide with each other. The essential design features of micro-fluidizers include a double acting intensifier pump and an interaction chamber. The intensifier pump, which is either air-driven or electric-hydraulic driven, forces milk/product at high pressure through the interaction chamber. The interaction chamber has fixed-geometry micro-channels, which divides the product into streams.These streams, which accelerate to a very high velocity, are made to collide against each other. Shear and impact that occur lead to homogenization effect. These micro-fluidizers

are capable of generating pressures upto 2800kg/cm$^2$. As in case of conventional valve homogenizers, micro-fluidizers too bring about changes in the fat and protein fractions of milk thereby altering some physico-chemical properties of milk.

## VACUUM HOMOGENIZATION

This innovation in the homogenization technology is based on the discrete pulse energy input theory. In vacuum homogenization, energy is introduced discretely into the liquid (milk) through powerful short-time impulses. The homogenizer unit, placed in the HTST pasteurization line has two condensing chambers. The chilled milk is first pumped to condenser-I where it is heated to 20°C and subsequently to 30°C in condenser-II. Milk then enters regeneration section of the pasteurizer where it is heated to 65°C. It is then delivered to 1st stage vacuum (homogenization) chamber through a special nozzle. As a result of flashing effect, bubbles are formed in the milk as it falls in the vacuum chamber maintained at 0.15 to 0.2 kg/cm$^2$. Due to the pressure changes taking place, the bubbles either show high frequency pulsation and release energy or collapse producing shockwave effect in the product. The bubbles therefore burst into smaller units and the fat globules are divided into smaller globules. As the milk enters 2nd stage homogenization chamber, further breakdown of fat globules takes place. The outgoing milk passes through regeneration section followed by chilling section to finally attain 5°C temperature. Besides the generally accepted homogenization effect, the other major advantages offered by this vacuum homogenizer are deodourization, reduced acidity and partial suppression of microbial activity. These systems also claim to be economical as it consumes almost 2.5 times less power than the valve type high pressure homogenizer.

## CHECKING THE EFFICIENCY OF HOMOGENIZATION

The method recommended by the United States Public Health Service has been the most widely used for checking the homogenization efficiency. It is performed by subjecting a specified volume (one quart) of milk to quiescent storage for 48 hours and then testing the fat in upper 100 ml and the remainder of milk. For properly homogenized milk, the percent difference in both the top 100 ml and the remainder milk should not be more than 10 per cent.

Homogenization is applied for any of the following reasons:

1. **Counteracting creaming:** To achieve this, the size of the fat globules should be greatly reduced. A cream layer in the product may be a nuisance for the user, especially if the package is nontransparent.

2. **Improving stability toward partial coalescence**: The increased stability of homogenized fat globules is caused by the reduced diameter and by the acquired surface layer of the fat globules. Moreover, partial coalescence especially occurs in a cream layer, and such a layer forms much more slowly in homogenized products.

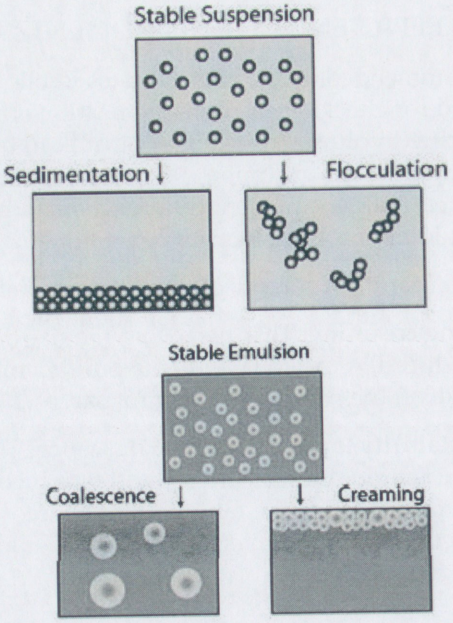
3. **Creating desirable rheological properties**: Formation of homogenization clusters can greatly increase the viscosity of a productsuch as cream. Homogenized and subsequently soured milk (e.g., yogurt) has a higher viscosity than unhomogenized milk. This is because the fat globules that are now partly covered with casein micelles in the aggregation of the casein micelles.
4. Recombining milk products: At one stage of the process, butter oil must be emulsified in a liquid suchas reconstituted skim milk. A homogenizer, however, is not an emulsifying machine. Therefore, the mixture should first be pre-emulsified, for example, by vigorous stirring; the formed coarse emulsion is subsequently homogenized.

## COLLOIDS APPLICATIONS

A colloid is typically a two phase system consisting of a continuous phase (the dispersion medium) and dispersed phase (the particles or emulsion droplets). The particle size of the dispersed phase typically ranges from 1 nanometer to 1 micrometer. Examples of colloidal dispersions include solid/liquid (suspensions), liquid/liquid (emulsions), and gas/liquid (foams).

### Particle Interactions

As particle size decreases, surface area increases as a function of total volume. In the colloidal size range there is much interest in particle-particle interactions. Most colloidal commercial products are designed to remain in a stable condition for a defined shelf life. Milk is an example where homogenization is used to reduce



droplet size to delay the onset of phase separation (i.e., creaming with the fat rising to the surface). Commercial suspensions may be formulated to keep particles in suspension without sedimenting to the bottom. Examples of phase separation mechanisms are shown below.

### *Colloidal Stabilization*

Stabilization serves to protect colloids from aggregation and/or phase separation. The two main mechanisms for colloid stabilization involve steric and electrostatic modifications. Electrostatic stabilization is based on the mutual repulsion of like electrical charges. By altering the surface chemistry to induce a charge on the surface of particles it is possible to enhance the stability of the colloidal dispersion.

## ZETA POTENTIAL

Zeta potential refers to the potential in the interfacial double layer (DL) at the location of the slipping plane versus a point in the bulk fluid away from the interface. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. A classic example of colloid chemistry is to measure zeta potential vs. pH to determine the conditions where the zeta potential reaches zero, known as the isoelectric point.

## EFFECT OF HOMOGENIZATION

| | No Homogenization | 15 MPa (2500 psig) |
|---|---|---|
| Av. diam. (μ m) | 3.3 | 0.4 |
| Max. diam. (μ m) | 10 | 2 |
| Surf. area ($m^2$/ml of milk) | 0.08 | 0.75 |
| Number of globules (μ $m^{-3}$) | 0.02 | 12 |

### Surface Layers

The milk fat globule has a native membrane, picked up at the time of secretion, made of amphiphilic molecules with both hydrophilic and hydrophobic sections. This membrane lowers the interfacial tension resulting in a more stable emulsion. During homogenization, there is a tremendous increase in surface area and the native milk fat globule membrane (MFGM) is lost. However, there are many amphiphilic molecules present from the milk plasma that readily adsorb: casein micelles (partly spread) and whey proteins. The interfacial tension of raw milk is 1-2 mN/m, immediately after homogenization it is unstable at 15 mN/m, and shortly becomes stable (3-4 mN/m) as a result of the adsorption of protein. The transport of proteins is not by diffusion but mainly by convection. Rapid coverage is achieved in less than 10 sec but is subject to some rearrangement.

Surface excess is a measure of how much protein is adsorbed; for example 10 $mg/m^2$ translates to a thickness of adsorbed layer of approximately 15 nm.

## CREAMING

Cream is produced by the centrifugal separation of milk. During separation, two streams are created, a highly concentrated milk fat stream termed cream and a non-fat stream of skim milk that can be further processed into evaporated or dried milk products. The fat content of the cream resulting directly from the separator typically ranges from 35-45%. Creams of different fat levels can be produced by standardizing (addition of a low fat stream to a higher fat stream to achieve a desired fat level) with skim milk. Higher fat creams can also be produced by further separating the high-fat stream (these creams are usually industrial products).

Once the cream has been standardized to the desired fat level, it is pasteurized or ultra high temperature (UHT) pasteurized to ensure that it is free of pathogenic bacteria. Depending on the cream type, it may also be homogenized.

Creams are oil-in-water emulsions and therefore when it is standardized to various fat levels with skim milk, it must be homogenized to reduce the fat globule size to increase the stability (prevent fat separation). Proteins from the milk migrate to the fat globule membranes to act as emulsifiers. During homogenization, various grades of viscosity in cream products can occur. Usually, lower pressures are used than those used for milk products. High fat whipping cream is not normally homogenized (unless UHT pasteurized), due to the excessive viscosity created and degraded whipping functionality. On the other hand, homogenization of high fat cream is utilized in the production of spoonable whipped cream products used as dessert toppings. Homogenization is conducted for UHT pasteurized whipping cream in order to prevent fat separation which increases with UHT pasteurization. Stabilizers such as mono-glycerides are added to improve whipping ability.

### Composition

Typical compositions of cream varieties are as follows:

**Typical Composition for Creams**

| Nutrient | Half and Half | Table Cream | Whipping Cream |
|---|---|---|---|
| Moisture | 80.50% | 73.8% | 57.70% |
| Protein | 3.00% | 2.70% | 2.00% |
| Fat | 11.50% | 19.30% | 37.00% |
| Carbohydrate | 4.30% | 3.60% | 2.80% |
| Ash | 0.70% | 0.60% | 0.50% |

**Lipid Profile of Creams per 15 mL serving**

| Nutrient | Half and Half | Table Cream | Whipping Cream |
|---|---|---|---|
| Saturated fatty acids | 1.07 g | 1.80 g | 3.43 g |
| Monounsaturated fatty acids | 0.50 g | 0.84 g | 1.59 g |
| Polyunsaturated fatty acids | 0.06 g | 0.11 g | 0.20 g |
| Cholesterol | 6 mg | 10 mg | 20 mg |

**Vitamin and Mineral Content of Creams per 15 mL serving**

| Nutrient | Half and Half | Table Cream | Whipping Cream |
|---|---|---|---|
| Sodium | 6 mg | 6 mg | 6 mg |
| Potassium | 20 mg | 18 mg | 11 mg |
| Calcium | 16 mg | 14 mg | 10 mg |
| Phosphorus | 14 mg | 12 mg | 9 mg |
| Magnesium | 2 mg | 1 mg | 1 mg |
| Zinc | 0.08 mg | 0.04 mg | 0.03 mg |
| Iron | 0.01 mg | 0.01 mg | 0.00 mg |
| Copper | 0.00 mg | 0.00 mg | 0.00 mg |
| Manganese | 0.00 mg | 0.00 mg | 0.00 mg |
| Selenium | 0.27 mcg | 0.09 mcg | 0.07 mcg |
| Vitamin A | 65 IU | 95 IU | 219 IU |
| Thiamin | 0.01 mg | 0.00 mg | 0.00 mg |
| Riboflavin | 0.02 mg | 0.02 mg | 0.02 mg |
| Niacin | 0.01 mg | 0.01 mg | 0.01 mg |
| Vitamin $B_6$ | 0.01 mg | 0.00 mg | 0.00 mg |
| Folate | 0.45 mcg | 0.30 mcg | 0.59 mcg |
| Vitamin $B_{12}$ | 0.05 mcg | 0.03 mcg | 0.03 mcg |
| Pantothenic Acid | 0.04 mg | 0.04 mg | 0.04 mg |
| Vitamin C | 0.14 mg | 0.12 mg | 0.09 mg |
| Vitamin E | 0.02 mg | 0.02 mg | 0.09 mg |

In terms of microbiological quality, cream has basically the same standards as those required for ordinary milk products.

## CHARACTERISTICS

### Appearance and Flavour

Cream should appear white to off-white with smooth consistency and varying degrees of viscosity depending on the fat content (typically increased viscosity as

the fat content increases). Cream should be homogeneous without separation. The flavour and odour of cream should be rich, clean and sweet with no off-flavours.

### Storage

Cream should be stored at refrigeration temperatures (4-7°C) in a closed container. The typical shelf life of pasteurized cream is one to two weeks and a few months for UHT pasteurized cream. However, once the container of UHT pasteurized cream is opened it should be treated as pasteurized cream. As cream ages, its lactic acid content increases and can result in curdling. Freezing destabilizes the oil-in-water emulsion of creams and therefore the fat will separate upon thawing. Separated cream cannot be used for whipping, but can be used in products such as "cream soups" where flavour is the critical factor.

- **Varieties**: There are a whole range of creams based usually on the milk fat content. For industrial purposes, creams can be produced at specific fat levels, however availability is dependent on the volume usage. Provincial legislation governs the retail and foodservice creams in the market. In Ontario the common cream products and their regulated compositions (Milk Act) are:
- **Half-and-Half**: shall contain not less than 10% milk fat and is pasteurized or UHT pasteurized and homogenized. Termed half-and-half as it is a mixture of milk and cream usually in equal parts. Half-and-Half cream may also contain pH adjusting agents and stabilizers.
- **Table Cream**: shall contain not less than 16% milk fat but less than 32% milk fat and may also contain pH adjusting agents and stabilizers. It can be pasteurized or UHT pasteurized and homogenized.
- **Whipping Cream**: shall contain not less than 32% milk fat but less than 40%; may contain pH adjusting agents, stabilizers, skim milk powder, glucose solids, calcium sulphate, xanthan gum and microcrystalline cellulose in regulated amounts. Whipping cream is pasteurized but not usually homogenized unless UHT pasteurized.

Another cream product found in the retail market is light cream which typically contains 5-6% fat in relation to the fat content of half-and-half cream (nutritional information must be on label when making a "light" claim). Light cream is pasteurized or UHT pasteurized and homogenized.

### Various Uses

Cream is a very versatile product and can be used straight from the package or as an ingredient in many food products. One of the most common uses of cream (such as half-and-half cream) is adding it to coffee, tea, hot chocolate and other beverages where a richer flavour and texture and a characteristic colour are desired. Table cream and whipping cream are often used as toppings to fresh fruit, desserts and cereals.

Cream can be found as an ingredient in a wide variety of products. Some common uses of creams are:

- **Soups and sauces** – fresh, dry-mix and canned
- **Sour cream** – pasteurized cream (typically 18% milk fat) that is cultured with lactic acid bacteria or acidified with suitable acidifiers
- Casseroles and other creamy main meals
- **Sweet syrups** – caramels, fudges and fruit syrups
- **Ice Cream** – cream is a key component in ice cream, providing the source of milk fat and functionality
- **Cream fillings** – whipping cream is typically used in decadent cream fillings for desserts
- Cheesecakes
- **Confectionary products** – such as fondant centers and truffles
- **Bakery products** – fresh and dried bakery mixes
- Alcoholic cream liqueurs and beverages
- **Piping cream** – whipping cream is often piped onto cakes and desserts
- Other dairy products such as cheeses and yogurts – added to standardize fat content

**Functional Properties**

Cream and other milk fat products (such as butter) are very different than fat from other animal and vegetable sources due to the presence of an aqueous phase, proteins and phospholipids. Milk fat exists in the form of globules dispersed in an aqueous phase of milk. Surrounding the fat globules is a membrane made up of varying proportions of protein, phospholipids and glycerides. The phospholipids in the membrane are of primary importance and account for much of the emulsifying properties of the fat globule membrane. The absorption of casein, proteins which are within the aqueous phase of cream, onto the milk fat globule surface when the globule membrane is disrupted (e.g. as a result of shear or homogenization), allows for production of variations in viscosity which can impart different functionality in food products. Caseins promote interaction with other molecules such as calcium, creating a new structure within the cream and therefore changing the textural properties.

The principle functional attributes of cream are:

- **Flavour**: Creams unique natural flavour adds richness to many foods and enhances other flavours in food products. Due the narrow melting range of the milk fat in cream, cream provides quick release of flavours and taste sensations.

- **Texture**: Primarily due to the milk fat present in cream and the fat-protein network created during homogenization, cream adds rich, smooth viscosity and mouthfeel to many food products such as soups and sauces.
- **Fat Soluble Ingredient Carrier**: The milk fat in cream acts as a carrier of fat soluble vitamins and aids in the even distribution of other fat soluble ingredients.
- **Emulsification**: Creams natural proteins act as emulsifiers and aid in emulsification, aeration, foaming and overrun to give products such as whipped cream and ice cream a smooth and stable texture.
- **Browning**: Cream contributes to the browning of cooked foods through the Maillard browning reaction of the proteins and lactose found in cream. The Maillard reaction will also contribute to brown flavours in cooked food products containing cream.
- **Whitening Colour**: Cream adds a whitening effect in products such as coffee. The colouring power of cream depends on the suspension of particles within the cream. Large particles in cream such as fat globules and casein reflect and scatter light creating a whitening effect.

# 13

# Milk Fat Globule Structure and Function

**Topics**

*Introduction • Importance of Food Structure in Milk • Milk Fat Globules as Products of Evolution • Milk Fat Globule Composition, Synthesis, and Size Distribution • Health Properties of Milk Lipid Macrostructure • Industrial Applications*

## INTRODUCTION

Food science is moving from foods developed to simply deliver essential nutrients to foods that promote health of the individual consumer. In the rush to develop this new product category, the major emphasis has been on adding single bioactive ingredients to more traditional foods. However, little attention has been paid to the overall molecular and macromolecular structures in food materials and their importance to the health properties of the foods. Therefore, there is a genuine need to gain knowledge of food structure and the association between structure and function. We have used mammalian milks as genetic, molecular and structural models to understand nourishment using the basic underlying principle of Life Sciences, Darwinian evolution. Milk and all of its components emerged through evolution under the relentless selective pressure to be nourishing. Like any other milk ingredient and structures, the assembly of milk fat globules was evolutionary evolved in order to promote the mammalian infants' health.

The traditional objectives of milk processing are to ensure a long shelf life, to produce satisfactory sensory properties, and to eliminate pathogens. Increasingly, processing strategies have been developed to separate milk components into discrete molecule classes (fat, protein, carbohydrate) to be used as ingredients in a broader range of food applications. An important question to ask as these processing methods are developed is what are the milk properties that are lost as a result?

The nutritional value of any commodity can be studied under two perspectives- the delivery of essential components and the presence of non-essential but beneficial structures and activities. The food industry is still challenged by the need to acquire knowledge regarding structures involved in food assembly. This knowledge will eventually be used to develop foods with special characteristics that will deliver maximal health properties to the consumer. From this perspective, milk provides a compelling opportunity to study the nanostructures that self-assemble in milk to maximize mammalian infants' health.

Of special interest are the milk fat globules. In addition the recent identification of small, non-triglyceride particles are of particular interest for their health properties. Therefore the mechanisms controlling the synthesis, assembly and size distribution of the small particles in milk should be studied separately from the large fat delivering particles. Pathways used to facilitate phospholipids discharge such as lamellar bodies or membrane derived phospholipid exosomes should be assessed as possible pathways elaborated by the mammary gland to assemble the milk fat globule macrostructure.

In the USA alone over 30 billion gallons of milk are consumed as liquid milk annually. The past 30 years were characterized by significant advances and novel techniques that equip factories to cope with more than 70 billion gallons of milk produced in the USA every year. One of the ways to facilitate milk processing is homogenization. Throughout the last two and a half decades, homogenization improvement has focused on producing the same fat globule size distribution applying lower operation pressure with standard homogenized valves.

Homogenization has little effect on the composition but important effects on milk structure and moreover on milk bioactivities as was previously shown. As the most complex entity in milk, the milk fat globules are the macrostructure most affected by homogenization.

In this chapter, milk lipid structures are reviewed emphasizing on milk fat globule composition in addition to the health properties related to its consumption. Implications for the manipulation of the milk fat globule by the milk production and processing industry are discussed.

## IMPORTANCE OF FOOD STRUCTURE IN MILK

The traditional nutritional view of food as a delivery system for individual nutrients is evolving due to the augmented understanding that diet affects health in more ways than simply through providing essential nutrients. It is now more apparent that food is a multi-component, multi-phasic ensemble of biomaterials that is dynamically altered during the processes of digestion and absorption. Because food is not consumed as individual nutrients, the structure of food influences how nutrient components are digested and absorbed in particular in the temporal dimension. Food matrices with complex structures generally slow the rate of digestion and absorption, a property which may be advantageous or deleterious depending on the biological context. In the context of vitamin bioavailability in an

individual who is deficient in that vitamin, a rapid and complete absorption may be favorable. However, in the context of glucose bioavailability in a diabetic individual, a slow and incomplete absorption might be preferred. In both cases, food processors have the opportunity to create products that influence the health of individual consumers via the manipulation of food structure.

Nowhere is the importance of food structure more evident than in evolution's recipe for the nutrition of mammalian infants: milk. Structural properties of milk regulate the digestion and absorption of each of the major macronutrients - carbohydrates, proteins, and fat. The remainder of this section will explore each in detail.

Glucose is the metabolic fuel of the brain and a central component of metabolic and biosynthetic pathways. Studies have recently shown that the rate of glucose absorption and delivery to the bloodstream affects subsequent endocrine regulation, energy homeostasis and long term health. Milk contains considerable quantities of glucose molecules; yet none is present as simple glucose. In milk, glucose is present as lactose, a dissacharide that can only be cleaved by the enzyme lactase. This allows the rate of glucose delivery to be directly regulated by the lactase-producing consumer of the milk, a process which inevitably slows the delivery of glucose.

Amino acids are the required building blocks of proteins and a necessary natural supply for all mammals especially growing infants. Amino acids are provided in milk mainly via casein proteins. Generally, the greater the degree of protein structure, the more resistant the protein is to proteases and the more difficult the digestion. Because casein proteins have little secondary or tertiary structure, they are effectively built for rapid, easy digestion. In vitro studies of casein proteins confirm that they are highly susceptible to proteolysis. However, the protein structure of milk has an additional layer of complexity to regulate the speed at which these easily digested proteins are made available to the infant gut. Casein proteins aggregate to form micelles that are soluble in water. In the infant stomach, chymosin cleaves a surface-stabilizing peptide from the kappa-casein molecule and enables a self assembly process of the casein micelles into insoluble curds. The curds are released in turn more slowly to the intestinal lumen. This system suggests that the easily digested casein proteins are delivered to the infant's small intestine in a sustained release manner to slow the speed of digestion while maintaining high digestibility.

The lipid fraction within milk was oversimplified by previous research by referring to this fraction as relatively pure triacylglycerols (TG) and disregarding the unique and complex macrostructures inherent in this unusual biological colloidal system. Actually, this unique macrostructure and its biochemical assembly process distinguish the milk fat globule from both simple plant derived oils and other animal fat systems. While milk carbohydrates and proteins structures were extensively studied and their applications in digestion and absorbance rates were described, such studies for the lipid entities within milk have not been conducted. Lipids are provided in milk via a unique delivery system: milk fat globules. Prior

to the evolution of mammals, insects, reptiles, fish, and amphibians had already elaborated a highly functional system of lipoproteins for the movement of lipids through aqueous biofluids (e.g. blood). Lipoproteins consist of a single layer of amphipathic phospholipid and cholesterol molecules that surround the non-polar lipids. Given this simple established system available early in animal evolution, it is surprising that fats from milk are not transferred in the same manner. Rather, fat transfer in milk is much more resource intensive because portions of the mammary epithelial cell membrane are sacrificed to secret the milk fat globules.

## MILK FAT GLOBULES AS PRODUCTS OF EVOLUTION

Milk is the only whole food which was evolved in order to completely meet the nutritional needs of the respective suckling mammalian neonates. This early nutritional strategy of mammals produced a remarkable Darwinian engine for driving the nutritional values of milk according to their abilities to maximize the survival chances of infants. Any components or structures added to milk cost the resources of the mother and hence would place her at a selective disadvantage. They are only of value then, to the extent that they protect her or encourage the success of her infant. Hence, why should the mammary gland elaborate such a unique bioprocess and highly energy consuming pathways to synthesize, assemble and secrete lipids into milk?

To understand the uniqueness of the structure and the forces driving the emergence of such an exclusive particle the milk fat globule should be studied under a broader perspective than the common approach. The intuitive notion that milk fat globules only role is to deliver fat is conspicuously wide of the mark since at least in some lactation stages, large portion of the lipid globules by number are in the submicron length scale and therefore are relatively triacylglycerol poor.

Lipid related research up to date has focused on understanding and managing to alter mainly fatty acid composition and concentration of milk lipid fraction which was found to be highly associated with the mother's dietary fatty acid composition. Nonetheless, very few studies looked at the possibility that these alterations might modify the milk fat globules (MFG) and milk fat globular membrane (MFGM), and almost none examined what industrial and subsequent nutritional impact such change, might have.

If indeed the dietary change influences the milk lipid macrostructure, it is not clear what outcomes those alterations have on different nutritional and digestion parameters. Will it enhance lipid digestion by gastrointestinal enzymes? Will it improve absorption by intestinal entrocytes? it might have implications on the lipid and fat absorption rate and subsequently on lipid metabolism. The MFG secretion mechanism evolved in order to optimize the lipid delivery to the suckling mammal by means of nutritional value. Thus the MFG macrostructure should be studied in order to further understand its role in enabling advantageous absorption (not necessarily rapid and complete absorption) and optimizing succeeding lipid tissue distribution. It should be recalled that the Darwinian selective pressure was exerted on the mammary gland and milk thousands of years before heart disease became

the main epidemiological health issue in the western word. Therefore, milk lipid macrostructure might affect health issues that are currently not assessed with respect to fat.

## MILK FAT GLOBULE COMPOSITION, SYNTHESIS, AND SIZE DISTRIBUTION

The lipid composition of a MFG is traditionally considered to be solely a function of the length and degree of unsaturation of the fatty acids. Fatty acids tend to be esterified to different lipid fractions depending on their carbon chain length and degree of unsaturation. In addition, milk fat globules span a wide range of sizes with quite different size classes having different TG/PL composition. Hence, differences in the TG/PL ratios in the MFGs of different diameters also indicate different FA compositions. The composition of the membrane phospholipids and the fatty-acid profile within the phospholipid fraction certainly play important roles in the milk fat globule membrane's physical and functional properties. Nonetheless, our ability to control membrane properties and the processes of milk fat globule assembly, need to extend beyond simply fatty acid composition.

The MFGs triglycerides are synthesized at the rough endoplasmic reticulum, accumulate into triglyceride-rich domains varied in size, which are released as discrete droplets to the cytoplasm, coated with polar lipids and proteins derived from the ER. From their site of origin, lipid droplets migrate to the apical pole of the cell from which they will be secreted. Milk fat globules follow a distinct export process in which the plasma membrane itself enrobes the emerging globule. In the secreted milk, lipid globule diameters range from 0.2 to more than 15 μm which implies that some lipid droplets grow substantially between the time of their formation and the time of their secretion. Furthermore, post-secretion, some of the larger milk fat globules may fuse with smaller ones and by that increase their diameter. However, whether this process is explicitly directed by cellular processes or whether the globules spontaneously self-assemble according to the composition of the respective surfaces, is unknown. The fact that milk lipid fraction, consist of MFG in various diameters implies that their size distribution within milk may have additional role than simply deliver fat to the infant. Moreover the diameter distribution might be altered under different physiological conditions since it has been shown to change in different lactation stages and subjected to nutrition administrations. Because MFG synthesis and secretion pathway is unique, major studies were conducted during the last two decades, pursuing ways to understand the MFG biological mechanisms enabling their synthesis and secretion. However the intracellular mechanisms remain illusive. If the basic mechanisms of the processes that dictate globule size distribution were known, and if indeed the nutritional importance is established, they could be controlled, and the size of particles, the compositions of surfaces and net composition of milk lipids be explicitly guided to desired goals.

The most studied lipid of the entire cream fraction of milk is the triacylglycerol core of the droplets which typically consist of more than 99% of milk lipids.

Nonetheless, this overwhelming predominance of neutral lipids obscures the complexity of the processes, structures and functions of actual milk fat particles. Once those globules are secreted into the milk they are completely coated with the cellular bilayer membranes of the epithelial cells of the mammary gland. Therefore all the apical membrane physical, chemical and functional features are carried over to milk by the milk fat globule membrane. Although extensive proteomics studies were conducted in order to characterize the proteins in the MFGM it seems that a parallel lipidomic study has not yet been conducted. In the nutritional, as well as industrial point of view, this kind of research might be highly valuable. Because protein distribution is dictated by genes, the ability to alter their distribution within milk is somewhat restricted. However, the lipid fraction of the milk can be modified nutritionally Therefore in order to enable control of the milk lipid macrostructure, further understanding of the regulating factors as well as the bioactivities of the macrostructure, is required.

## HEALTH PROPERTIES OF MILK LIPID MACROSTRUCTURE

As was previously reviewed milk fat globules in their native macromolecular structures have a wide range of speculated health benefits. Since technologies necessary to describe more complex lipid structures in smaller length scales are being applied in other lipid research fields (i.e. lipoproteins), those same techniques should be applied to characteriznig the smallest milk fat globules.

Researchers have recently investigated whether the source of dietary fatty acids, mostly long chain poly-unsaturated fatty acids (PUFA) such as EPA, DHA and AA, influences the regulation of their incorporation into tissues and erythrocyte membranes. Whether the chemical form of PUFA administration affects its bioavailability is unresolved. The natural delivery of milk fatty acids is the MFG where the PUFA are principally attached to the polar head group of phospholipids that form the surface layers of the MFGM. Thus, the possibility that MFG structure has a role of delivering bioactive advantageous nutrients, and moreover, essential compounds to the suckling neonate, should be considered when the bioavailability of the PUFA is examined.

Plasma lipoprotein distributions, cholesterol distribution within different lipoprotein classes, and the composition of various lipids within the lipoproteins of adults and children are influenced by normal or specifically enriched milk consumption. Discouragingly, relatively few studies have examined the role of milk in altering infant lipoprotein distributions, even human breast milk. Nonetheless, those reports that have emerged have indicated that breast feeding versus formula feeding does influence the distribution of lipoproteins and by inference alters the overall lipid metabolism of infants. The obvious fact is that the unique lipid macrostructure within milk is exceedingly difficult to replicate or imitate. Therefore, while most abundant milk proteins and carbohydrates (i.e. caseins and lactose) are found in both milk and infants' formula, the lipid structure factor is found only in milk. Consequently, this unique structure should be considered when whole body lipid metabolism is evaluated after milk and various dairy products are consumed.

The ability of intestinal epithelial cells to synthesize a variety of apoproteins and assemble and secrete lipoproteins has been extensively studied. Subsequent to lipid digestion, triacylglycerols are hydrolyzed within the intestinal lumen to form free fatty acids, which diffuse across the apical membrane of enterocytes. In the enterocytes, fatty acids re-esterify to form TG, PL and cholesterolesters. Two distinct pathways by which fatty acids are incorporated into TG have been described The main variation between those two pathways is the transport rate of re-esterified TG to the intestinal mucosa and the liver. The possibility that the rate of TG efflux from the digestive tract to the rest of the body tissues could be nutritionally regulated by up/downregulating each of the above pathways, is not new. In this context, the milk fat globule native structure and the different sizes of the globules might be differently affected by processes in the infants' gastrointestinal tract as well as subsequent digestion and absorbance rates. Therefore, we hypothesize that under different circumstances in which different nutrient delivery rates are valuable, milk fat globule production and their size distribution are subjected to the lactating mother's physiological state.

## INDUSTRIAL APPLICATIONS

The milk lipid fraction is known to contribute to the textural characteristics of many dairy products. Lipid globules of different diameters exhibit different thermal and physiological properties and different lipid compositions. In order to reduce the size distribution of MFG industrially, technologies have been developed to disrupt the native structure of globules, most commonly by homogenization. Homogenization is achieved by artificially increasing the number and net surface area of MFGs. At the end of the homogenization process, the large amount of the new milk fat globules surface area created by homoginazation must be covered by protein. Cano Ruiz and Richter estimated that only of 10% of the total MFGM after homogenization is covered by the original MFGM material. The newly formed fat globules exibit different physical and chemical properties. In addition, the MFG special structure is disrupted and interactions between different milk constituents like caseins and whey proteins occure, which in turn will influence the dairy products properties. Therefore, the development of adequate protocols and nondestructive methods which will facilitate naturally MFG usage would enable a new dimension of milk functionality to be explored, leading to dairy products with altered functional, nutritional and physical properties.

From a nutritional prospective, It has been previously shown that some fractions of the MFGM may be shed into the skim milk and spontaneously assemble into liposomes, or those liposomes might be artificially formed due to homogenization. Through disruption of the native macrostructure by homogenization, some bioactivities or at least bioactive molecules might be carried over to the skim milk. Hence, the possible affects on digestion, absorption and plasma lipid distribution should be assessed in relation to the small milk fat globule macrostructure and skim milk liposomes.

Due to the nature of the biological processes of MFG assembly combined with its physio-chemical properties of native milk fat globules that vary according to their size, it is possible to imagine producing milks with highly controlled and nonetheless highly native MFG's.

## CONCLUSION

The milk fat globule remains the least understood component of one of the world's most valuable agricultural commodities, milk. Darwinian selective pressure drove the emergence of a remarkable lipid delivery system in which the particles and their surface properties are unique to any other biological lipid export system. Recent nutrition research is identifying multiple factors associated with the milk fat globule membrane and distinct health properties. Unfortunately, there are no available data regarding the outcome of the genetic, screening and breeding in bovine milk in the past 100 years on the milk lipid macrostructures. We propose to understand the key biochemical and physical steps associated with fat globule formation and secretion with the explicit goal to control them. With this basic knowledge in place we shall then be able to manipulate the size, abundance and very importantly the amount and composition of the surfaces of these particles. One obvious application of this research would be to produce milk naturally enriched with small globules. Bringing to practice such a bioguided process of milk homogenization would explicitly increase the number and surface of MFGs and preserve their native structure.

# 14

# Concentration Processes

**Topics**

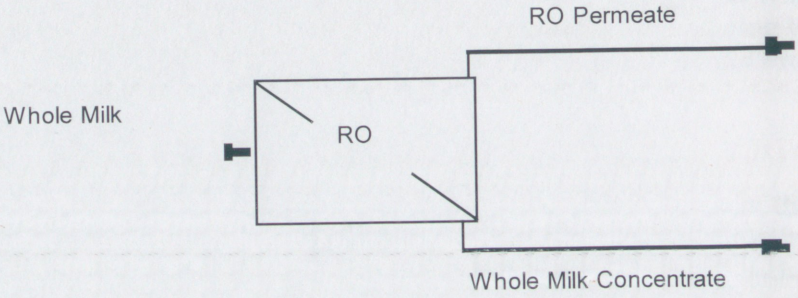
*Skim Milk • Whey • Other • WPC Concentration • Concentration of UF Permeate • Phase diagram / lactose / glass transition / crystallisation / kinetics • Evaporating*

Whole milk can be concentrated with Reverse Osmosis (RO) to levels of 25-30% total solids (TS). This represents a 2-2.5x volume concentration factor (VCF).

RO refers to a pressure driven membrane separation technique in which a membrane is employed to separate different components in a fluid mixture. RO membranes have pore sizes less than 0.001 micron. Separation occurs based on molecular size and chemical interactions between the membrane and fluid components that are in contact with the membrane. In this process, pressure is used to push water molecules through the pores of a membrane while retaining the colloidal solids and salts. Typical operating pressures range from 450-600 psi.

Permeate quality from RO systems depends on the quality and composition of the feed and the level of concentration. The higher the TS, the greater the amount of constituents in the permeate. Typically, RO permeate will contain small (but measurable) amounts of organic solids. The process performance is greatly affected by operating parameters such as feed flow rate, pressure, temperature, pH, microbiological quality of feed stream, feed concentration, and fouling characteristics of the membrane for various components.

Reverse Osmosis, which avoids the phase change seen with evaporation, preserves the functionality of proteins and the product does not have a cooked flavor. Processing temperature of whole milk is typically ~45°F. Systems are properly designed and operated to maintain the integrity of the fat molecules. Doing so prevents the rancidity caused by damaged or ruptured fat molecules.

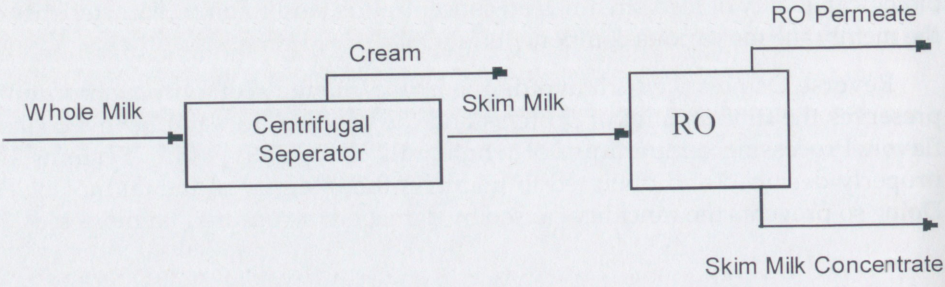



## SKIM MILK

Buttermilk concentration by cold reverse osmosis (RO) is frequently used as an efficient and economical way to increase capacity of a skim milk evaporation operation. MSS' cold milk experience has shown it technically feasible to pre-concentrate raw cold (45°F) whole milk without fat damage, excessive fouling, or any rancidity. By locating the cold RO ahead of the existing HTST/separator, the existing equipment does not need to be revised. Up to 25% increase in plant capacity can be achieved with the addition of a cold whole milk RO without increasing separator or pasteurizer capacity. Although the cost for a cold whole milk RO is somewhat higher that a warm skim RO, the savings by not increasing the pasteurizer and not adding to the separation capacity results in an overall lower investment. Generally, membranes operated colder at lower concentration last longer. The cold RO permeate can effectively be utilized for cream pre-cooling or other energy saving applications.

Permeate quality from RO systems depends on the quality and composition of the feed and the level of concentration. The higher the TS, the greater the amount of constituents in the permeate. Typically, RO permeate will contain small (but measurable) amounts of organic solids. The process performance is greatly affected by operating parameters such as feed flow rate, pressure, temperature, pH and micro-biological quality of feed stream, feed concentration and fouling characteristics of the membrane for various components.

Reverse Osmosis, which avoids the phase change seen with evaporation, preserves the functionality of proteins and the product does not have a cooked flavor. Processing temperatures of skim milk are typically <45°F or ~120°F. Skim



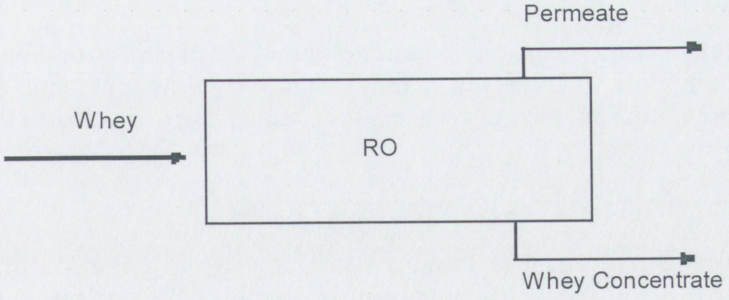
milk can be concentrated for several reasons: reduced volume for transportation, increased production capacities, or increased TS for use in fortification. Cream skimmed from the milk prior to concentration with RO can be added to the concentrated skim in order to obtain "concentrated whole milk".

## WHEY

### Sweet/Acid Whey

Whey concentration by reverse osmosis (RO) is frequently used to reduce volumes and increase solids content prior to transportation or further processing. RO refers to a pressure-driven membrane separation technique in which a membrane is employed to separate different components in a fluid mixture. A Reverse Osmosis membrane has pore sizes less than 0.001 micron and separation occurs based on molecular size and chemical interactions between the membrane and fluid components that are in contact with the membrane. In this process, pressure is used to push water molecules through the pores of a membrane while retaining the colloidal solids and salts. Typical operating pressures range from 450-600 psi.

Preconcentration of sweet whey before evaporation allows for more energy efficient removal of water at lower solids and increased capacity of existing evaporators. Total solids (TS) levels of 10-25% can be obtained in an efficient, practical manner. Reverse Osmosis membranes are used to remove water and will not alter the relative composition of the sweet whey components. Permeate quality from RO systems depends on the quality and composition of the feed and the level of concentration. The higher the TS, the greater the amount of constituents in the permeate. Typically, RO permeate will contain small (but measurable) amounts of organic solids. The process performance is greatly affected by operating parameters such as feed flow rate, pressure, temperature, pH and micro-biological quality of feed stream, feed concentration and fouling characteristics of the membrane for various components.




## OTHER

### Rinse Recovery

Reclaiming water from condensate, rinsing operations, and low-solid effluents allows you to find hidden value from your process and waste streams. RO

technology will allow you to recover up to 90% of the volume of waste waters that would be lost through discharge. Water recovery benefits you buy: lowering water costs, reducing energy costs, decreasing raw water treatment costs, reusing valuable resources, and compliance with regulatory standards.

## WPC CONCENTRATION

A combination of RO and NF can be used to concentrate whey product streams. Through these system applications levels of 30-40% TS are obtainable. These applications allow UF operation at lower solids for better efficiency and higher protein yield. These processes improve drying operations by lowering energy consumption and improving bulk density.

### Polishing of Cow Water (Evaporator Condensate Polishing and RO Permeate)

Water recovered from processes such as evaporation or membrane processing can be subsequently handled and treated in such a manner that it can be considered a safe water supply ("acceptable process water"). Acceptable process water is suitable for intermixing with products for human consumption in certain specified applications.

Reverse osmosis (RO) polishing of the streams can produce water that can be used directly for washing applications excluding final rinses of processing equipment. Polisher permeate can be further conditioned to meet potable water standards. Potable water, which may be used for final rinses.

Maximum Chemical Oxygen Demand 12 mg/l (ppm)

Mechanisms must be in place to assure that the permeate is maintained at or below these levels. Approved chemicals, such as chlorine, with a suitable retention period may be used to suppress the development of bacteriological growth and maintain a COD value of less than 12 ppm. The addition of chemicals shall be by an automatic proportioning device prior to the water entering the storage tank to assure satisfactory quality water.

State regulatory agencies will require submission of paperwork for the process water. Paperwork includes but is not limited to; a flow schematic of intended distribution, intended uses and application, and consent to perform the required tests of water quality.

## CONCENTRATION OF UF PERMEATE

Permeate from the ultrafiltration of whey is a stream that is low in total solids (TS), typically 3.5 - 5.5% TS. UF permeate is usually 75-85% lactose on a dry basis, and is utilized for harvesting lactose or as cattle feed. Removal of water from this stream prior to further processing or transporting is essential to the economic feasibility. Membranes can be used to effectively increase the solids levels to 15% to 25%. Temperature and pH are critical factors determining the final concentration level. Due to the concentration of salts and sugars, UF permeate has osmotic pressure much higher than most other dairy streams.

At concentration >20% TS, nanofiltration membranes are occasionally used. Nanofiltration membranes will negate the effect of increasing osmotic pressure found at these TS levels. The volumes of permeate required to increase the TS levels in this range are quite small. Permeate from nanofiltration membranes at this point in a system tends to be higher in organic material.

The NF-70 nanofiltration membranes exhibit excellent performance for concentration factors up to the range 2.0 - 2.5

## PHASE DIAGRAM / LACTOSE / GLASS TRANSITION / CRYSTALLISATION / KINETICS

### Introduction

The spray-drying process of milk has been developed using an approach based more on empirical and technological concepts than on food material science. However, optimisation of the concentration and the spray-drying steps require a detailed consideration of material phase transitions, especially concerning the state of lactose. Milk powder manufacture should preserve emulsion functionality throughout the process down to reconstitution. In particular, fat globules should be well covered with membranes and encapsulated in a fully amorphous matrix to limit free fat. Therefore, lactose crystallisation, either during concentration or spray-drying, should be avoided. Since 1990, due to meaningful use of the glass transition concept, exploitation of the state diagram has spread gradually in various food processing applications. This paper deals with the phase diagram of milk, focusing on phase transitions of lactose, and includes some kinetic considerations. It is both a critical review of the literature and a summary of some personal results that will not be presented in detail.

### The Various States of Lactose in Milk

Lactose is the major ingredient of milk. When it is dissolved in water or raw milk at a pH between 2 and 7, lactose exists in two anomeric forms: alpha and beta. The beta:alpha ratio (governed by mutarotation) is not strongly dependent on temperature and concentration, but may be influenced by the presence of salts. Lactose is more stable in the crystalline alpha monohydrate form, containing 5.0% water chemically bound to the crystal lattice structure. Crystalline monohydrate alpha lactose is not very soluble (7.4% w/w water over total matter at 20°C; 18.5% at 60°C), compared to sucrose (66.7% at 20°C; 74.3% at 60°C). However, the beta form is more soluble (16.1% at 20°C; 36.0% at 60°C) and mutarotation of the alpha fraction into the beta form (equilibrated beta:alpha ratio is about 1.6 at 20°C and 1.4 at 90°C) increases the final solubility. When a non-crystallised milk concentrate is spray-dried, the lactose is rapidly solidified in an amorphous solid structure. The beta:alpha ratio in this powdered glassy state (typically 1.2 to 1.4) is not thought to significantly change during the end of drying. In a glassy powder particle, the amorphous lactose coats milk proteins and fat globules. Amorphous lactose is metastable, and may undergo glass transition and crystallisation under certain conditions, e.g. water uptake or heat treatment.

## The Glass Transition in Milk Powders

The onset glass transition temperature ($T_g$) of any milk powder, characterised by the water content (W) or the water activity ($a_w$) is mainly an effect of the amorphous lactose. It can be determined by differential scanning calorimetry (DSC). However, in whole milk powders (WMP) Tg may be difficult to detect; depending on the W, the glass transition may more or less overlap the end point of fat melting. Therefore, DSC is easier to use in skim milk powders (SMP). The procedure for determining the Tg and its relationship to W and aw in WMP or SMP requires:

- The accurate determination of W and $a_w$ (at a given temperature) of the sample. In the next examples, W values were determined in duplicate (difference < 0.1%) using a home-made method (2 h at 102°C at 25 mbar initial pressure, in presence of $P_2O_5$), which fully removes the water (proved by NIR) and gives results in very good agreement with KarlFischer. The $a_w$ values were determined at 25°C in duplicate (difference < 0.005) using a Rotronic Hygroscop DT calibrated with saturated salts.
- Doing a first DSC scan to eliminate the relaxation of the matrix by heating to the end of the relaxation mechanism (but avoiding any other physical or chemical reactions). Otherwise, the onset of the relaxation peak will dramatically affect determination of the onset $T_g$.
- The rapid cooling of the sample (at the rate of – 25°C. $min^{-1}$) in the glassy solid state, to typically 50°C below the expected Tg, and then heating it a second time to a higher temperature, in order to detect successively the temperature of the true glass transition, lactose crystallisation and onset of non-enzymatic browning. Tg determinations were done in duplicate (differences < 2°C).

The DSC procedure is for a typical industrial spray-dried SMP (canned at 5.1% W), using a Seiko 220C DSC instrument with heating at 5°C.$min^{-1}$. Onset with relaxation (1st scan) is at 44°C and the true $T_g$ (2nd scan) is 4°C lower at 40°C. The end point of relaxation is at 57°C, but the end point of the glass transition is at 48°C.

## Lactose Crystallisation in Milk Powders

Lactose crystallisation may occur in milk powders exposed to high relative humidities or submitted to heat treatments after a period of storage. However, the state of crystallisation seems to be different in SMP and WMP. The storage period before significant nucleation is supposed to be ($T - T_g$) dependent, according to the glass transition theory. However, the kinetics of crystallisation is highly dependent on the experimental conditions.

### *Isothermal Heat Treatment at Constant Water Content*

A rather large amount of powder (e.g. a can or a tube) with a well-known W and $T_g$ is exposed to a constant storage temperature above $T_g$ (in an oven). If the packaging material is not permeable to water, no water exchange with the outside

environment is possible. Under such conditions, lactose crystallisation will induce a very significant increase in the aw due to the release of water from the amorphous lactose during crystallisation (most often present in an anhydrous form). This water is available to the remaining amorphous ingredients (e.g. the proteins) and may provoke reactions such as Maillard browning. The RH first slightly increases due to relaxation of the amorphous matrix and the early stages of the Maillard reaction (no browning), which produce a small amount of water (max 0.1%). Then onset of lactose nucleation (after 34 h) provokes the first major water release, which plasticises the surrounding matrix, depresses the Tg and accelerates the propagation of nucleation and crystal growth. Therefore, the maximum crystallisation level is reached in a rather short time (here within 9 h, between 34 and 43 h). It is suggested to use the midpoint of the RH step (here 38 h) to define the time required for crystallisation.

*Isothermal Heat Treatment at a Constant Relative Humidity*

A rather small amount of powder of known initial W is evenly spread in a dish and exposed to a constant RH imposed by a saturated salt at a given temperature (most often room temperature). The weight of the powder is recorded on a regular basis and the kinetics of water uptake and release (after the onset of crystallisation) with time follows. Water uptake occurs during the first 4 h up to 12% W. Lactose nucleation occurs between 4 and 5 h. Then the W decreases to below 10% after 8 h. A Near Infrared Reflectance (NIR) fingerprint (InfraAlyzer 500, Bran and Luebbe) collected each hour allows the onset of crystallisation to be detected and the state of lactose to be predicted.

*Heating at a Constant Rate at a Constant Water Content*

This is the standard DSC procedure and it uses a very small sample (10 to 20 mg). Here, the onset crystallisation temperature is very dependent on the heating rate, and the initial W. The glass transition with the onset of the relaxation at 44°C, the onset lactose crystallisation (102°C), the full peak and the onset of Maillard browning for a SMP initially at 5.1% W heated at $5°C.min^{-1}$.

These different experiments allow data for modelling of the time period of lactose crystallisation to be collected.

**The Phase Diagram of Whole Milk**

The establishment of the milk state diagram, restricted to description of the state of lactose, requires knowledge of three main lines described by the temperature versus the concentration in milk total solids (TS) at thermodynamical equilibrium:

- **The freezing point depression line ($T_f$)**: well known and described in the literature, up to a certain TS.
- The solubility line ($T_s$): established based on the solubility of lactose in water, and according to the hypothesis on the partition of water to dissolve milk

ingredients (mainly the proteins and the salts). The thermodynamical equilibrium requires a consideration of the mutarotation kinetics.

- **The glass transition line ($T_g$)**: consisting of a number of data points for milk at various TS, especially in the solid state zone. The reliability of the $T_g$ line depends on the accuracy of the determination of the TS [0–100%] in milk solids and of the $T_g$. It also depends on the significance of the model used.

This chapter focuses on the establishment of the glass transition line. The dynamic lines, which describe the time period crystallisation as a function of $(T - T_g)$, depend on the reliability of the $T_g$(TS) line. A procedure for obtaining a coherent model equation for both whole milk (WM) and skim milk (SM) will be presented.

### The glass transition line of WM and SM

The Gordon and Taylor equation (1) may be applied to most amorphous soluble food products to describe the plasticising effect of water. This model requires adjustment of the three following constants:

- The glass transition $T_{gw}$ of pure water.
- The glass transition $T_{gm}$ of the fully dried material (influenced by the amorphous carbohydrates).
- A k coefficient, which express the non-linearity of the plasticising effect of water.

$$T_g(TS) = [TS\ T_{gm} + k\ (100 - TS)\ T_{gw}] / [TS + k\ (100 - TS)] \quad (1)$$

As water is the solvent, $T_{gw}$ may be considered as well defined (the reference value of –135°C is commonly used). Therefore, only the $T_{gm}$ and k parameters need to be optimised experimentally. A small number of data points are sufficient for adjusting the coefficients. Here, the quality of the data is more important than their number. Two procedures may be considered:

- A first procedure consists of determining $T_{gm}$ by measuring a fully dehydrated product, and then adjusting k by a curve fitting using the $T_g$ of powders of known W. However, it is not easy to dehydrate an amorphous milk powder to 0.0% W. There is a risk of chemical change or partial recrystallisation by heating at too high a temperature, and diffusion of water throughout the matrix is slowed down as water is removed, thereby increasing the $T_g$. Moreover, it is difficult to prove that there is no residual water (NIR is an option) and such a fully dehydrated amorphous powder will rapidly take up moisture from the environment during any manipulation. Therefore, procedures described in the literature based on desiccation of freeze-dried samples over $P_2O_5$ at room temperature for some days probably allow significant residual water content.
- Another procedure consists of adjusting both $T_{gm}$ and k simultaneously by curve fitting (e.g. applying the Excel solver) using a set of experimental data. Most often in the literature, the TS are obtained by equilibrating dried

powders at different RH (over saturated salts). The W reached after an arbitrary equilibration time is calculated based on the initial W and the weight exchange. Therefore, the initial W values will affect all other determinations. The powders are presumed to be in equilibrium at the aw corresponding to the saturated salt solutions at the sorption temperature, and this is not necessarily true.

As already mentioned, the $T_g$ may be difficult to detect for WMP between 4 and 8.5% W. The glass transition more or less overlaps fat melting (usually between –5 and 40°C). Consequently, determination of the onset $T_g$ of WMP can only be done for powders with less than 4%, or more than 8.5% W. In any case, it is easier to determine onset $T_g$ for SMP, and this implies a new adjustment of the k parameter for the Gordon and Taylor equation.

In this secrion, the Gordon and Taylor equations for WM and SM will be established based on a minimum amount of data from four well-selected samples: two WMP (A and B) and two SMP (C and D). WMP-A (3.8% W), SMP-C (4.0%) and SMP-D (5.1%) were obtained from conventional spray-drying by adapting after-drying fluidised bed parameters. These amorphous powders were canned and stored at room temperature for one month to allow water distribution and aw equilibration. WMP-B at 9.8% W was obtained directly from the output of the drying tower with extreme spray-drying conditions and a rapid cooling in liquid nitrogen to keep the powder fully amorphous. This frozen powder was stored at –40°C for one month, and heated at 20°C just before analysis.

The first two $T_g$(TS) determinations of WMP-A and WMP-B allowed the exact calculation of both $T_{gm}$ and k values of the Gordon and Taylor equation for WM. The fitted parameters are: $T_{gm}$ = 100.6°C and k = 8.57. This $T_{gm}$ value agrees with the value of 101°C given in the literature for pure lactose. In the following sections, we will use the Gordon-Taylor equation for WM with the values $T_{gm}$ = 101°C and k = 8.6. The line for SM was obtained using a similar procedure with the second two $T_g$(TS) determinations, fixing $T_{gm}$ = 101°C and optimising k = 6.5. The same Tgm value was used for SM as the protein: lactose ratio of WM and SM are the same. Here the k value for SM agrees very well with the value of 6.7 obtained by Jouppila and Roos for skim milk powder.

The sorption isotherm of whole milk at 25°C Most sorption isotherms described in the literature are obtained by equilibrating a spray-dried amorphous powder over saturated salts at a range of RH at a fixed temperature. The W was either determined after sorption by a reference method, or calculated from weighing the water exchange and using the initial W. The powders are often equilibrated without recording the kinetics of sorption. In addition, the whole series of powders is supposed to be equilibrated after the same period of time. Then the data are used for fitting a mathematical model such as the Brunauer-Emmet-Teller (BET) or Guggenheim-Andersson-DeBoer (GAB), which is sometimes extrapolated. However, the glass transition theory and the plasticising effect of water clarify the limits of this procedure:

- For samples with a low $a_w$, and especially in the case of desorption, $T_g$ increases as water is removed from the surface of the particles. The matrix becomes more and more solid and the mobility of the molecules more and more difficult. Therefore, it may take weeks or months for dried particles to equilibrate at aw below 0.2.
- For samples with a high $a_w$, $T_g$ decreases below the sorption temperature as water is adsorbed and diffuses into the matrix. The matrix is more and more rubbery and may collapse, often becoming like syrup. Then phase transitions and chemical reactions may occur rapidly, and the sample does not tend to the expected equilibrium.

The adjustment of the BET or GAB coefficient is very sensitive to such extreme data. In particular, the monolayer water content value ($W_m$) is strongly influenced by non-equilibrated low aw data. and immediately cooling the powders below their $T_g$. Those with W higher than 5% ($T_g$ below 30°C) must be frozen. The data may be treated either by the physical BET (2 parameters: C and Wm) or the mathematical GAB (3 parameters) model. The GAB model is more flexible and fits the data better. However, it may diverge for high aw values and does not necessarily fit the reality for low TS. Therefore, the physical BET model is preferred here, according to equation (2) with Wdb expressed on a dry basis

$$W_{db} / W_m = C_{aw} / [(1 - a_w)(1 - a_w + C_{aw})] \quad (2)$$

We collected such a spray-dried WMP (28% milk fat) up to about 10% W, as well as concentrated milk with TS between 45 and 55% total solids.

The BET parameters allow one to fit the low W values (W < 11%) very well. With C = 5.21 and $W_m$ = 4.69% (dry basis), the residual standard deviation between the model and the first ten data points is 0.11%. However, the predicted data for the concentrate are systematically biased. By fitting all the data together the constants become C = 6.70 and Wm = 4.28%, but the model fits the low W data less well, especially the three data points ranged between 0.45 and 0.65 aw, which were the most difficult to establish (frozen samples which rapidly crystallise at room temperature). A compromise may be obtained by fixing the monolayer at the intermediate value $W_m$ = 4.5% and optimising C using the whole set of data. The best fit for WM is obtained for C = 5.79 and the BET equation now fits all the data rather well, and may be used for the whole range of TS from liquid to solid. This BET desorption line of WM (28% fat) is shown in Figure 9. The BET line for SM (< 1% fat) was obtained by calculating Wm according to the solid not fat (SNF) and adjusting C for another series of SMP powders ranged between 0.08 and 0.34 aw. Thus the BET values for SM are Wm = 5.76% and C = 6.344. The monolayer value of 5.76% for SM is a little higher than the value of 5.47% that can be calculated from the skim milk powder.

### Glass Transition and Water Activity

In a previous section, $T_g$ of skim, half skim and full cream milk powders versus the powder aw measured at 25°C were presented. It was shown that the

relationship was linear in the range of aw between 0.1 and 0.3, and a unique $T_g(a_w)$ line allowed the description of the behaviour of the whole range of fat content, with the same lactose to protein ratio. Moreover, this line was rather similar to the line established for pure amorphous lactose and for spraydried whey powders with various degrees of lactose pre-crystallised in the alpha hydrate form. It was concluded that the aw may be considered as the reference parameter for more generally describing the glass transition of any kind of amorphous material that only contains lactose (no other low molecular weight carbohydrate). Similarly, a linear behaviour was also found for maltodextrins of various dextrose equivalents in a larger range of aw between 0.2 and 0.75.

Now, the $T_g(a_w)$ relationship of WM may be described over the full aw range (0 to 1) by solving the $T_g$(TS) Gordon-Taylor relationship (1) and the robust BET description (2) of the sorption isotherm of WM.

It may be done by reversing the BET equation in order to express aw versus the W or the TS, then plotting Tg and aw versus TS. The $T_g(a_w)$ relationship of SM may be calculated using the Gordon-Taylor and the BET equations for SM and the same procedure. Both WM and SM related Tg(aw) lines are plotted in Figure 10. It can be seen that the lines do not differ significantly.

By analysing the curvature we can see that the $T_g(a_w)$ relationship is very linear between 0.12 and 0.65 aw and well predicted by the equation (3):

$$Tg[°C] = -143.6\ aw + 77.8 \quad (3)$$

Such a linear behaviour may be compared to data for WMP, partially skimmed milk and SMP powders previously determined by DSC 1st scan at $5°C.min^{-1}$.

The following linear regression was calculated from these 1st scan data: $T_g$ [°C] $= -110\ a_w + 74$. However, most data above 0.2 aw resulting from the 1st scan determinations averaged up to 4°C above the predicted line using the second scan. Therefore the slope of the linear regression should be lower.

This is a typical effect of the relaxation using the onset $T_g$ determination at the first scan. This very good fit between the new model and the previous data validates both equations (1) and (2) in the low aw range between 0.1 and 0.3. Finally, the full $T_g(a_w)$ line, including the non-linearity in the very dry and wet regions, may be approximated well by a third degree polynomial:

$$T_g\,[°C](a_w) = -425\ a_w^3 + 545\ a_w^2 - 355\ a_w + 101 \quad (4)$$

This "universal" equation may be very useful for estimating the $T_g$ of any kind of milk (powder or concentrate with any fat content and any degree of lactose crystallisation) by determining only the aw of the product at 25°C.

## Lactose Crystallisation in the Rubbery Region

### *Lactose crystallisation forms in whole milk powders (WMP)*

WMP (2.5 to 4% W) stored under adverse conditions (some weeks at 40°C or subjected to heat shocks of some hours at temperatures up to 70°C) always exhibits

a large amount of beta anhydrous crystals. This may be checked either by NIR or by capillary gas chromatography (CGC) analysis. Short treatments (less than 1 h) at temperatures up to 100°C give the same results. For instance, an initially fully amorphous WMP with 3.5% W ($a_w$ = 0.24) became very crystallised (and slightly yellow) in less than 1 h at 90°C (but not at 80°C): the total beta:alpha ratio increased from 1.32 to 4.2.

*Lactose crystallisation forms in skimmilk powders (SMP)*

The state of crystallisation was found to be rather different for SMP. After storing powders with a high W (5 to 6%) at 60°C for some days, or with a lower W (3 to 4%) at 90 to 110°C, a mixture of alpha and beta anhydrous crystals was found (detected by NIR and confirmed by CGC). As we can determine the beta/alpha ratio in the initial amorphous powder (Ra) as well as in the final treated powder (R) by CGC, it is possible to calculate the degree of lactose crystallisation in any form if the crystalline ratio (Rc) is known. The fraction of crystallised lactose Fcr (total of alpha and beta crystallised, referred to as total lactose) may be calculated by the following equation (5):

$$F_{cr} = (R_a - R)\,(1 + R_c) \,/\, [(R_a - R_c)\,(1 + R)] \qquad (5)$$

SMP with between 4 and 6.5% W and treated for between 30 min and 1 week at temperatures between 60 and 110°C was analysed by NIR and CGC. NIR shows crystallisation states, including both alpha and beta forms, but without crystallisation water. Coherent results on the fraction of crystallised lactose between 0 and 50% may be calculated using equation (5) by adjusting the crystalline beta: alpha ratio Rc to 0.6, which exactly confirms the 5:3 alpha:beta found elsewhere.

**Conclusion**

The $T_g$ (2nd DSC scan) versus TS relationship may be described by the GordonTaylor equation. Simply changing the k value differentiates both WM and SM. The sorption isotherm BET equations were also adapted for WM and SM and may be applied in the whole TS range (0 to 100%). A universal description of $T_g$ versus aw (measured at 25°C) was obtained. It is very linear between 0.12 and 0.65 aw. The nonlinearity at low and very high TS is well predicted by a 3rd order polynomial. The phase diagram of milk was therefore adapted to WM or SM. The WLF model allows the prediction of the kinetics of lactose crystallisation (from minutes to days) within the whole rubbery region ($T_g$ + 100°C). Crystallisation in the beta anhydrous form was induced by subjecting WMP to heat shocks. A mixture of anhydrous alpha and beta lactose was found in SMP.

## EVAPORATING

Evaporated milk, also known as dehydrated milk, is a shelf-stable canned milk product with about 60% of the water removed from fresh milk. It differs from sweetened condensed milk, which contains added sugar. Sweetened condensed milk requires less processing since the added sugar inhibits bacterial growth.

Evaporated milk is fresh, homogenized milk from which 60 percent of the water has been removed. After the water has been removed, the product is chilled, stabilized, packaged and sterilized. It is commercially sterilized at 240-245 °F (115-118 °C) for 15 minutes. A slightly caramelized flavor results from the high heat process, and it is slightly darker in color than fresh milk. The evaporation process alsoconcentrates the nutrients and the food energy. Thus, for the same weight, undiluted evaporated milk contains more food energy than fresh milk.

The product takes up half the space of its nutritional equivalent in fresh milk. When the liquid product is mixed with a proportionate amount of water, evaporated milk becomes the rough equivalent of fresh milk. This makes evaporated milk attractive for shipping purposes as it can have a shelf life of months or even years, depending upon the fat and sugar content. This made evaporated milk very popular before refrigeration as a safe and reliable substitute for perishable fresh milk, which could be shipped easily to locations lacking the means of safe milk production or storage.

The process involves the evaporation of about half the water from the milk, after which the product is homogenized, canned, and sterilized.

## Manufacturing Process

### *Evaporated milk*

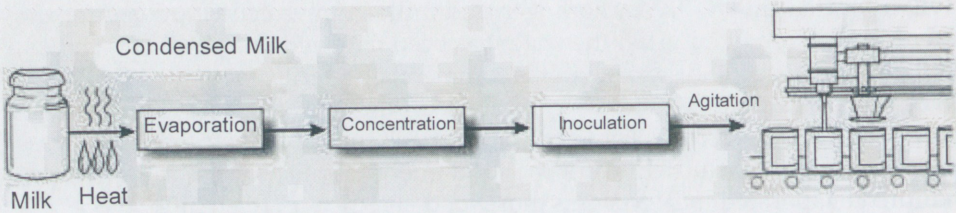
1. The raw milk is transported from the dairy farm to the plant in refrigerated tank trucks. At the plant, the milk is tested for odor, taste, bacteria, sediment, and the composition of milk protein and milk fat. The composition of protein and fat is measured by passing the milk under highly sensitive infrared lights.

2. The milk is piped through filters and into the pasteurizers. Here, the milk is quickly heated in one of two ways. The High Temperature Short Time method (HTST) subjects the milk to temperatures of 161 °F (71.6°C) for 15 seconds. The Ultra High Temperature (UHT) method heats the milk to 280°F (138°C) for two seconds.

   Both methods increase the milk's stability, decrease the chance of coagulation during storage, and decrease the bacteria level.

3. The warm milk is piped to an evaporator. Through the process of vacuum evaporation, (exposing a liquid to a pressure lower than atmospheric pressure) the boiling point of the milk is lowered to 104-113°F (40-45°C). As a result, the milk is concentrated to 30-40% solids. Also, the milk has little or no cooked flavor.

4. The milk is then homogenized by forcing it under high pressure through tiny holes. This breaks down the fat globules into minute particles, improving its color and stability.

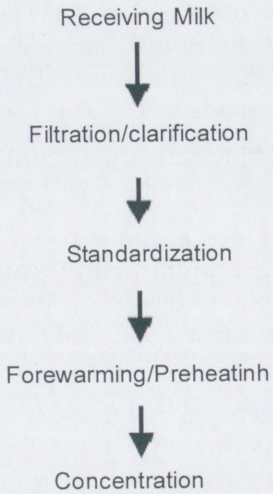



**A diagram showing the manufacturing steps involved in making condensed milk.**

5. Pre-measured amounts of a stabilizing salt, such as potassium phosphate, are added to the milk to make it smooth and creamy. This stabilization causes the milk to turn a pale tan.
6. The milk is passed under a series of ultraviolet lights to fortify it with Vitamin D.
7. The milk is piped into pre-sterilized cans that are vacuum-sealed.

Unsweetened concentrated milk is known as evaporated milk and manufactured practically in the same manner as the sweetened condensed milk. In this product no sugar is added as preservative. To achieve preservation the product is sterilized by heat after concentration and sold in the hermetically sealed container in which it has been sterilize.

i) **Receiving milk/filtration/clarification/standardization:** The raw milk used in the preparation of evaporated milk is treated in a similar manner to that which applies in case of sweetened condensed milk as discussed earlier. It is important that the raw milk should be of very good quality in order to ensure that it is free from any heat resisting microorganisms. Only fresh




**Flow Diagram of Manufacture of Evaporated Milk**

milk can be used since, if the acidity of evaporated milk is above normal it will clot or curdle during sterilization and will thus be rendered un-saleable.

ii) **Prheating/Forewarming of milk:** To improve the heat stability of concentrated product and for imparting optimum viscosity to the finished product the fluid milk is preheated before it is condensed. Milk may be preheated either at 95-100° C for few minutes (5-10) or at 140-145° C with not holding time.

iii) **Concentration:** Preheated milk is concentrated in an evaporator. Multiple effect evaporators are used for handling large amount of milk to have continuous operation.

iv) **Homogenization:** After concentration the milk is homogenized. Fat separation in evaporated milk during storage is a major defect. This defect can be reduced or eliminated by homogenization of condensed milk. Homogenization refers to a process of forcing milk under pressure through equipment called homogenizer. This is an essential part of production of evaporated milk. The homogenizer reduces the mean size of the fat globules in the milk so that they are uniformly distributed in milk and do not rise to the top to form the creamy layer during storage. In raw milk, the diameters of the fat particles (globules) vary from 1 to 20 micron while a diameter of about 2 micron or less is required to keep the fat distributed uniformly. In this process milk is forced through a small orifice at a high pressure by means of a positive displacement pump. The mechanical forces, which are set up as the fat particles pass quickly through the orifice, cause the particles to split. Condensed milk removed from evaporator is passed through two stage homogenizer at about 50°C with a pressure of 175 kg/sq. cm at first stage and 35 kg/sq cm at second stage.

v) **Cooling**: After homogenization concentrated milk is cooled to 5°C and held in storage tank. Stabilizing salts may be added to concentrated milk in tank as indicated by pilot sterilization test.

vi) **Pilot Sterilization**: The purpose is to determine the amount and type of chemical stabilizers to be added to any given batch of condensed milk for most satisfactory heat stability. Heat coagulation of milk is influenced by many factors such as initial quality of raw milk, fore warming, concentration and sterilization. In raw milk it has been suggested that salt balance of milk i.e. ratio of Calcium + Magnesium to Citrate + Phosphate is important for the stability of milk towards heat. If the ratio of Ca + Mg/ Citrate + Phosphate is disturbed the heat stability may be affected. The more common cause of heat coagulation is the disturbed salt balance. Depending on the location of pH of maximum stability with respect to natural pH of concentrated milk either sodium salt of orthophosphate (disodium phosphate) or calcium chloride/monosodium phosphate are used as stabilizers. This is the basis of a process of pilot-sterilization in which various quantities of selected stabilizers are added to the concentrated milk

in 170 ml (60 oz) tins which are then sterilized and the condition of the product carefully examined in order to determine whether any stabilizer is necessary and if so in what amount it should be used. Depending upon the type of milk and the processing condition used 100 to 300 g of stabilizing salts per 100 kg of evaporated milk may be needed to improve the heat stability. Calculated amount of salt is added to the evaporated milk in the form of a solution using just enough water to dissolve it.

vii) **Packaging**: The evaporated milk is filled in suitable cans or containers either manually or mechanically. It is essential that the packaging is done as quickly as possible. After cans have been filled they should be sealed immediately. The sealing process requires care, as the seal has to withstand the heat and pressure of sterilization.

viii) **Sterilization**: The object is to destroy all bacteria their spores and enzymes thereby preserve the product for a longer time. In addition sterilization process is also used to increase the viscosity and improve body and texture to give a creamy consistency to the product.

Two important factors in sterilization process are:

- The temperature attained
- The period during which this temperature is maintained.

The temperature time of heating during sterilization should be such as to ensure sterility consistent with sufficient body and texture without causing objectionable discolouration or excessive cooked flavour.

Two systems are in common use for sterilization:

- **Batch Sterilization:** This method is suitable for small scale operation and is also useful for cans of various sizes. In this system the cans leaving the filling machine are placed in racks. The racks are then loaded into a steam-tight boiler. The racks are moved by a revolving mechanism, which keeps them in motion throughout the process,the speed of rotation varies between 6-12 revolutions per minute. Steam is admitted in to the sterilizer until the temperature reaches 118°C and the sterilizer is maintained at this temperature for not less than 15 min. After sterilization is completed, the steam is shut off and cans are cooled to 25-30°C either by means of water spray or by immersing in cold water. After cooing the cans are passed through an oscillating machine, which breaks up any curd formed during the process of sterilization and retains the smooth homogeneous texture of evaporated milk.
- **Continuous Sterilization:** This is used for large scale continuous operation. This system consists of many compartments, which are maintained at different temperatures/ pressures. The filled cans entering the apparatus roll through the chambers by means of spiral track, which ensures that the heat distribution to each can is uniform. This rolling motion of cans takes

the place of the methods of agitation provided in the batch method. The filled cans are preheated before they pass to the main sterilizing section. The movement of cans is controlled so that they remain in the sterilizing chamber for 15 min. After sterilization process is complete the cans pass to the cooling section, which under similar pressure conditions to those of the sterilizer. Both systems have their merits and demerits. During sterilization the temperature of evaporated milk is raised rapidly to 116-118°C and held at this temperature for 15 minutes and their cooled.

- **Storage:** After sterilization cans of evaporated milk are unloaded from the sterilizer, and cooled by water spray. The cans are then shaken mechanically to break any curd or lump which might have formed during sterilization to have homogenous consistency. Excessive shaking is avoided as it may decrease viscosity. As evaporated milk is a sterilized product it may be stored at or below room temperature. The present trend is to store at below room temperature to check deterioration in quality and thereby prolong keeping quality.

**Condensed milk**

1. The milk is flash-heated to about 185°F (85°C) for several seconds. It is then piped to the evaporator where the water removed.
2. The milk is then concentrated under vacuum pressure until it measures between 30-40% solid. It now has a syrupy consistency.
3. The milk is cooled and then inoculated with approximately 40% powdered lactose crystals. The milk is then agitated to stimulate crystallization. It is this sugar that preserves the condensed milk.
4. The milk is piped into sterilized cans that are then vacuum-sealed.

*Quality Control*

The milk industry is subject to stringent regional and federal regulations regarding the prevention of bacteria and the composition of solids and fats. According to the United States Food and Drug Administration (FDA), sweetened condensed milk must contain at least 28% by weight of total milk solids and at least 8% by weight of milk fat. Evaporated milk must contain at least 6.5% by weight of milk fat, at least 16.5% by weight of milk solids that are not fat, and at least 23% by weight of total milk solids. The evaporated milk must also contain 25 International Units (IUs) of vitamin D.

# 15

# Drying of Milk

**Topics**

*Separation / Standardization • Preheating • Evaporation • Packaging and storage • Agglomerated Powders • Instant Whole Milk Powder • Energy and Environmental Considerations • Applications • Spray drying • Atomization • Nano spray dryer • Mixing of spray and drying medium (air) with heat and mass transfer • Selected Definitions*

Milk powder manufacture is a simple process now carried out on a large scale. It involves the gentle removal of water at the lowest possible cost under stringent hygiene conditions while retaining all the desirable natural properties of the milk - colour, flavour, solubility, nutritional value. Whole (full cream) milk contains, typically, about 87% water and skim milk contains about 91% water. During milk powder manufacture this water is removed by boiling the milk under reduced pressure at low temperature in a process known as evaporation. The resulting concentrated milk is then sprayed in a fine mist into hot air to remove further moisture and so give a powder. Approximately 13 kg of whole milk powder (WMP) or 9 kg of skim milk powder (SMP) can be made from 100 L of whole milk. The milk powder manufacturing process is shown in the following schematic and is described in detail below.

## SEPARATION / STANDARDIZATION

The conventional process for the production of milk powders starts with taking the raw milk received at the dairy factory and pasteurising and separating it into skim milk and cream using a centrifugal cream separator. If WMP is to be manufactured, a portion of the cream is added back to the skim milk to produce a milk with a standardised fat content (typically 26- 30% fat in the powder). Surplus cream is used to make butter or anhydrous milkfat.

## PREHEATING

The next step in the process is "preheating" during which the standardised milk is heated to temperatures between 75 and 120°C and held for a specified time from a few seconds up to several minutes (cf. pasteurisation: 72°C for 15 s). Preheating causes a controlled denaturation of the whey proteins in the milk and it destroys bacteria, inactivates enzymes, generates natural antioxidants and imparts heat stability. The exact heating/holding regime depends on the type of product and its intended end-use. High preheats in WMP are associated with improved keeping quality but reduced solubility. Preheating may be either indirect (via heat exchangers), or direct (via steam injection or infusion into the product), or a mixture of the two. Indirect heaters generally use waste heat from other parts of the process as an energy saving measure.

## EVAPORATION

In the evaporator the preheated milk is concentrated in stages or "effects" from around 9.0% total solids content for skim milk and 13% for whole milk, up to 45-52% total solids. This is achieved by boiling the milk under a vacuum at temperatures below 72°C in a falling film on the inside of vertical tubes, and removing the water as vapour. This vapour, which may be mechanically or thermally compressed, is then used to heat the milk in the next effect of the evaporator which may be operated at a lower pressure and temperature than the preceding effect. Modern plants may have up to seven effects for maximum energy efficiency. More than 85% of the water in the milk may be removed in the evaporator. Evaporators are extremely noisy because of the large quantity of water vapour travelling at very high speeds inside the tubes.

## SPRAY DRYING

Spray drying involves atomising the milk concentrate from the evaporator into fine droplets. This is done inside a large drying chamber in a flow of hot air (up to 200°C) using either a spinning disk atomiser or a series of high pressure nozzles. The milk droplets are cooled by evaporation and they never reach the temperature of the air. The concentrate may be heated prior to atomisation to reduce its viscosity and to increase the energy available for drying. Much of the remaining water is evaporated in the drying chamber, leaving a fine powder of around 6% moisture content with a mean particle size typically of $< 0.1$ mm diameter. Final or "secondary" drying takes place in a fluid bed, or in a series of such beds, in which hot air is blown through a layer of fluidised powder removing water to give product with a moisture content of 2-4%. Precautions must be taken to prevent fires and to vent dust explosions should they occur in the drying chamber or elsewhere. Such explosions can be extremely dangerous to life, property and markets.

## PACKAGING AND STORAGE

Milk powders are immensely more stable than fresh milk but protection from moisture, oxygen, light and heat is needed in order to maintain their quality and

shelf life. Milk powders readily take up moisture from the air, leading to a rapid loss of quality and caking or lumping. The fat in WMPs can react with oxygen in the air to give off-flavours, especially at higher storage temperatures (> 30°C) typical of the tropics.

Milk powder is packed into either plastic-lined multi-wall bags (25 kg) or bulk bins (- 600 kg). WMPs are often packed under nitrogen gas to protect the product from oxidation and to maintain their flavour and extend their keeping quality. Packaging is chosen to provide a barrier to moisture, oxygen and light. Bags generally consist of several layers to provide strength and the necessary barrier properties. Shipments of milk powder should never suffer prolonged exposure to direct sunshine especially in tropical countries. A few hours at elevated temperatures (> 40°C) during transhipment can negate many weeks of careful storage.

## AGGLOMERATED POWDERS

Standard powders, because of their fine dusty nature, do not reconstitute well in water. "Agglomerated" and "instant" powders were specifically developed to counter this. The manufacture of an agglomerated powder initially follows the standard process of evaporation and drying, described above. However, during spray drying small particles of powder leaving the drier (the "fines") are recovered in cyclones and returned to the drying chamber in the close proximity of the atomiser. The wet concentrate droplets collide with the fines and stick together, forming larger (0.1-0.3 mm), irregular shaped "agglomerates". Agglomerated powders disperse in water more rapidly and are less dusty and easier to handle than standard powders.

## INSTANT WHOLE MILK POWDER

With WMP, an extra step is required after agglomeration to make the product truly "instant" and overcome the hydrophobic (water-hating) nature of traces of free fat on the surface of the particles. This extra step consists of spraying minute quantities of the natural surfactant or wetting agent, soy lecithin, on to the powder in a fluid bed. Soy lecithin is extracted from soy bean oil. Lecithins are widespread in nature and they occur naturally in milk.

## ENERGY AND ENVIRONMENTAL CONSIDERATIONS

Large amounts of energy are expended in the process of removing water and so plants developed over the years have become increasingly more energy efficient. Evaporators are much more energy efficient than driers, using only a fraction of a kilogram of steam (or the energy equivalent) per kilogram of water removed. Driers on the other hand use several kilograms of steam (or steam equivalent) per kilogram of water evaporated. Spray drying provides a means of rapidly and gently removing the bulk of the remaining water but, ideally, spray driers have short residence times. Hence fluid beds are used for the final stages of drying. The

powder remains for several minutes in fluid beds allowing time for the last of the water to be removed.

Milk powder manufacturing plants tend to be very large, few in number and located in rural areas. If modern and well managed, they have only relatively small effects on the environment. They are moderately energy intensive, burning coal or gas and consuming substantial electricity. There are strong economic pressures to reduce energy consumption but there is little scope for further major improvement. Milk storage silos, cream separators and the evaporators and associated plant must be cleaned daily, and driers less often. Sodium hydroxide and nitric acid are used as cleaning agents. The spent cleaning fluids must be disposed of by suitable means. There can be emission of milk powder dust into the local environment during plant malfunctions but this is rare. Noise is a problem mainly within the plant buildings but fans can affect close neighbours. Unbleached paper and plastic laminate packaging must be disposed of in overseas markets.

## APPLICATIONS

Plant manufactures a wide range of spray dried milk powders (> 100) to meet the diverse and special needs of customers. Milk powders may vary in their gross composition (milkfat, protein, lactose), the heat treatment they receive during manufacture, powder particle size and packaging. Special "high heat" or "heat stable" milk powders are required for the manufacture of certain products such as recombined evaporated milk. Some powders are agglomerated and they may be instantised for easy use in the home. Instant powders must wet, sink and disperse quickly, with minimal stirring, when added to water. The resulting liquid should closely resemble fresh milk and be free from undissolved particles. Some powders are fortified with vitamins and minerals. It is very important to use a powder suited to the intended application. Milk powders of various types are used in a wide range of products including the following.

- Baked goods, snacks and soups
- Cheese milk extension (powder is added to local fresh milk to increase the yield of cheese) Chocolates and confectionery (e.g. milk chocolate)
- Dairy desserts Direct consumer use (home reconstitution)
- Ice cream Infant formulae
- Nutritional products for invalids, athletes, hospital use etc.
- Recombined "fresh", UHT, evaporated and sweetened condensed milks
- Recombined cheeses, mainly "soft" or "fresh"
- Recombined coffee and whipping creams
- Recombined yoghurts and other fermented products.

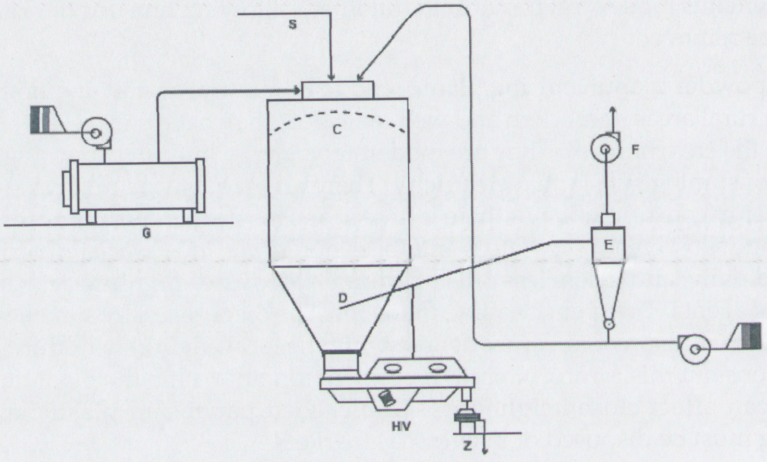



**Fig. 15.1. Two-stage spray dryer**

## SPRAY DRYING

The discovery of spray drying constituted a very important advance in the production of heat sensitive dry products, allowing the drying of solutions in a very short time and with relatively low product temperatures. Large scale application of this process began in the 1920´s, mainly focused in the dairy industry and the production of detergents. Since then, there have been a series of improvements such as the vibrating fluid bed dryer to finish the drying process outside the chamber and the fines reinjection for simultaneous agglomeration and drying, which helps obtain high dispersability products. Nevertheless, one of the most important advances has been the incorporation of a static fluid bed at the bottom of the drying chamber, giving birth to the so-called 3 stage-drying process.

### Description and working principle of a conventional 2 stage spray dryer

An air current heated in hot air generator G enters the drying chamber C through the top (Fig. 15.1). Previously, it flows through a circular duct and it is distributed in the chamber by means of a maze duct system. As a consequence of following this path, the hot air flows with a rotational movement and is evenly distributed in the whole chamber. Simultaneously, the drying solution is introduced, which in most cases is a liquid solvent with a defined amount of solids dissolved in it. The main function of the drying chamber is to eliminate the solvent by evaporation and to retrieve the powder formed by the dissolved solids.

The drying solution (S) enters the chamber through an atomizing device that consists of a rotating disc spinning at very high speed (around 10.000 RPM) that sprays the liquid feed. The plant configuration is such that the hot air meets the liquid mist. This happens at the top of the chamber, very close to the roof. The mix of hot air and thinly pulverized liquid is the fundamental principle of a 2 stage drying chamber, given that under these conditions a fast evaporation of the solvent is produced (water in most cases).

As a consequence of this fast evaporation process, the solids contained in the solution are dried at a low temperature despite the air is entering the chamber at temperatures ranging 160°C to 250°C, depending on the product that is being processed. Heat sensitive elements, such as milk solids, vegetable extracts, proteins, colorants, preservants, etc. are dried at temperatures around 50°C to 60°C and with a very short residence time (15 to 30 seconds). Solids continue to dry inside the chamber, dragged by the air heading for the exit duct towards the cyclone. From there the air is eliminated to the atmosphere by a suction blower.

As for the powder produced, it is divided into two flows:

A) The coarser and heavier particles in an advanced drying state, fall into a vibrating fluid bed dryer (Hv) where they reach their final moisture. The exit of the fluid bed dryer is the end of the drying process where the powder is collected after going through a screener (Z).

B) The fines are dragged by the air current on its way to the cyclone that separates them by centrifugal action. The fines fall to the bottom of the cyclone where it is dragged by another air current and reinjected into the chamber for agglomeration and increased wettability, given that the powder exiting the cyclone is too fine and this undermines some of the its qualities like dispersability.

As described before, in this type of chamber the drying process is Split in 2 stages. The first stage happens in the proper drying chamber and the second in the fluid bed dryer that gathers the powder exiting the chamber. This fluid bed receives a hot air current that has two functions: fluidizing the product and finish the drying process (2nd stage).

The drying in the chamber includes the drying stage at constant speed and temperature very close to wet bulb temperature and in most part a drying process at a diminishing speed by diffusion which forces to keep high temperatures for the exiting air. As it will be explained when addressing the 3 stage spray dryer, this hot

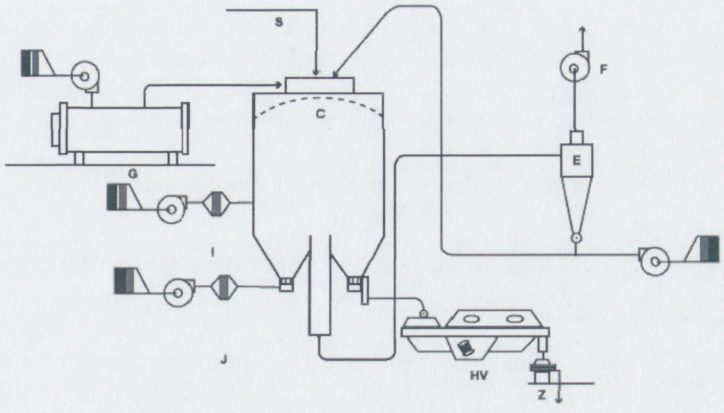



**Fig. 15.2. Three-stage spray dryer**

air exit current is one of the main differences between them that favours lower energy consumption given that exit temperature for air is lower in 3 stage drying.

**Description and working principle of a conventional 3 stage spray dryer (Fig. 15.2)**

The general configuration of a 3-stage spray drying plant is similar to the 2 stage dryer regarding:

- Hot air generation (G)
- Hot air inlet into the chamber
- Liquid inlet through the rotary atomizer

The main difference is the addition of a static fluid bed (K) inside the chamber where the poder coming from the 1st stage falls with high moisture. Until then, only the outer moisture of the particles is evaporated in a very short time of around 20 seconds, where drying temperature is constant an very close to wet bulb temperature of the air which is very low (approximately 40°C). The second stage of drying consists in the injection of hot air into the chamber at the point where the first stage ends. In the static fluid bed the core moisture if the particles is evaporated.

This process requires more time, and the still humid fluidized powder is agglomerated while it is being dried. In the third stage, the powder finishes the drying process in a vibrating fluid bed dryer and is cooled to packaging temperature. With the combination of these 3 stages, drying is split into two systems specifically adequate for the water content of the particle at each moment. The surface moisture is eliminated during the path of the powder inside the chamber and the core moisture is eliminated while the powder is in the static fluid bed.

The air required to fluidize the powder is produced by a hot air generator (J). This air current enters the fluid bed through a specially perforated sheet. Once the drying is complete the powder can be cooled by means of a vibrating fluid bed dryer (Hv).

1. **Energy Balance**: the heat energy required by a 3 stage chamber is approximately 10 % to 20 % less than the necessary for a conventional 2 stage dryer with the same output capabilities. To analyze the thermal issue a global thermal yield for an adiabatic process is defined as:

   Yield : = $(T_{in} - T_{sat\ out}) \times 100 / T_{in} - T_{amb}$

   $T_{in}$ = inlet temperature of drying air

   $T_{sat\ out}$ = outlet adiabatic saturation temperature for the drying air

   $T_{amb}$ = ambient temperature

   As it can be appreciated, for the same inlet temperature and constant ambient conditions, when lowering the outlet temperature of the drying air the thermal yield is increased. In a 2 stage plant the outlet temperature is around 85-90°C while in a 3 stage process it is reduced to 75-80°C

2. **Characteristics of the powder**: in a 3 stage system high density agglomerated powders can be obtained, with free-flowing and high dispersability qualities.

3. **Equipment size**: For the same production capacity, a 3 stage chamber should be shorter than a conventional one, reducing costs in civil works.

4. **Operational flexibility**: Both rotary and spraying nozzles can be used in 3 stage chambers, allowing a higher control of the product qualities.

5. **Operational stability**: Given that drying is produced in 3 stages that are designed for each different moisture content of the product, this type of system offers high operational stability.

6. **Cleaning of the equipment**: the internal geometry of a 3 stage chamber is especially apt for the use of CIP cleaning nozzles or spraying systems.

## ATOMIZATION

Spray drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with a hot gas. This is the preferred method of drying of many thermally-sensitive materials such as foods and pharmaceuticals. A consistent particle size distribution is a reason for spray drying some industrial products such as catalysts. Air is the heated drying medium; however, if the liquid is a flammable solvent such as ethanol or the product is oxygen-sensitive then nitrogen is used.

All spray dryers use some type of atomizer or spray nozzle to disperse the liquid or slurry into a controlled drop size spray. The most common of these are rotary disks and single-fluid high pressure swirl nozzles. Atomizer wheels are known to provide broader particle size distribution, but both methods allow for consistent distribution of particle size. Alternatively, for some applications two-fluid or ultrasonic nozzles are used. Depending on the process needs, drop sizes from 10 to 500 μm can be achieved with the appropriate choices. The most common applications are in the 100 to 200 μm diameter range. The dry powder is often free-flowing.

The most common spray dryers are called single effect as there is only one drying air on the top of the drying chamber. In most cases the air is blown in co-current of the sprayed liquid. The powders obtained with such type of dryers are fine with a lot of dusts and a poor flowability. In order to reduce the dusts and increase the flowability of the powders, there is since over 20 years a new generation of spray dryers called multiple effect spray dryers. Instead of drying the liquid in one stage, the drying is done through two steps: one at the top (as per single effect) and one for an integrated static bed at the bottom of the chamber. The integration of this fluidized bed allows, by fluidizing the powder inside a humid atmosphere, to agglomerate the fine particles and to obtain granules having commonly a medium particle size within a range of 100 to 300 μm. Because of this large particle size, these powders are free-flowing.

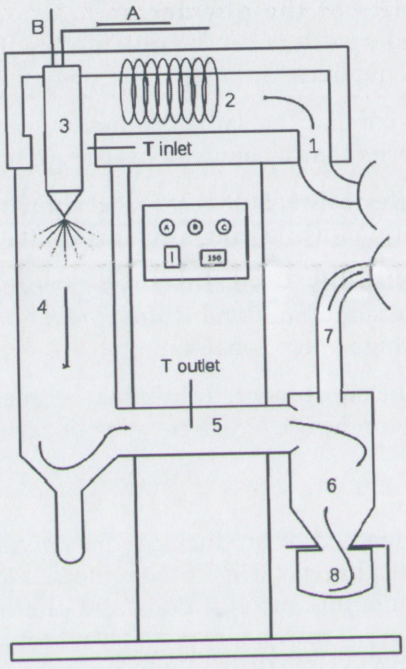



**Fig. 15.3. Laboratory-scale spray dryer.**

A=Solution or suspension to be dried in, B=Atomization gas in, 1= Drying gas in, 2=Heating of drying gas, 3=Spraying of solution or suspension, 4=Drying chamber, 5=Part between drying chamber and cyclone, 6=Cyclone, 7=Drying gas is taken away, 8=Collection vessel of product, arrows mean that this is co-current lab-spraydryer

The fine powders generated by the first stage drying can be recycled in continuous flow either at the top of the chamber (around the sprayed liquid) or at the bottom inside the integrated fluidized bed. The drying of the powder can be finalized on an external vibrating fluidized bed.

The hot drying gas can be passed as a co-current or counter-current flow to the atomiser direction. The co-current flow enables the particles to have a lower residence time within the system and the particle separator (typically a cyclone device) operates more efficiently. The counter-current flow method enables a greater residence time of the particles in the chamber and usually is paired with a fluidized bed system.

**Alternatives to Spray Dryers are:**

1. **Freeze dryer**: a more-expensive batch process for products that degrade in spray drying. Dry product is not free-flowing.
2. **Drum dryer**: a less-expensive continuous process for low-value products; creates flakes instead of free-flowing powder.

3. **Pulse combustion dryer**: A less-expensive continuous process that can handle higher viscosities and solids loading than a spray dryer, and that sometimes gives a freeze-dry quality powder that is free-flowing.

### Spray Drying Applications

Food: milk powder, coffee, tea, eggs, cereal, spices, flavorings, starch and starch derivatives, vitamins, enzymes, stevia, colourings, etc.

Pharmaceutical: antibiotics, medical ingredients, additives

Industrial: paint pigments, ceramic materials, catalyst supports, microalgae

## NANO SPRAY DRYER

The nano spray dryer offers new possibilities in the field of spray drying. It allows to produce particles in the range of 300 nm to 5 µm with a narrow size distribution. High yields are produced up to 90% and the minimal sample amount is 1 mL.

## MIXING OF SPRAY AND DRYING MEDIUM (AIR) WITH HEAT AND MASS TRANSFER

The manner in which spray contacts the drying air is an important factor in spray dryer design, as this has great bearing on dried product properties by influencing droplet behaviour during drying.

This mixing is an important aspect and defines the method of spray drying:

### Co-Current flow

The material is sprayed in the same direction as the flow of hot air through the apparatus. The droplets come into contact with the hot drying air when they are the most moist. The product is treated with care due to the sudden vaporization (Fig. 15.4).

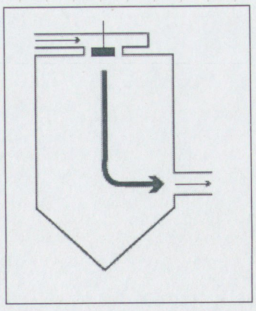


**Fig. 15.4. Co-Current flow**

### Counter-Current flow

The material is sprayed in the opposite direction of the flow of hot air. The hot air flows upwards and the product falls through increasingly hot air into the collection tray. The residual moisture is eliminated, and the product becomes very hot. This method is suitable only for thermally stabile products (Fig. 15.5).

### Combined

The advantages of both spraying methods are combined. The product is sprayed upwards and only remains in the hot zone for a short time to eliminate the residual moisture. Gravity then pulls the product into

the cooler zone. Due to the fact that the product is only in the hot zone for a short time, the product is treated with care (Fig. 15.6).

### Disk atomizer (rotary wheel)

The material to be sprayed flows onto a rapidly rotating atomizing disk and is converted to a fine mist. The drying air flows in the same direction. The product is treated with care, just as in the co-current flow method (Fig. 15.7).

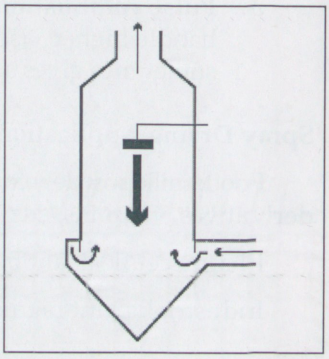


Fig. 15.5. Counter-Current flow

## SELECTED DEFINITIONS

**Atomiser**: A device for producing fine droplets of liquid. Usually either a high pressure nozzle or a perforated spinning disk through which the liquid is pumped.

**Concentrate**: Milk concentrated by evaporation, typically containing around 48% total solids.

**Cyclone**: A device for separating air and powder particles.

**Effect**: A single unit in an evaporator operating at a particular pressure and temperature. Evaporators commonly have three to seven effects to allow heat to be reused several times.

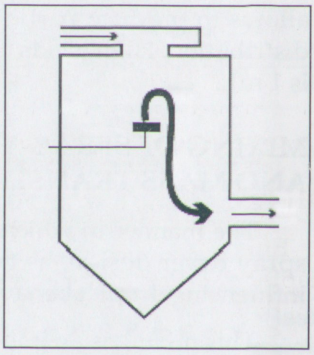


Fig. 15.6. Combined

**Fluid bed**: A piece of equipment used for drying or cooling milk powder. Air is blown through the powder from below, causing the powder particles to separate and behave rather like a fluid. Alternatively, a layer of fluid-like powder in which the particles are kept apart by an air flow.

**Recombined**: Liquid milk or other "fresh" product made by mixing skim milk powder, milkfat, water and possibly other components.

**Reconstituted**: Liquid milk or other "fresh" product made by mixing milk powder and water.

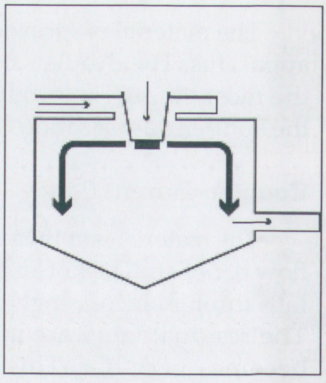


Fig. 15.7. Disk atomizer (rotary wheel)

# 16

# Cooling and Freezing of Milk

***Topics***

Milk Cooling • Cooling milk during storage • Freezing

## MILK COOLING

Effective milk cooling is essential to ensure the quality of the product. This chapter describes the key components and features of the common systems use to cool their milk.

### Features of Cooling Milk

A basic understanding of milk cooling systems can help farm managers ask good questions and make informed decisions about their milk cooling components. When it comes to cooling milk there is no one solution for all farms.

Milk can be entirely cooled in the vat or chilled before it hits the vat or be cooled using a combination of precooling and vat cooling.

### Pre-cooling Milk

Most farms now pre-cool milk before it enters the storage vat. Pre-coolers remove heat from milk very quickly due to the large surface area available for heat exchange. A plate cooler consists of a series of very thin stainless steel plates. Water flows along one side of each plate while milk flows along the other. Heat is transferred from the milk to the water via the plate. The capacity of a plate cooler is adjusted by adding or subtracting plates. A plate cooler

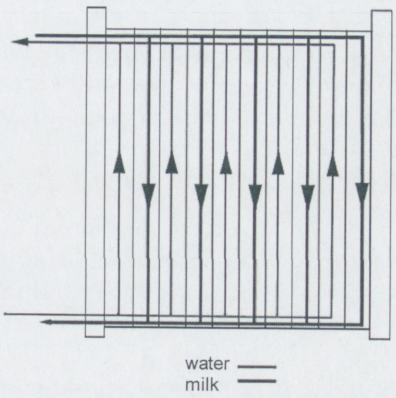



**Fig. 16.1: Single bank plate cooler**

that is working well should cool milk to within 2° C of the temperature of the incoming cooling fluid. A typical plate cooler is depicted in Fig. 16.1. The 'M-series' plate coolers are the most common type found in Australian dairies and are available in 'single' bank' and 'double' bank configurations. The 'M-series' is designed to handle milk flow rates of up to 4,500 litres per hour. Industrial plate coolers are more suitable in situations where milk flow rates exceed 4,500 litres per hour. Industrial plate coolers are much bigger in size than the 'M-series' and are designed for flow rates up to 12,000 litres per hour.

**Factors affecting performance of pre-coolers**

There are several factors which can impact on the efficiency of the pre-cooler. These are:

*The ratio of the flow rate of cooling fluid to milk*

The system should be designed for the peak flow of milk expected from the milk pump. An even flow of milk will help make the plate cooler system easier to size and make efficient use of the cooling water. 'M-series' plate coolers work most efficiently with a water to milk flow ratio of 3:1, whilst 2:1 or even 1.5:1 is adequate for the newer industrial models.

*Available surface area*

The peak flow rate of milk expected from the milk pump will determine the type of pre-cooler (size of the plates) and number of plates required.

*Plate compression*

Allow about 3mm per each plate and gasket. If too tight the fluid flow will be restricted. Measure the overall thickness of the plates before disassembly. Never take the plates completely off the guide rails.

*Plate cleanliness*

Contaminants in either the water or milk that adhere to the plates will affect heat exchange capacity and reduce performance. Blockages to the flow can restrict flow over the heat exchange surfaces.

*Source water temperature*

Ideally plate coolers should use water that is the coldest available on the farm. Although some fluctuation in source temperature can be expected over the year, this is an inexpensive way to initially reduce the temperature of the milk from around 35°C to 18 - 20°C, reducing the load on the refrigeration system significantly.

**Chilling fluids for use in pre-cooling**

Many larger farms have a two staged plate cooler that first uses bore or surface water and then in a second stage uses chilled water or a chilled water-glycol mix.

The chilled water can either be refrigerated on demand (instant chilling) or it can be chilled overnight on off-peak power and stored until required (cooling tower, refrigerated thermal storage or ice bank).

Two stage pre-cooling has the advantage of delivering milk to the vat at close to 4°C. Some of these systems also allow the bulk of milk cooling to be undertaken using off-peak power, reducing the operating costs and spreading the demand for farm electricity use.

*Cooling towers*

Cooling towers can be very effective at cooling water especially in areas of low humidity. Water can be cooled to within 5°C of the wet bulb temperature in a properly designed plant. The most effective plants are fan forced and turn over a large store of water every hour. They operate overnight to cool a large volume of water, usually 4½ times the volume of the daily (peak) milk yield. There are restrictions in Victoria on the operation of fan-forced cooling towers. Cooling towers must be registered and have an audited operations and maintenance plan.

*Ice banks*

Ice banks generate ice along evaporator coils using night-rate power. The ice is used to chill water for the precooler. The warm water is then returned from the pre-cooler to the top of the ice bank and cooled again as it runs down the ice. These systems can require more maintenance than other systems and are not as energy efficient as a direct expansion vat. If working on night rate electricity rates they may save money even though they use more energy. Ice banks take up less space than storage of chilled water.

*Instant chillers*

Another option is to use a refrigeration system to cool water or a glycol/water mixture. Glycol systems tend to use a very small volume of fluid and create the chilled fluid on demand (at milking time). Note that a system that is designed to chill milk to 4°C in line (ie prior to vat entry) will need a much larger (and therefore expensive) compressor than an in-vat system.

*Thermal stores*

Thermal storage systems chill water using off peak power and require an insulated storage tank to hold a large (enough for a day's milking) volume of chilled water. Using more energy than a direct expansion vat they have advantages relating to installation and maintenance procedures.

## COOLING MILK DURING STORAGE

### Direct Expansion

In refrigerated / direct expansion vats the refrigerant (commonly R22 in Australia) is pumped into the jackets (evaporators commonly referred to as 'dimple

plates') on the internal surfaces of the milk vat. Here the refrigerant expands as it takes heat from the milk, is pumped out of the jackets, compressed, then pushed into the condenser.

*The hot refrigerant is cooled by air (or water) flowing through the condenser fins.* The cooled gas condenses into a liquid and is pumped back in to the jackets around the milk vat to start the cycle again. Direct expansion is the simplest and most energy efficient way of cooling milk to the required storage temperature. It has the disadvantage of maximum power draw during and after milking which is generally peak rate. If large electric motors are used there can be problems in areas of poor power supply or where generators are used.

Direct expansion refrigeration systems are pressurised, which means they require a skilled technician when there is need for maintenance.

*Low-pressure / glycol systems*

Another common option is to replace the vat's pressurised gas refrigerant system with a low-pressure system that supplies cooled liquid to the dimple plates. The refrigeration is not attached to the vat but works to cool the fluid that is pumped through the vat. This fluid can either be cooled at the time of demand or be refrigerated off-peak and stored for use at milking.

An advantage of this system is that the cooling fluid circulating through the vat is not under high pressure so the risks of vat failure are reduced. This makes this system ideal for recycled vats. It is also possible to cool the fluid at off peak rate or during a time of lower power demand.

**Ice Builders**

This is a separate ice store which supplies chilled water to a plate heat exchanger. An ice builder is most commonly used in combination with a Direct Expansion tank. The benefits are:

- Where space is limited they can be located in a nearby room.
- Smaller compressors are required.
- Enable maximum benefit to be taken of cheap rate electricity.
- Lower milk cooling costs.

## FREEZING

When milk is cooled, it starts to freeze at about -0.54°C, if no undercooling occurs. Concentrated milk naturally has a lower freezing point. Pure ice is formed, and the remaining milk thus becomes concentrated, thereby further decreasing the freezing point. The lower the temperature, the higher the proportion of the water that freezes. If there is equilibrium among water, ice, and moist air, the water activity is a function of temperature only; this is because the vapor pressure of ice, and therefore its $a_w$, is a function of temperature only (for constant pressure).

The change in composition of the remaining solution is considerable. At -8°C, for example, $Q^*$ is approximately 13, corresponding to about 60% dry matter when skim milk is frozen. Making an ultrafiltrate of the remaining liquid (at -8°C), it has a pH (at room temperature) of about 1 unit lower than that of an ultrafiltrate of the skim milk before freezing. The ionic strength may be almost tenfold higher, the calcium ion activity is significantly increased, and the quantity of calcium phosphate in the casein micelles is also increased, despite the decrease in pH.

Upon freeze concentration, the remaining liquid can attain a glassy state. When a solution of, say, 5% lactose is cooled to below 0°C, ice will form. The liquid will thus be concentrated and the temperature decreases, as given by curve $T_f$; note that the values of $x$L now refer to the lactose solution. At about -0.75°C and 12% lactose, the eutectic point is reached, and if equilibrium would be attained, lactose then starts to crystallize. However, lactose exhibits rather slow nucleation and crystal growth, and upon rapid cooling its crystallization will fail to occur. Freeze concentration, then, will proceed according to curve $T_f'$, although its course can slightly depend on cooling rate. The viscosity of the liquid will markedly increase, whereby lactose crystallization becomes ever less likely, and finally the curve for $T_g$ is reached.

Now, the liquid has changed into a glassy state and further ice crystallization is prevented. This *special glass transition* at maximum freeze concentration is characterized by a special glass transition temperature $T'_g$ and unfrozen water content $x'_w$. For lactose these values are approximately -28°C and 15%, respectively. (*Note*: The description given in the preceding text is, to some extent, an oversimplification because of the mutarotation of lactose and of its crystallization as a monohydrate.)

When lactose does not crystallize, it acts as a *cryoprotectant*. Because the equilibrium water activity is determined by temperature only, less water will freeze. Consequently, the other substances, notably salts, are concentrated less; hence, ionic strength and $Ca^{2+}$ activity increase less. When lactose does crystallize and the frozen milk is thawed, aggregation of casein micelles is often observed, presumably due to salting out and to deposition of calcium phosphate in the micelles. When the aggregation is not too strong, it can often be undone by stirring at low temperatures (e.g., 5°C), but frozen milk that has been stored for a long time at, say, -18°C shows irreversible aggregation upon thawing. If lactose crystallization has been prevented by freezing rapidly, aggregation does not occur.

Freezing and thawing of whole milk, and especially of cream, generally causes partial coalescence (clumping) of fat globules because of the growing ice crystals pressing the globules together. It can be prevented by prior homogenization and rapid freezing.

In practice, rapid freezing is achieved in a scraped-surface heat exchanger. The liquid flows through a tube that is deeply cooled at the outside, causing ice to form at the inner surface. The ice is immediately removed by a rotating stirrer that is provided with knives that scrape over the surface. In this way, a mixture of

concentrated milk product and ice crystals results. By removing the crystals, concentrated milk can be obtained. This process of freeze concentration is, however, much more expensive and more limited in possibilities than removing water by evaporation; it is occasionally used to concentrate a liquid containing aroma compounds because these may become lost during concentration by evaporation.

## FREEZING DAIRY PRODUCTS

**Butter** - Freeze only high-quality butter made from pasteurized cream. Over-wrap store wrap with freezer wrapping. Unsalted butter loses flavor so its storage time is shorter. Flavored butter freezes well.

**Cheese** - Hard or semi-hard cheeses can be frozen. Frozen cheese will be crumbly and a little dry and will not slice as well, but the flavor will be just as good as fresh cheese. Freeze cheese in small pieces - no more than ½ pound per chunk. Seal it in foil, freezer wrap, plastic film or bag.

**Cottage cheese** - Cream style and dry cottage cheese and ricotta cheese can be frozen for a month. Cream style may separate when thawed.

**Cream cheese** - can be frozen for later use in cooking, dips or as icing.

**Cheese food products,** such as sauces, dips, processed cheese — flavored or plain — usually freeze fine. If in real doubt, freeze a small quantity and check after 24 hours by thawing it. If pleased with the results, freeze the rest. Otherwise, do not freeze.

**Cream** - Freeze only heavy cream containing 40 percent or more butter fat. Lighter cream and half and half do not freeze well. Cream that has been frozen will not whip to the usual volume. Whipped cream can be sweetened to taste and frozen in individual sized portions. Tray-freeze in mounds.

**Ice cream** - A plastic wrap laid tightly on the surface of partially used containers of ice cream helps prevent sur-face changes. Homemade ice cream is difficult to store for any length of time because it becomes grainy. Commercial products have added milk solids and gelatin to prevent this.

**Milk** - Pasteurized homogenized milk may be frozen, including low and non-fat. Some quality change may be noted upon thawing. Stirring or shaking may help restore smoothness.

**Sour cream, yogurt and buttermilk** - All of the cultured, soured dairy products lose their smooth texture when frozen. They become grainy and sometimes separate out their water. They can still be used for cooking. Flavored yogurts may be more stable because of the fruit and sugar. It may taste more acidic when thawed.

# 17

# Membrane Processes

**Topics**

*Types of Membrane Processes • Reverse Osmosis • Dead End Filtration: Theory and Equations • Membrane Transport Mechanism • Membrane Transport Mechanism • Characteristics of Membrane • Application of membranes in different fields of dairy technology • Fouling of Membranes • Future Trends*

Membrane technology has revolutionized the dairy sector. Different types of membranes are used in the industry for various purposes like extending the shelf life of milk without exposure to heat treatment, standardization of the major components of milk for tailoring new products as well increasing yield and quality of the dairy products, and concentrating, fractionation and purification of milk components especially valuable milk proteins in their natural state. In the cheese industry, membranes increase the yield and quality of cheese and control the whey volume, by concentrating the cheese milk.

With the advancement of newer technology in membrane processes, it is possible to recover growth factor from whey. With the introduction of superior quality membranes as well as newer technology, the major limitation of membranes, fouling or blockage has been overcome to a greater extent.Membrane processes are applied to separate a liquid into two liquids of different composition. The primary aim may be to remove a substance such as water, which amounts to concentrating milk or whey, etc. Other components to remove are salts or bacteria, for example.

Another aim is to accumulate a (group of) components such as protein. Membrane separations are increasingly applied in dairy processing, particularly to whey. They often replace older separation processes, but they also give rise to new or improved products.

## TYPES OF MEMBRANE PROCESSES

### Ultrafiltration

Ultrafiltration (UF) is the process of separating extremely small particles and dissolved molecules from fluids. The primary basis for separation is molecular size, although in all filtration applications, the permeability of a filter medium can be affected by the chemical, molecular or electrostatic properties of the sample. Ultra filtration can only separate molecules which differ by at least an order of magnitude in size. Molecules of similar size can not be separated by ultra filtration. Materials ranging in size from 1K to 1000K molecular weight (MW) are retained by certain ultrafiltration membranes, while salts and water will pass through. Colloidal and particulate matter can also be retained. Ultrafiltration membranes can be used both to purify material passing through the filter and also to collect material retained by the filter. Materials significantly smaller than the pore size rating pass through the filter and can be depyrogenated, clarified and separated from high molecular weight contaminants. Materials larger than the pore size rating are retained by the filter and can be concentrated or separated from low molecular weight contaminants. Ultrafiltration is typically used to separate proteins from buffer components for buffer exchange, desalting, or concentration. Ultrafilters are also ideal for removal or exchange of sugars, non-aqueous solvents, the separation of free from protein-bound ligands, the removal of materials of low molecular weight, or the rapid change of ionic and/or pH environment. Depending on the protein to be retained, the most frequently used membranes have a nominal molecular weight limit (NMWL) of 3 kDa to 100 kDa. Ultrafiltration is far gentler to solutes than processes such as precipitation. UF is more efficient because it can simultaneously concentrate and desalt solutes. It does not require a phase change, which often denatures labile species, and UF can be performed either at room temperature or in a cold room.

## REVERSE OSMOSIS

Reverse osmosis (RO) separates salts and small molecules from low molecular weight solutes (typically less than 100 daltons) at relatively high pressures using membranes with NMWLs of 1 kDa or lower. RO membranes are normally rated by their retention of sodium chloride while ultrafiltration membranes are characterized according to the molecular weight of retained solutes. Millipore water purification systems employ both reverse osmosis membranes as well as ultrafiltration membranes. Reverse osmosis systems are primarily used to purify tap water to purities that exceed distilled water quality. Ultrafiltration systems ensure that ultrapure water is free from endotoxins as well as nucleases for critical biological research.

There are various configurations of operating a filtration process:

### Dead-end Filtration

The most basic form of filtration is dead-end filtration. The complete feed flow is forced through the membrane and the filtered matter is accumulated on the

surface of the membrane. The dead-end filtration is a batch process as accumulated matter on the filter decreases the filtration capacity, due to clogging. A next process step to remove the accumulated matter is required. Dead-end filtration can be a very useful technique for concentrating compounds.

### Cross-flow Filtration

With cross-flow filtration a constant turbulent flow along the membrane surface prevents the accumulation of matter on the membrane surface. The membranes used in this process are commonly tubes with a membrane layer on the inside wall of the tube. The feed flow through the membrane tube has an elevated pressure as driving force for the filtration process and a high flow speed to create turbulent conditions. The process is referred to as "cross-flow", because the feed flow and filtration flow direction have a 90° angle. Cross-flow filtration is an excellent way to filter liquids with a high concentration of filterable matter.

### Hybrid-flow Filtration

The hybrid flow process combines the dead-end and the cross-flow principle. As in the cross-flow filtration tubular membranes are with the filtration layer on the inside wall are used. The filtration process has two phases: the production phase and the flushing phase. During the production phase, the tubes are closed on one side and a dead-end filtration is performed. During the flushing phase, the tube is open on both sides and the fraction that did not pass through the membranes is removed in order to clean the membrane surface as in cross-flow filtration. This filtration technique is especially suitable for treating water streams containing suspended solids in low concentrations (polishing).

### Submerged Filtration

With submerged membrane filtration the membranes are submerged in the liquid that has to be filtered. The filtration is performed from the outside to the inside of the membrane (filtering layer is on the outer side of the tube or plate). Sheer forces along the membrane surface are created by a flow of air bubbles along the surface. In some cases the airflow also results in a liquid flow created by the airlift principle. The driving force is a vacuum applied on the inner side of the membrane.

## DEAD END FILTRATION: THEORY AND EQUATIONS

The dead end filtration is the one where the flow of water is perpendicular to the membrane surface. The water is pushed through the membrane by pressure. All the water that is introduced in the dead-end-cell passes through as the permeate, in other words there is no rejected water. In dead end filtration the retained particles build up with time one the membrane surface or within the membrane. In either case, the particle builds results in an increased resistance to filtration and causes the permeate flux to decline, as a result dead end filtration requires the stopping of

filtration in order to clean or replace the membrane therefore this type of filtration is also called batch filtration.

There are two types of filtration which can be employed in a dead end cell unit; dead-end microfiltration with constant flux and dead end microfiltration with constant pressure drop. The dead end microfiltration with constant flux ensures that the permeate flux through the filter remains constant, this filtration can be achieved by positive displacement pump. As the cake build-up increases with time, the pressure drop must be increased to maintain constant flux. In dead end microfiltration with constant pressure, as the cake build-up with the time the permeate flux decreases.

## MEMBRANE TRANSPORT MECHANISM

Membranes provide absolute barrier to particles greater than their pore size. A membrane process requires two bulk phases physically separated by a third phase, the membrane (Fig. 17.1). The membrane phase interposed between the two-bulk phases controls the exchange of mass between the two bulk phases in a membrane process. The process allows the selective and controlled transfer of a certain species from one bulk phase to another bulk phase separated by the membrane.

The flow of water through membranes can be modeled using empirical equations.The Hagen-Poiseuille or Carman-Kozeny models can be used to demonstrate this flow of water. The use of these equations depends on the shapes and sizes of the pores.

### Hagen-Poiseuille Equation

The Hagen-Poiseuille equation considers that the membrane has a number of parallel cylindrical pores,which are parallel or oblique to the membrane surface. It assumes that the capillaries are uniform and cylindrical.

$$J = \left( \frac{\varepsilon_m \cdot r^2}{8\eta\tau} \right) (\Delta P) / \Delta X_m)$$

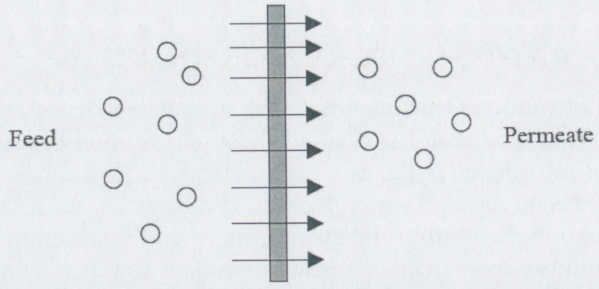



**Fig. 17.1: Schematic representation of the different phases in membrane separation**

Where,

J is the flux($L/m^2.hr$)

$\Delta P$ is the pressure difference ($N/m^2$)

$\Delta X_m$ is the membrane thickness (m)

$\eta$ is the viscosity of liquid ($N.s/m^2$)

$\varepsilon_m$ is the surface porosity

*Carman-Kozeny Equations*

This equation is used for membranes which consist of closely packed spheres.

$$J = \left( \frac{\varepsilon_m^3 \cdot r^2}{K \Delta X_m S_m^2 (1-\varepsilon_m)} \right) (\Delta P / \eta)$$

Where,

J is the flux($L/m^2.hr$)

$S_m$ is the pore internal surface area/unit volume (1/m)

$\Delta P$ is the pressure difference ($N/m^2$)

K is the Carman-Kozeny constant

$\Delta X_m$ is the membrane thickness (m)

$\eta$ is the viscosity of liquid ($N.s/m^2$)

$\varepsilon_m$ is the surface porosity

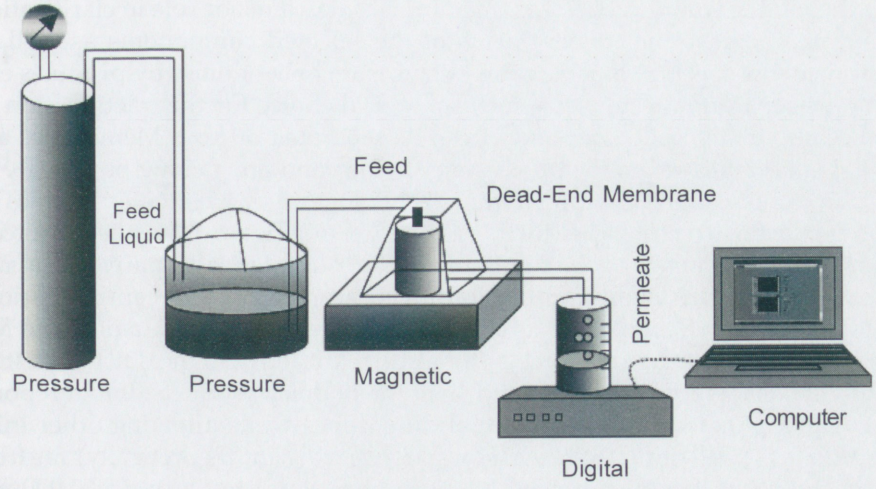



**Fig. 17.2 A typical process configuration for batch filtration using an inert gas blanket on the feed reservoir.**

## CHARACTERISTICS OF MEMBRANE

Microfiltration (MF) is a membrane separation process similar to ultrafiltration (UF) but with even larger membrane pore size allowing particles in the range of 0.2 to 2 micrometers to pass through. The pressure used is generally lower than that of UF process. MF membranes are especially well suited for the separation of fine particles in the size range of 0.1 to 10.0 μm whereas UF membranes with 1 to 100 nm pore size were designed to provide high retention of proteins and other macromolecules. UF is a variety of membrane filtration in which hydrostatic pressure forces a liquid against a semi-permeable membrane. Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane. It is a separation/fractionation process using a 10,000 MW cutoff, 40 psig, and temperatures of 50 to 60°C with polysulfone membranes. Nanofiltration (NF) is a type of reverse osmosis where the membrane has a slightly more open structure allowing monovalent ions to pass through the membrane and rejecting the divalent ions to a great extent. RO is a high pressure-driven membrane filtration process which is based on a very dense membrane. The Reverse osmosis (RO) membrane pore size is very small allowing only small amounts of very low molecular weight solutes to pass through the membranes. It is a concentration process using a 100 MWCO (molecular weight cutoff), 700 psig, temperature lower than 40°C with cellulose acetate membranes and 70–80°C with composite membranes. MWCO is the equivalent molecular weight of the smallest protein that would exhibit above 90% rejection. Diafiltration (DF) is a specialized type of ultrafiltration process in which the retentate is diluted with water and re-ultrafiltered, to reduce the concentration of soluble permeate components and to further increase the concentration of retained components.

### Use of Membrane in Dairy Industry

In the modern dairy processing, membranes play a major role in clarification of the milk, increase the concentration of the selected components as well as separation of the specific valuable components from milk or dairy by-products etc. Membrane separation technology seems a logical choice for the fractionation of milk, because many milk components can be separated on size. Membranes are already well established in the processing of whey and are gaining popularity in other dairy applications. The membrane technology improves the economics of dairy by reducing the cost of production as well generating new revenue resources. Membrane technology proves a suitable and economical alternative to many important processing stage of milk in dairy industry such as centrifugation, bactofugation, evaporation, demineralization of whey, etc. The $CO_2$-aided cold MF process has the potential to become economically attractive to the dairy industry, with direct benefits for the quality and shelf life of dairy products. Initial reports have indicated that MF will reject fat and microbes while allowing other milk constituents to pass through the membrane, resulting in (theoretically) fat-free, bacteria-free milk. The membrane area of RO has stabilized around 60,000 $m^2$, mainly for whey concentration. MF is developing due to its capability to retain, partly or totally, particles (microorganisms, casein micelles, fat globules), and NF

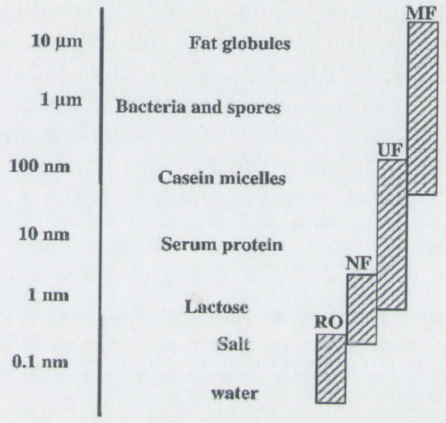



**Fig. 17.3. Components in milk: size indication and membrane processes. MF = Microfiltration, UF = Ultrafiltration, NF = Nanofiltration, RO = Reverse osmosis**

has a large field of applications due to its intermediate selectivity (200 to 1,000 Da) between UF and RO (demineralization, de-ionization, purification) in particular for whey protein valorization.

## APPLICATION OF MEMBRANES IN DIFFERENT FIELDS OF DAIRY TECHNOLOGY

Various types of membrane filters with different properties are available in market and commonly used in the dairy industry. Membranes have been applied in different fields of dairy technology including shelf life of milk, whey processing, cheese industry, milk protein processing, fractionation of milk fat and desalting or demineralization.

### Application of Membranes in Extending Shelf Life of Milk

Extended shelf life (ESL) milk products are products that have been treated in a manner to reduce the microbial count beyond normal pasteurization, packaged under extreme hygienic conditions, and have a prolonged shelf life under refrigerated conditions. Microfiltration constitutes an alternative to heat treatment to reduce the presence of bacteria and improve the microbiological safety of dairy products while preserving the taste. It is a non thermal method of removing bacteria and spores from milk, whey and cheese brine and extending shelf life without damaging sensory attributes. The significant reduction of mesophilic, *Salmonellae* and *Listeria* count have been reported upon using MF with 1.4 µm pore size. Cold MF could also minimize microbial fouling of the membrane and prevent the germination of thermophilic spores. performed a study to analyse microbiological, chemical, and somatic cell count to evaluate the effect of MF on the composition of skim milk and found that permeate flux increased drastically when velocity was increased from 5 to 7 m/s.

By the suitable modifications in the membranes structure, design and composition, it is possible to make milk free from bacteria. ESL milk has been prepared by removing bacteria from milk by MF without causing any compositional change or negligible decrease in the total protein by 0.02 to 0.03%. A thermal treatment or combination of heat treatment and membrane filtration are involved in the production of ESL milk.

By adding a filtrate circulatory system in the concentrate circulatory system of membrane filters (better known as UTMP concept Uniform Transmembrane Pressure concept), up to 99.70% of the bacterial load in skim milk has been achieved by Alfa Laval. Tetra Alcross Bactocath of Alfa Laval removes the bacteria and spores from milk by separating milk into cream and skim milk and then separates the skim milk from bacteria and spores by microfiltration, while retaining all bacteria and spores in retentate which is about 0.5% of the original milk volume. The retentate is mixed with suitable amount of cream and mixed in filtered milk after pasteurizing it. As the heat treatment is given to a very small amount of milk, the organoleptic and sensory attributes of milk are retained. It extends the shelf life of milk by 12 to 45 d at 4°C. The major hitch is that it does not remove all pathogenic bacteria from milk, thus still necessitate the heat treatment.

The somatic cell counts (SCC) increase in the milk of lactating cows suffering from mastitis and thus severely affecting the composition and quality of milk. This somatic cell count can be reduced up to 100% by the application of combined processes of MF (microfiltration) followed by HHT (high heat treatment). reported the extension of shelf life of refrigerated milk from 12 d to 18 d at 8°C by the combined application of MF and HHT without compromising sensory attributes by reducing the number of psychrotrops. The MF process is more efficient in removing bacteria and spores than the bactofugation. With the direct epifluorescent filter technique (DEFT) technology, keeping quality of milk can be judged in short time by concentrating the bacterial cells and their spores as well as somatic cells by membranes followed by quantification of these by epifluorescent microscopy.

Recently narrow pore sizes in smooth inert silicon nitride surface microsieves have been prepared by micro-machining technology to increase the selectivity and permeability to pass liquids at lower transmembrane pressure. The microsieves also reduce the fouling as well as microbial count significantly in milk.

### Application of Membranes in Whey Processing

Whey is a dairy by-product which is obtained during the preparation of milk products viz. cheese, paneer and casein. Paneer is an Indian dairy product similar to soft cheese prepared by coagulating casein with citric acid, lactic acid or tartaric acid. Whey is simply drained in most of the cases in developing countries. This causes huge loss of valuable nutrients as well as creating environmental hazards. The separation or concentration of whey nutrients by traditional method is cumbersome and time consuming. By application of different membrane filtration technology, the nutrients in whey are concentrated, fractionalized or purified into valuable products such as whey protein concentrate/isolates, α-lactalbumin, β-

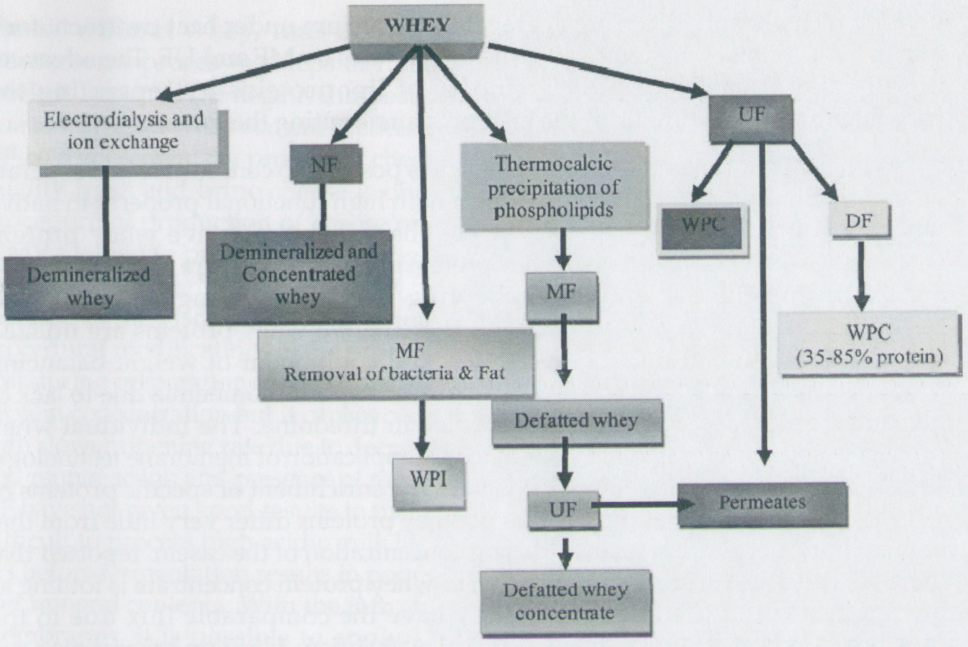



**Fig. 17.4. Diagrammatic representation of use of membrane in whey processing.**

lactoglobulin, lactose and salts. Up to 60% more saving on fuel has been reported in whey concentration by applying RO over the traditional evaporation methods. By application of UF and DF, the protein content of the whey protein concentrate can be increased by 35 to 85% of the total solids whereas by removing bacteria and fat by passing whey through MF, the protein content of whey protein isolates can be increased to 90% of the total solid content.

The membrane separation of bacteria and spores also leads to production of high quality whey protein concentrate (WPC) and whey protein isolates (WPI) with inherent functional properties from whey as it avoid the denaturation of whey or serum proteins. These whey proteins are used in food industry for their high biological value as well as their ability to improve the functional properties (emulsifying, foaming and gelling) of the food products. The removal of as much lipids as possible from whey by efficient membrane function produces whey protein concentrate with good keeping quality. At present the WPC are prepared on industrial scales by using UF, however fouling of membranes still remains a concern. UF and RO have application in production of concentrated whey and whey protein concentrates.

The development of off-flavour in these whey products due to the impurities of lipids causing rancidity remain the major concern. A number of studies have suggested the removal of residual fat from whey by thermocalcic precipitation

under which phospholipids are aggregated by calcium under heat treatment for 8 min at 50°C followed by removal of the precipitates by MF and UF. The advances in MF make it easier to direct removal of lipoproteins by separating the transmembrane pressure from the inlet pressure limiting the fouling.

With the use of membrane technology, it is possible to concentrate and separate whey proteins in their un-denatured form with high functional property in native whey as compared to traditional sweet cheese whey. Native whey protein concentrates (NWPC) and native whey protein isolates (NWPI) are produced by UF concentrating the native whey, showing excellent gelling, foaming and reconstituability on drying. At present, these native whey proteins are utilized extensively in human nutrition as an integral component of weight balancing products and in baby foods due to lower risk of hyperthreoninemia due to lack of glycomacropeptides which in turn are rich in threonine. The individual whey protein can be concentrated or fractionated by application of membrane technology. This facilitates the production of WPC with the enrichment of specific proteins or with single proteins. The composition of whey proteins differ very little from that of membrane permeates obtained during concentration of the casein. reported that the main concern during the preparation of whey protein concentrate is fouling as membranes with different permeability have the comparable flux due to the pressure independent flux regime. A typical whey micro-filtration process may run in long cycles of about 24 h, which offer great potential for the formation of bacterial biofilms leading to reduced membrane performances.

## Application of Membranes in Cheese Industry

Membrane filtration technology has number of applications in cheese industry such as improving the nutritive quality, better compositional control and yield of cheese by increasing the total solid content, utilization of whey during preparation of cheese, comparatively reducing the requirement of rennet and starter culture. Concentrating the milk prior to cheese making opens a new arena in cheese industry thereby reducing the cost as well as hasten the whole procedure of cheese making. The quality of cheese brine is maintained easily by membrane filters of pore size of 100 μm to removing bacteria and spores as well as other extraneous matters and maintaining the chemical balance of brine which results in production of high quality cheese with superior flavour and shelf life.

The UF and MF are most commonly used in the cheese industry.

### *Use of UF in Cheese Making*

UF technology in cheese processing is highly remunerative by concentrating milk by a factor of 1.2 to 2.0 times and increase in the casein: protein ratio leading to fewer requirements of processing equipments, better compositional and quality control as well as improving the yield of cheese. A 13 to 14% saving of skim milk has been reported during preparation of quarg cheese with the application of UF process as compared with traditional process. By removing the water during concentration of milk by the application of membrane filtration technology, it is possible to make

cheese without whey, thus do not require the use of cheese vat and avoid cumbersome processing steps of removal and draining of whey. However, some workers reported the negative impact on the ripening of semi-hard and hard cheese due to increased whey content in cheese prepared by concentrated milk. rightly called UF as complementary process of cheese making. With the application of UF, good quality fresh and brine cheese is obtained with higher yield. first patented the procedure of production of cheese on industrial basis from UF retentate by using MMV method (a process named after its inventors Maubois, Mocquot and Vassal) by concentrating the milk by 5 to 7 times and getting the high quality curd. The increase in the viscosity and buffering capacity of such cheese milk needs certain procedural modifications in the process of cheese making. UF of milk is done to save cost during preparation of semi-hard and hard cheese by increasing the salt and whey protein concentration but it causes compromised sensory and functional properties with slower ripening rate due to decreased proteolysis caused by reduction chymosin and rennin action and presence of more whey proteins. Some reports mentioned that UF have not given good results in making of fresh cheese from acidified curd as it is difficult to process high acidic milk by these traditional membranes. Increasing the pH before fermentation results in poor sensory attributes with enhanced proteolysis and mineral contents. With the introduction of advanced ceramic and polysulfone membranes, it is possible to apply UF to acidic milk with pH 4.4 to 4.6 in the production of better quality of fresh cheese.

Based on the concentration factors and increase in the protein content, there are three different types of retentate obtained in the UF viz. low concentrated retentate (LCR), medium or intermediate concentrated retentate (MCR) and liquid pre-cheese (LPC).

*Use of MF in Cheese Making*

The MF casein concentrated milk is very suitable for various cheese preparations due to its superior microbial quality achieved after removing bacteria and spores from milk, as well as optimization of the different major milk components. The MF pre-treatment of cheese milk improves the firmness of curd, accelerate ripening, reduce the amount of additives e.g. $CaCl_2$ and facilitates heating at higher temperature. Further the UHT milk need higher amount of rennet for coagulation. By application of UF, UHT milk is coagulated with lesser amount of rennet by compensating the hindrance to coagulation arising from the formation of complexes between k-casein and β-lactoglobulin by reduction of the zeta potential of the casein micelle.

The MF has future possibilities in standardization of protein in cheese milk and fortification with casein micelle powder. The MF followed to HTT (high temperature treatment) is more efficient than single stage bactofugation in terms of microbial quality. But this treatment of MF followed by HTT modifies rennet coagulation and increases the water holding capacity, necessitating adjustments in the parameters during cheese preparation.

## Application of Membranes in Milk Protein Processing

Milk proteins especially caseins play important role in white turbid appearance and viscosity of milk. By application of ultra filtration, the level of protein content in milk is adjusted without adding any extraneous protein source by removing the water. This causes the maintenance of the composition, nutritive value, physico-chemical properties and sensory attributes of the milk irrespective to the variation caused by the genetic as well as environmental factors. Adding of 1% ultra filtered protein enriched milk in skim milk has improved the viscosity and sensory attributes to similar to full cream milk. The milk protein concentrate (MPC) containing 50 to 58% proteins of good functionality are prepared by application of MF, UF and diafiltration (DF) technologies either alone or in combinations.

### *Fractionalization of Milk Protein*

Separating out the various milk components of different sizes from milk by applying membrane technology will transform dairy industry into very efficient and profitable enterprises. described membrane pore size homogeneity, concentration polarization phenomena and membranes fouling as main factors determining the fractionalization of milk components. The milk protein concentrates (with 50 to 85% protein in the products) and milk concentrates (with more than 85% protein in the products) are increasingly prepared by using MF. The composition, heat stability, rheological and textural properties of these products depend upon the type of membranes used and prevailing processing conditions such as, temperature, pH, time of processing. The judicious use of these factors should be selected based on the ultimate use of the MPC (milk protein concentrate) and MP (milk protein). suggested UF processing at 50 to 60°C with high flow rate to minimize the whey protein denaturation for desired rheological properties.

A lot of work has performed to recover valuable proteinacious components from dairy waste streams. Dairy proteins are valuable products and used as high-value food additives, neutriceutical and therapeutics. The milk protein can be recovered by using MF, UF and NF processes. MF of milk separates the casein micelles and whey proteins. The various milk proteins such a casein and serum or whey proteins are separated in a simple and economic method by applying membrane filters of 0.2 μm pore size at constant TMP, without the need of application of heat and chemicals.

### *Casein*

Membrane technology has revolutionized the casein industry. Application of different membrane types with desired pore size either alone or in combination with enzymatic action, chromatography lead to the concentration, fractionalization and purification of milk proteins. The physico-chemical properties of casein in relation to membranes largely depend upon the ionic strength as well as temperature. At suitable ionic strength, it is possible to concentrate and fractionate casein as β-casein at 5°C, skim milk at 4°C and pH 4.2 to 4.6 using MF and UF separating β-casein in permeates and α and κ-casein in retentate. The native casein

can be concentrated in retentate by passing the skim milk into MF with 0.2 μm pore size. Up to 90% casein concentration is possible by DF and it can be used for industrial, pharmaceutical as well as edible purposes. The concentrated casein and native casein micelles can be prepared through ceramic, ceraflow, membralox membrane with different flux. The significant improvement was recorded in flux with 65 kPa transmembrane pressure having flow velocity of 12.5 metre/second.

Researchers found that although there was higher profit in the production of cheddar and mozzarella cheese by MF milk in North America but the profit changed little with the increase in concentration of casein from 2 to 3 times. The fouling is major concern than selectivity during the concentration of casein in milk as some whey proteins are there in casein and vice versa and thus affecting the yield of cheese as well as whey concentrates. Use of membrane process accompanied with other advance technology as liquid chromatography, leads to purification of casein derived bioactive peptides showing morphine-mimicking, cardiovascular and immunostimulating activities.

**Fractionalization of Whey Protein**

Whey proteins are obtained by concentrating and purification from whey. and separated the whey protein from skimmed milk by polymeric microfiltration in permeate flux. Mass flux form of permeated whey protein with permeates flux, mainly determine the whey protein concentration and fractionalization. With the advancement of newer technology in membrane process, it is possible to recover growth factor from whey. Attempts have been made to tap the growing market of health foods by separating immunoglobulins and growth factors from colostrum by applying MF and UF with suitable modifications.

The β-lactoglobulin is separated by the membrane process followed by heat treatment. The β lactoglobulin can be fractionized from defatted whey proteins by using MF and centrifugation followed by purification by UF with electrodialysis (ED) or DF. β-lactoglobulin is purified by diafiltration of supernatant, while immunoglobulins are separated from whey protein concentrates by using membrane technology. The α-lactalbumin is separated and purified by applying UF on solubilised MF retentate at neutral pH. With the introduction of inorganic membranes with polyvinylimidazole derivates, the pure α-lactalbumin can be obtained in filtrate but the fouling proves major constraint. The whey proteins can be separated from WPI by applying UF/DF with more than 99% purity. The use of hydrophilic cellulose membrane made it possible to separate low molecular weight compounds from high molecular weight compounds. Lactoferrin and lactoperoxidase are recovered from defatted WPC on industrial scale by using ion-exchange chromatography.

**Application of Membranes in Milk Fat Processing**

Traditionally, cream is separated from whole milk by energy intensive centrifugation in which lighter fat globules moves towards the centre and heavier skim milk move towards periphery under the centrifugal force. The cream

separation is also possible by energy saving membranes technology producing skim milk with good storage quality and improved sensory attributes of cream without causing any damage to fat globular membranes. reported the fractionation of fat globules by application of membranes of 2 μm size. The size of fat globules have profound impact on textural and sensory attributes as the cream with smaller fat globules have fine texture and improved flavour than cream with large fat globules.

**Role of membranes in desalting or demineralization**

The removal of minerals from whey increases its value. The cheese whey is rich in salt and acids and the reduction or demineralization of whey is essential before its use and to alleviate the environmental hazards. The demineralization is done by electrodialysis and ion exchange process in the dairy industry to get up to 60% reduction of minerals. The efficiency of electrodialysis can be increased by pre-concentrating up to 20% dry matter by RO or evaporation. By ion exchange, desalting of whey is done by passing it over ion exchange column and the rate of removal of ions depends upon the resin used in the column as well as the type of ions. The need of lots of water and chemicals for regeneration of resins is the major limitations of this technology.

The NF membranes with molecular weight cutoff (MWCO) ranging between 200 to 1,000 Daltons are best suited for demineralization of whey as these membranes are permeable to salt and monovalent ions but impermeable to organic compounds. The carboxyl group of organic compounds bound to these membranes under prevailing acidic conditions. This technology simultaneously demineralises whey at the time of concentrating, helping in savings in term of cost, time and water disposal. The NF is more economical than electrodialysis and a method of choice for partial desalting of whey. NF membranes are highly permeable to water and monovalent ions. The mineral contents of the whey is reduced by 35% and ash content by 3 to 4 times in addition to increase concentration of whey by applying nanofiltration (NF), making it suitable for the people having cardiovascular diseases. The mineral content of whey is further reduced by 45% by applying DF. The reduction of mineral content in whey, liquor and brines on industrial scale can be done mainly by NF. Daifiltration also causes the removal of ions to a greater extent.

**Other applications**

The use of different membranes accompanied with suitable heat treatments leads to the development of several dairy products with unique characteristics as better compositional control, binding, texture, juiciness, etc. With the help of membrane technology, several novel products are introduced in market as lactose free milk for lactose intolerant people, low calcium milk, non fat yoghurt, high protein low lactose ice cream, protein fortified low fat milk, whey based beverages, etc. By applying RO (reverse osmosis), milk is condensed by removing about 70% of its water and retaining all other components of milk without undergoing any thermal processing. During preparation of fermented dairy milk such as cheese,

yoghurt and curd, the protein and total solids of standardized UF milk show better textural and nutritional properties than those of prepared by either adding milk powder or evaporated milk. It is easier to control the textural and compositional properties of milk by membrane filtration technology, but it necessitates better attention to the selection of starter and incubation/ripening conditions such as temperature, time and pH. noted that such changes is due to the increasing content of lactose and minerals, higher osmotic pressure, change in the pH, change in ionic strength as well accumulation of some inhibitory compounds in the UF treated milk.

The transport cost of milk is reduced by membrane technology by pre-concentrating it, removing the water and lactose or dewatering it at farm level and thus decreasing the volume of transported milk without damaging its sensory attributes. RO of milk results in permeate containing protein and minerals whereas UF of milk produces permeate consisting of water and lactose. Membrane technology is used to separate κ-casein-glycomacropeptide (GMP), which have pharmaceutical uses in controlling the adhesion of *E. coli* cells to the intestine walls, protects against influenza and prevents adhesion of tartar to teeth.

## FOULING OF MEMBRANES

Membranes are having some limitations such as fouling of membranes due to blockage of membrane pores, adsorption of particles on the pores, deposition of protein and minerals, cake formation and depth fouling, and bacterial biofilms. The fouling is caused by the accumulation of particles, bacteria and sediments present in milk leading to remarkable loss in the efficiency of membranes. The fouling of membrane remains the major concern in dairy industry. The fouling of membranes is mainly affected by several factors such as backpulsing, crossflushing, backwashing, particle size, membrane surface chemistry and ionic strength.

This major issue of fouling in membranes can be trounced by regular cleaning of membranes at appropriate time intervals, use of low fouling membranes, membrane modules with suitable channel heights, by applying high pressure, application of electric potential, ultrasound waves, microturbulence, ceramic membranes, uniform transmembrane pressure (UTP), vibrating and rotating disc modules, use of turbulent flow of liquids and high frequency back pulsing.

## FUTURE TRENDS

There is need for progressive development of innovative applications based on the technique. Currently, the membranes for dairy processes have rather a low capacity owing to strong flux decline by fouling and processes are energy demanding because of the high cross-flow velocity that is required to control fouling. Furthermore, methods to control fouling have increased the complexity in equipments and operation. Besides fouling, selectivity is an important issue for membrane fractionation of milk. Therefore, we need innovative techniques for the development of cheap, easily available, superior and long lasting membranes.

Consumers are looking for products with increased freshness and higher quality and the retailers require products with extended shelf life and the demands of both can be fulfilled by the use of the membrane technology.

## DESALTING

Desalting is for the most part applied to whey. It can be achieved in various ways. In all of these, ions are removed and not undissociated salts. This implies, for instance, that at a low pH organic acids are hardly removed because the carboxyl group is incompletely dissociated. The presence of proteins, which generally have a negative charge, impedes the removal of counterions (especially $Ca^{2+}$) and enhances that of co-ions (for the most part $Cl^-$).

### Electrodialysis (ED)

Here the ions are removed from the whey due to an electric potential gradient and the presence of two kinds of membranes, one kind being permeable to anions and the other to cations. Transmembrane pressure is zero. Generally, the whey is preconcentrated by RO or evaporation to about 20% dry matter before ED; this increases the ion concentrations and decreases the costs. Besides desalted whey, two salt solutions are produced that must be regenerated. The rate of removal varies with the kind of ions. Examples of the percentages removed when desalting whey are:

| Removal by | ED 40% | ED 60% | NF 45 % |
|---|---|---|---|
| K + Na | 42 | 64 | 45 |
| Ca | 24 | 35 | 6 |
| Cl | 71 | 89 | 54 |

where a result for nanofiltration is included for comparison; the percentage figures of ED and NF refer to the overall proportion of salts removed. ED can achieve 80% removal but one generally stops at 60% for economic reasons. To achieve further desalting, ED can be followed by ion exchange.

### Ion Exchange

Whey can be passed over ion exchange columns for desalting. The removal rate of the various ions depends on the properties of the resins applied, but very low salt contents can be reached. A disadvantage is that the resin must be frequently regenerated, which requires large quantities of chemicals and water.

### Nanofiltration

Although this method is less efficient for salt removal than ED, it has the advantage that the liquid is also concentrated, which often is desired anyway. Consequently, it is generally more cost-effective than ED, and it has become the method of choice for partial desalting of whey.

Nanofiltration evolved because membrane materials were developed that have a high solubility - therefore, a high permeability - for monovalent ions and water but not for other components. Generally, the permeability to other salts is low, whereas proteins, lactose, urea, and other small nonionic molecules are almost totally reflected. Examples of the percentage removed for some ions as a function of pH are given in the following table:

| pH of whey | 4.6 | 5.8 | 6.6 |
|---|---|---|---|
| Ca + Mg | 4 | 2 | 2 |
| Na + K | 62 | 46 | 35 |
| Anions | 40 | 37 | 29 |

The anions removed consist for the most part of $Cl^-$. It is seen that desalting works better at a lower pH.

In most cases, the whey is concentrated to 20% dry matter and an overall salt removal of 40 to 50%. Considerably greater removal of monovalent ions can be reached by diafiltration; most other ions are still retained. Also, the whey obtained from Cheddar curd after salting, which contains a very high NaCl content, can be effectively desalted.

The membranes are comparable to those used for RO in structure and composition. However, the difference in osmotic pressure between retentate and permeate is not very high. This is because the concentration factor reached is not very high, and the monovalent ions passing the membrane make up a significant part of the osmotic pressure of whey. Hence, the transmembrane pressure need not be as high as for RO; it is generally 0.2 to 0.4 MPa. This means that the technical operation is similar to that applied in ultrafiltration. Nanofiltration is generally done at a low temperature, e.g., 25°C, because the selectivity of the separation decreases with increasing temperature.

# 18

# Lactic Acid Bacteria

**Topics**

*Metabolism • Homolactic Fermentation • Heterolactic Fermentation • Fermentation of Foods by Lactic Acid Bacteria • Starter cultures • Probiotics and Lactic Acid Bacteria • Lactic acid-producing Bacteria Used as Probiotics*

Lactic Acid Bacteria (LAB) are Gram-positive, non-sporeforming cocci, coccobacilli or rods with a DNA base composition of less than 53mol% G+C. They generally are non respiratory and lack catalase. They ferment glucose primarily to lactic acid, or to lactic acid, $CO_2$ and ethanol. All LAB grow anaerobically, but unlike most anaerobes, they grow in the presence of $O_2$ as "aerotolerant anaerobes". Although they lack catalase, they possess superoxide dismutase and have alternative means to detoxify peroxide radicals, generally through peroxidase enzymes.

Although many genera of bacteria produce lactic acid as a primary or secondary end-product of fermentation, the term **Lactic Acid Bacteria** is conventionally reserved for genera in the order *Lactobacillales*, which includes *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus* and *Streptococcus*, in addition to *Carnobacterium, Enterococcus, Oenococcus, Tetragenococcus, Vagococcus*, and *Weisella.*

Because they obtain energy only from the metabolism of sugars, lactic acid bacteria are restricted to environments in which sugars are present. They have limited biosynthetic ability, having evolved in environments that are rich in amino acids, vitamins, purines and pyrimidines, so they must be cultivated in complex media that fulfil all their nutritional requirements. Most are free-living or live in beneficial or harmless associations with animals, although some are opportunistic pathogens. They are found in milk and milk products and in decaying plant materials. They are normal flora of humans in the oral cavity, the intestinal tract and the vagina, where they play a beneficial role.

A few LAB are pathogenic for animals, most notably some members of the genus *Streptococcus*. In humans, *Streptococcus pyogenes* is a major cause of disease

(strep throat, pneumonia, and other pyogenic infections, scarlet fever and other toxemias), *Streptococcus pneumoniae* causes lobar pneumonia, otitis media and meningitis; some viridans and nonhemolytic oral streptococci play a role in dental caries and may be an insidious cause of endocarditis. The pathogenic streptococci are dealt with elsewhere in the text. This chapter deals primarily with LAB in association with food and dairy microbiology, to a lesser extent with LAB as beneficial components of the human normal flora and probiotics.

Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid. As agents of fermentation LAB are involved in making yogurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives and sauerkraut, but some species may spoil beer, wine and processed meats.

**Differential characteristics of lactic acid bacteria based on morphology and physiology**

| Genus<br>Characteristic | Lacto-bacillus | Entero-coccus | Lacto-coccus | Leuco-nostoc | Pedio-coccus | Strepto-coccus |
|---|---|---|---|---|---|---|
| Morphology | rods | cocci | cocci | cocci | cocci in tetrads | cocci |
| $CO_2$ from glucose* | ± | – | – | + | – | – |
| Growth | | | | | | |
| at 10°C | ± | + | + | + | ± | – |
| at 45°C | ± | + | – | – | ± | ± |
| in 6.5% NaCl | ± | + | – | ± | ± | – |
| at pH 4.4 | ± | + | ± | ± | + | – |
| at pH 9.6 | – | + | – | – | – | – |
| Lactic acid configuration | D, L, DL | L | L | D | L, DL | L |

+ positive; - negative; ± varies between species

*test for homo- or heterofermentation of glucose: - homofermentation

+ heterofermentation

## METABOLISM

The essential feature of LAB metabolism is efficient carbohydrate fermentation coupled to substrate-level phosphorylation. Adenosine triphosphate (ATP) generated is subsequently used for biosynthesis. LAB as a group exhibit an enormous capacity to degrade different carbohydrates and related compounds. Generally, the predominant end product is lactic acid (>50% of sugar carbon). However, LAB adapt to various conditions and change their metabolism accordingly. This may lead to significantly different end-product patterns.

Based on sugar fermentation patterns, two broad metabolic categories of LAB exist: homofermentative and heterofermentative. The first category, **homofermentative** LAB, includes some lactobacilli and most species of enterococci, lactococci, pediococci, streptococci, tetragenococci, and vagococci, that ferment hexoses by the Embden-Meyerhof (E-M) pathway. The second category, **heterofermentative** LAB, includes leuconostocs, some lactobacilli, oenococci, and weissella species. The apparent difference on the enzyme level between these two categories is the presence or absence of the key cleavage enzymes of the E-M pathway (fructose 1,6-diphosphate) and the PK pathway (phosphoketolase).

## HOMOLACTIC FERMENTATION

Under conditions of excess glucose and limited oxygen, homolactic LAB catabolize one mole of glucose in the Embden-Meyerhof pathway to yield two moles of pyruvate. Intracellular redox balance is maintained through the oxidation of NADH, concomitant with pyruvate reduction to lactic acid. This process yields two moles of ATP per glucose consumed. Representative homolactic LAB genera include *Lactobacillus, Lactococcus, Enterococcus, Streptococcus* and *Pediococcus*species.

The transport and phosphorylation of sugars occur by (1) transport of free glucose and phosphorylation by an ATP-dependent hexose kinase (other sugars, such as mannose and fructose, enter the major pathways at the level of glucose-6-phosphate or fructose-6-phosphate after isomerization or phosphorylation or both); or (2) the phosphoenolypyruvate (PEP) sugar phosphotransferase system (PTS), in which PEP is the phosphoryl donor for the uptake of sugar. Some species of LAB use the PTS for transport of galactose only; others use the PTS for all sugars (Fig. 18.1).

## HETEROLACTIC FERMENTATION

Heterofermentative LAB utilize the phosphoketolase pathway (pentose phosphate pathway) to dissimilate sugars. One mole of glucose-6-phosphate is initially dehydrogenated to 6-phosphogluconate and subsequently decarboxylated to yield one mole of $CO_2$. The resulting pentose-5-phosphate is cleaved into one mole glyceraldehyde phosphate (GAP) and one mole acetyl phosphate. GAP is further metabolized to lactate as in homofermentation, with the acetyl phosphate reduced to ethanol via acetyl-CoA and acetaldehyde intermediates. Theoretically, end- products ($CO_2$, lactate and ethanol) are produced in equimolar quantities from the catabolism of one mole of glucose. Obligate heterofermentative LAB include*Leuconostoc, Oenococcus, Weissella*, and certain lactobacilli (Fig. 18.2).

Lactic acid bacteria have a very limited capacity to synthesize amino acids using inorganic nitrogen sources. They are therefore dependent on preformed amino acids being present in the growth medium as a source of nitrogen. The requirement for amino acids differs among species and strains within species. Some strains are prototrophic for most amino acids, whereas others may require 13–15 amino acids. Since the quantities of free amino acids present in their environment are not sufficient to support the growth of bacteria to a high cell density, they require a proteolytic system capable of hydrolyzing peptides and proteins in order to obtain

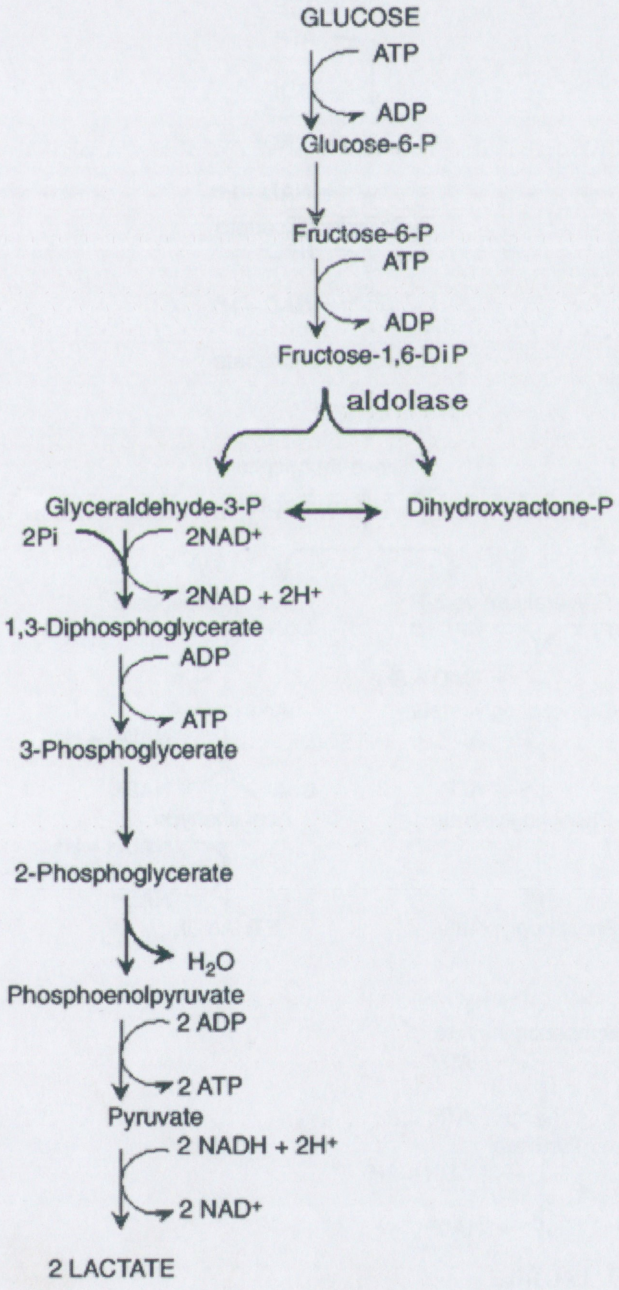



**Fig. 18.1. The pathway of homolactic acid fermentation in Lactic Acid Bacteria**

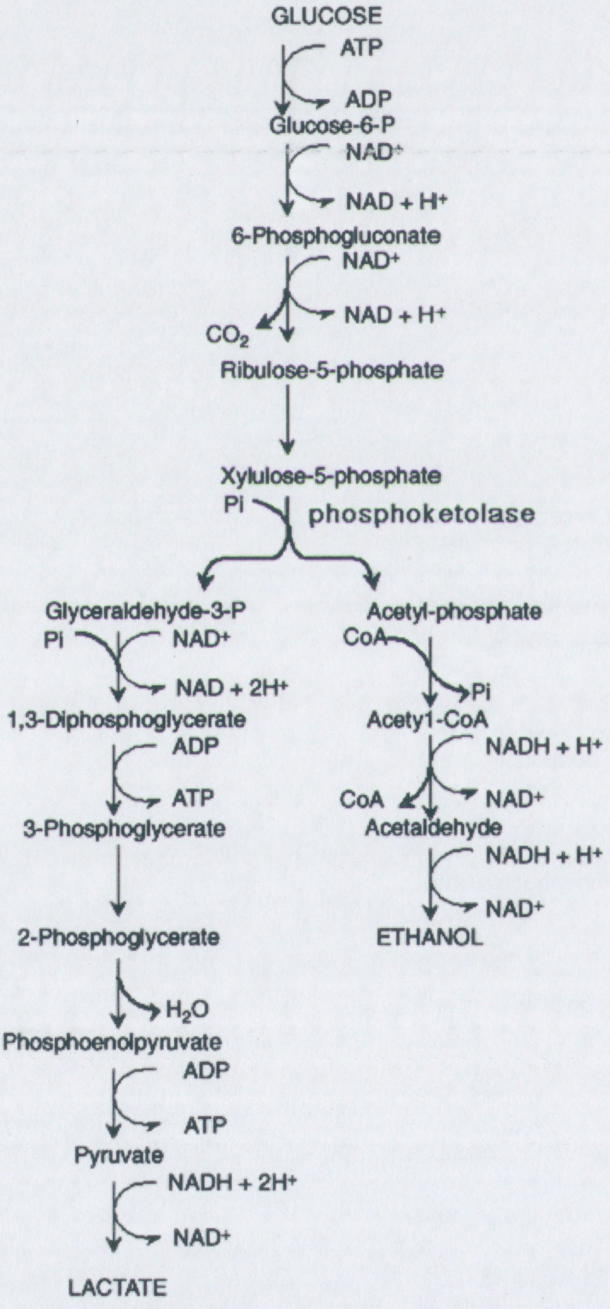



**Fig. 18.2. The pathway of heterolactic acid fermentation in Lactic Acid Bacteria**

essential amino acids. All dairy lactococci used for acidification of milk (e.g., in cheese manufacture) have proteolytic activity. The lactococcal proteolytic system consists of enzymes outside the cytoplasmic membrane, transport systems, and intracellular peptidases. The proteolytic activity of LAB contributes additionally to the development of the flavor, aroma and texture of fermented products. For many varieties of cheeses, such as Swiss and *Cheddar*, desirable "flavour tones" are derived by proteolysis.

## FERMENTATION OF FOODS BY LACTIC ACID BACTERIA

Many human foods are plants or animal products which have been fermented by lactic acid bacteria, since these bacteria possess properties that can benefit food production or conversion. The acidic and organoleptic properties of fermented foods result from the metabolic activities of these microorganisms. Foods such as ripened cheeses, fermented sausages, sauerkraut and pickles have not only a greatly extended shelf life compared to the raw materials from which they are derived, but also aroma and flavor characteristics contributed directly or indirectly by the fermenting organisms.

Fermented dairy products have been made for thousands of years, but only within the last century have the microbiological bases of these fermentations been elucidated. Lactic acid bacteria are the principal organisms involved in fermenting dairy products. Prior to the availability of starter cultures, milk fermentations relied on the LAB naturally present in raw milk. The first commercial starter cultures were unknown mixes of microbes from raw milk that were prepared in Denmark around end of the 19th century. In the 1930s and 40s, the idea of pure single-strain starter cultures evolved.

Fermented dairy products are enjoying increased popularity as convenient, nutritious, stable, natural, and healthy foods. Lactic acid bacteria are the principal organisms involved in the manufacture of cheese, yogurt, buttermilk, cottage cheese, sour cream and cultured butter. In some fermented dairy products, additional bacteria, referred to as secondary microflora, are added to produce carbon dioxide, which influences the flavor and alters the texture of the final product.

Sausage is one of the oldest processed meat products. The writings of ancient Egyptians described the preservation of meat by salting and sun drying. The ancient Babylonians, Greeks, and Romans used sausage as a food source during times of war. Microorganisms were recognized as being important to the production of sausages about 1921. In the 1940s and 1950s, pure microbial starter cultures consisting of lactic acid bacteria became available but their use was not widespread until the early 1980s.

The fermentation of vegetables, a practice that originated in the Orient, has been used as a means of preserving food for more than 2000 years. In the third century B.C., during the construction of the Great Wall of China, the Chinese produced fermented vegetables (cabbages, radishes, turnips, cucumbers, etc.) on a large scale.

The most common fermented vegetables available in the United States are pickles, sauerkraut, and olives. Carrots, cauliflower, celery, okra, onions, and sweet and hot peppers also are sold as fermented vegetable products.

Generally LAB that are important in the fermentation of food products (dairy, meat, vegetables, fruits, and beverages), include only certain species of the genera *Lactobacillus, Lactococcus, Streptococcus, Leuconostoc* and *Pediococcus*. Some of these species are also members of normal flora of the mouth, intestine, and vagina of mammals.

**Lactobacilli**

*Lactobacillus* is very heterogeneous genus, encompassing species with a large variety of phenotypic, biochemical, and physiological properties.

Most species of lactobacilli are homofermentative, but some are heterofermentative. The genus has been divided into three major subgroups and over 70 species are recognized. Group I lactobacilli are obligately homofermentative and produce lactic acid as a major end product (>85%) from glucose. They are represented by *L. delbrueckii* and *L. acidophilus*. They grow at 45°C but not at 15°C. Group II, also homofermentative, grow at 15°C and show variable growth at 45°C. Represented by *L. casei* and *L. plantarum*, they can produce more oxidized fermentations (e.g. acetate) if $O_2$ is present. Group III lactobacilli are heterofermentative. They produce lactic acid from glucose, along with $CO_2$ and ethanol. Aldolase is absent and phosphoketolase is present. Representative species include *L. fermentum, L. brevis* and *L. keferi*.

Lactobacilli are often found in dairy products, and some species are used in the preparation of fermented milk products. For example *L. delbrueckii* subsp*bulgaricus* are used in the preparation of yogurt; *L. acidophilus* is used in the preparation of acidophilus milk; *L. helveticus*, as well as *L. delbrueckii* subsp*bulgaricus*, are used to make Swiss, Mozzarella, provolone, Romano, and parmesan cheeses. Other species are used in the production of sauerkraut, silage and pickles. The lactobacilli are usually more resistant to acidic conditions than are other LAB, being able to grow at pH values as low as 4. This enables them to continue to grow during natural lactic fermentations when the pH has dropped too low for other LAB to grow, so they are often responsible for the final stages of many lactic acid fermentations.

Many sausage fermentations include a *Lactobacillus* species. *L. plantarum* is used in starter cultures for the manufacture of summer sausage, pepperoni and salami.

The natural fermentation of cabbage to make sauerkraut involves *L. brevis* and *L. plantarum* in the final succession of microbe. The starter cultures for manufacture of cucumber pickles includes *L. brevis* and *L. plantarum*. *L. plantarum* is the most essential species in pickle production, as it is for sauerkraut.

Like cucumbers, olives are fermented under conditions similar to those of other vegetable products. The microbial population responsible for the fermentation of olives differs from that of sauerkraut and pickles mainly because the higher salt

**Involvement of lactic acid bacteria in the manufacture of fermented dairy products**

| Product | Principal acid producers | Secondary microflora |
|---|---|---|
| **Cheese** | | |
| Colby, cheddar, cottage, cream | *Lactococcus lactis*subsp. *cremoris*<br>*Lactococcus lactis*subsp. *lactis* | None |
| Blue | *Lactococcus lactis*subsp. *cremoris*<br>*Lactococcus lactis*subsp. *lactis* | Cit$^+$ *Lactococcus lactis*subsp. *lactis*<br>*Penicillium roqueforti* |
| Mozzarella, provolone, Romano, parmesan | *Streptococcus thermophilus*<br>*Lactobacillus delbrueckii* subsp.*bulgaricus*<br>*Lactobacillus helveticus* | None |
| Swiss | *Streptococcus thermophilus*<br>*Lactobacillus delbrueckii* subsp.*bulgaricus*<br>*Lactobacillus helveticus* | *Propionibacterium freudenreichii* subsp.*shermanii* |
| **Fermented milk** | | |
| Yogurt | *Streptococcus thermophilus*<br>*Lactobacillus delbrueckii* subsp.*bulgaricus* | None |
| Buttermilk | *Lactococcus lactis*subsp. *cremoris*<br>*Lactococcus lactis*subsp. *lactis* | *Leuconostoc* sp. Cit+*Lactococcus lactis* subsp.*lactis* |
| Sour cream | *Lactococcus lactis*subsp. *cremoris*<br>*Lactococcus lactis*subsp. *lactis* | None |

concentration of the brine prevents many salt-sensitive strains from growing and provides an advantage to salt-tolerant strains. LAB become prominent during the intermediate stage of fermentation. *L. mesenteroides* and *P. cerevisiae* are the first lactics to become predominant, followed by lactobacilli, with *L. plantarum* and *L. brevis* being the most important.

### Streptococci and Lactococci

***Streptococcal Classification.*** Since 1985, members of the diverse genus *Streptococcus* have been reclassified into *Lactococcus, Vagococcus, Enterococcus,* and *Streptococcus,* based on biochemical characteristics, as well as ssRNA analysis. Historically, streptococci were segregated into serological groups based on the presence of specific carbohydrate antigens. Antigenic groups, or Lancefield groups (named for Rebecca Lancefield, a pioneer in Streptococcus taxonomy), are designated by letters A through O. Lancefield groups have proven to correlate well with the current taxonomic definitions. The beta-hemolytic streptococci found in humans contain the group A antigen, while "fecal streptococci" (enterococci) contain the group D antigen. Group B streptococci, usually found in animals, are a cause of mastitis in cows, and have been implicated in human infections. "Lactic streptococci" (streptococci and lactococci) contain the group N antigen and are nonpathogenic. However, *Lactococcus* has been defined as a genus separate from *Streptococcus*. As lactic acid bacteria, *Lactococcus lactis* and *Streptococcus thermophilus* are the cornerstones of cheese manufacture.

### *Streptococcus thermophilus*

*Streptococcus thermophilus* is an alpha-hemolytic species of the viridans group. The bacterium is found in milk and milk products. It is not a probiotic (it does not survive the stomach) and generally is used in the production of yogurt and the manufacture of several types of cheese, especially Italian and Swiss cheeses. The organism is a moderate thermophile with an optimal growth rate at 45 °C. Although *S. thermophilus* is closely related to other pathogenic streptococci (such as *S. pneumoniae* and *S. pyogenes*), *S. thermophilus* is classified as a non-pathogenic, alpha-hemolytic species that is part of the viridan's group. It is closely related to *S. salivarius* in the oral cavity.

### *Lactococcus lactis*

*Lactococcus* is a genus of of LAB with five major species formerly classified as Group N streptococci. The type species for the genus is *L. lactis,* which has two subspecies, *lactis* and *cremoris.* Lactococci differ from other lactic acid bacteria by their pH, salt and temperature tolerances for growth.

*Lactococcus lactis* is critical for manufacturing cheeses such as Cheddar, cottage, cream, Camembert, Roquefort and Brie, as well as other dairy products like cultured butter, buttermilk, sour cream and kefir. The bacterium can be used in single strain starter cultures, or in mixed strain cultures with other lactic acid bacteria such as *Lactobacillus* and *Streptococcus.*

## STARTER CULTURES

Starter cultures play an essential part in the manufacture of fermented dairy products. They produce the lactic acid that coagulates milk and they contribute to texture, moisture content, freedom from pathogenic microorganism, and taste of the product. The rate of acid production is critical in the manufacture of certain products, e.g. Cheddar cheese. Depending on the product, especially in mechanized cheese production units, starters may also be required to produce acid at a consistently fast rate through the manufacturing period each day and every day. The negative redox potential created by starter growth in cheese also aids in preservation and the development of flavor in Cheddar and similar cheeses. Additionally antibiotic substances, now referred to as bacteriocins, produced by starters, e.g. nisin, may also have a role in preservation.

### Ecology of Starter Bacteria

Most starters in use to today have their origins as lactic acid bacteria naturally present as part of the contaminating microflora of milk. These bacteria probably came from vegetation in the case of lactococci or the intestinal tract in the case of bifidobacteria, enterococci and *Lactobacillus acidophilus*.

Modern starter cultures developed from the practice of retaining small quantities of whey or cream from the successful manufacture of a fermented product on a previous day and using this as the inoculum or starter for the following day's production. In the foods industry his practice has been referred to as "back-slopping".

### Classification of Starter Cultures

While the microbes used in the manufacture of fermented dairy products are generally lactic acid bacteria, *Propionibacterium shermanii* and *Bifidobacterium* spp. which are not lactic acid bacteria, are also used. In addition, other bacteria including *Brevibacterium linens*, responsible for the flavor of Limburger cheese; and molds (*Penicillium* species) are used in the manufacture of Camembert, Roquefort and blue cheeses.

## PROBIOTICS AND LACTIC ACID BACTERIA

Probiotics are products designed to deliver potentially beneficial bacterial cells to the microbiotic ecosystem of humans and other animals. Strains of lactic acid bacteria are the most common microbes employed as probiotics, especially *Lactobacillus* and *Bifidobacterium* species, but lactococci, some enterococci and some streptococci are also included as probiotics.

## LACTIC ACID-PRODUCING BACTERIA USED AS PROBIOTICS

### *Lactobacillus*

*Lactobacillus* species are facultative anaerobes. They grow in the presence of $O_2$, however, and may convert it to $H_2O$ or $H_2O_2$. Lactobacilli normally predominate

in the small intestine, and they are known for their beneficial effects which may antagonize potential pathogens. Of the more than 100 *Lactobacillus* species, the following are commonly used probiotics:

- *L. acidophilus*
- *L. fermentum*
- *L. paracasei*
- *L. brevis*
- *L. gasseri*
- *L. plantarum*
- *L. bulgaricus*
- *L. helveticus*
- *L. reuteri*
- *L. casei*
- *L. jensenii*
- *L. rhamnosus*
- *L. crispatus*
- *L. johnsonii*
- *L. salivarius*



**Bifidobacterium longum.** Bifidobacteria are an obligately anaerobic bacteria, not classified with the lactic acid bacteria, but which occupy similar habitats and produce lactic acid as a sole end-product. They are a prominant Gram-positve bacterium in the large intestine (colon). *Bifidobacterium infantum* is the predominant bacterium in the intestine of breast-fed infants because mother's milk contains a specific growth factor that enriches for the growth of the bacterium.

### *Bifidobacterium*

*Bifidobacterium* is not included in the traditional Lactic Acid Bacteria due to its genetic unrelatedness, but the bacterium has a habitat that overlaps with LAB, and it has a metabolism that produces lactic acid as a primary end-product of fermentation. Bifidobacteria are strictly anaerobic and normally vie for predominance in the large intestine. Among 30 species, those recognized as probiotics include:

- *B. adolescentis*
- *B. breve*
- *B. longum*
- *B. animalis*
- *B. infantis*
- *B. thermophilum*
- *B. bifidum*
- *B. lactis.*

### *Streptococcus*

*Streptococcus* species are not typically associated with health benefits and some are highly pathogenic. However, one facultative anaerobic species, *Streptococcus*

*thermophilus*, is known to promote health. It is one of the two primary species found in yogurt cultures, the other being *L. bulgaricus*.

*Enterococcus*

Found in a number of probiotic products, the facultative anaerobe Enterococcus faecium is invariably a component of the normal intestinal microbiota and is considered a beneficial microbe. However, *E. faecium* has evolved from a relatively nonpathogenic commensal bacterium to the third most common cause of hospital-acquired infections and now accounts for over 10% of enterococcal clinical isolates. Furthermore, it has developed extensive resistance to antibiotics, which it is capable of transferring to other bacteria.

The human body, primarily the gastrointestinal tract, is home to a large number of different species of bacteria, and it is likely we could not survive without their presence. Two of the most common bacteria that comprise the intestinal microbiota ("normal flora") are *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. Hence, they are a main component of probiotics.

The indigenous bacteria of humans serve a wide range of functions, which include manufacture of some B vitamins and vitamin K, synthesis of some digestive enzymes (e.g. lactase), competition with pathogens for colonization sites, production of antibacterial and antifungal substances that protect against harmful organisms, production of chemicals that have been shown to be anti-carcinogenic, and stimulation of the development and activity of the immune system.

The natural balance of the body's bacteria can be upset by several factors such as certain medicines, antibiotics and steroids, increased acidity in the digestive system caused by stress, lack of sleep and poor diet, constipation or diarrhea, yeast overgrowth, fatigue, IBS and other intestinal conditions.

It has been been suggested, in a few cases proven, that one way to combat these conditions is by supplementation of the diet with probiotic bacteria in natural foods or artificial supplements.

Probiotics have been recommended or suggested for patients receiving radiation treatment, individuals who have recurrent thrush, vaginal yeast infections, or urinary tract infections, persons suffering from irritable bowel syndrome (IBS) or other bowel problems, for travelers abroad to protect against food poisoning and during any period where antibiotics may be taken.

*Lactococcus lactis* is a microbe classified informally as a Lactic Acid Bacterium because it ferments milk sugar (lactose) to lactic acid. Lactococci are typically spherical or ovoid cells, about 1.2 µm by 1.5 µm, occurring in pairs and short chains. They are Gram-positive, non motile, and do not form spores. Lactococci are found associated with plant material, mainly grasses, from which they are easily inoculated into milk. Hence, they are found normally in milk and may be a natural cause of souring. *Lactococcus lactis* has two subspecies, *lactis* and*cremoris*, both of which are essential in manufacture of many varieties of cheese and other fermented milk products.

*Lactococcus lactis* is related to other lactic acid bacteria such as *Lactobacillus acidophilus* in our intestinal tract and *Streptococcus salivarius* in the mouth. However, *Lactococcus* does not normally colonize human tissues and differs from many other lactic acid bacteria in its pH, salt, and temperature tolerances for growth, which are important characteristics relevant to its use as a starter culture in the cheesemaking industry.

*Lactococcus lactis* is vital for manufacturing cheeses such as Cheddar, Colby, cottage cheese, cream cheese, Camembert, Roquefort and Brie, as well as other dairy products like cultured butter, buttermilk, sour cream and kefir. It may also be used for vegetable fermentations such as cucumber pickles and sauerkraut. The bacterium can be used in single strain starter cultures, or in mixed strain cultures with other lactic acid bacteria such as *Lactobacillus* and *Streptococcus*species.

When *Lactococcus lactis* is added to milk, the bacterium uses enzymes to produce energy (ATP) from lactose. The byproduct of ATP production is lactic acid. The lactic acid curdles the milk that then separates to form curds, which are used to produce cheese and whey. Curdling the milk is not the bacterium's only role in cheese production. The lactic acid produced by the bacterium lowers the pH of the product and preserves it from the growth by unwanted bacteria and molds while other metabolic products and enzymes produced by *Lactococcus lactis* contribute to the more subtle aromas and flavors that distinguish different cheeses.

**Fermented dairy products wherein *Lactococcus lactis* is the primary organism involved in manufacture**

| Product Cheese | Principal acid producers | Secondary microflora |
|---|---|---|
| Colby, Cheddar, cottage, cream | *Lactococcus lactis*ssp. *cremoris*<br>*Lactococcus lactis*ssp. *lactis* | None |
| Blue | *Lactococcus lactis*ssp. *cremoris* | Citrate⁺ *Lactococcus lactis* ssp. *lactis Penicillium roqueforti* |
| | *Lactococcus lactis* ssp. *lactis* | |
| Fermented milk | | |
| Buttermilk | *Lactococcus lactis*ssp. *cremoris* | *Leuconostoc* spp. Citrate⁺ *Lactococcus lactis* ssp. *lactis* |
| | *Lactococcus lactis*ssp. *lactis* | |
| Sour cream | *Lactococcus lactis*ssp. *cremoris* | None |
| | *Lactococcus lactis*ssp. *lactis* | |

## Cheese

Cheese making is essentially a dehydration process in which milk casein, fat and minerals are concentrated 6 to 12-fold, depending on the variety. The basic steps common to most varieties are acidification, coagulation, dehydration, and salting. Acid production is the major function of the starter bacteria. Lactic acid is



***Lactococcus lactis*. Magnification 1500X. Phase micrograph**

responsible for the fresh acidic flavor of unripened cheese and is important in coagulation of milk casein, which is accomplished by the combined action of rennet (an enzyme) and lactic acid produced by the microbes. During the ripening process the bacteria play other essential roles by producing volatile flavor compounds (e.g. diacetyl, aldehydes), by releasing proteolytic and lipolytic enzymes involved in cheese ripening, and by producing natural antibiotic substances that suppress growth of pathogens and other spoilage microorganisms. For Cheddar and Colby cheese production, starter cultures include strains of *Lactococcus lactis* ssp. *cremoris* and/or *lactis*. Likewise, blue cheeses require *Lactococcus lactis* ssp. cremoris or *lactis*, but the mold*Penicillium roqueforti* is also added as a secondary culture for flavor and blue appearance.

### Cultured Butter, Buttermilk and Sour Cream

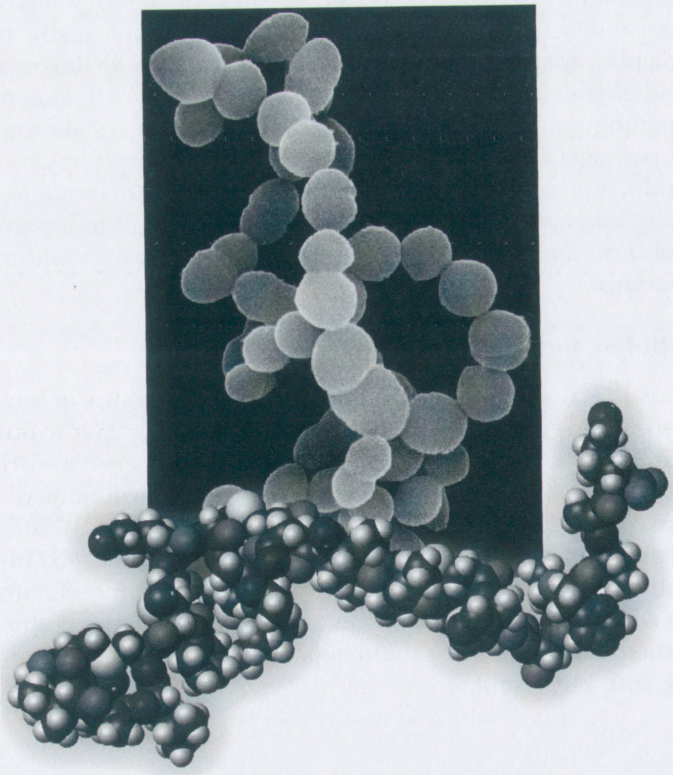
Sour cream is made from cream to which a starter culture of *Lactococcus lactis*has been added to coagulate the cream and to enhance its flavor. Buttermilk is also made with *Lactococcus lactis* in order to acidify, preserve and flavor the milk. Diacetyl, made from citrate by *Lactococcus*, gives buttermilk its distinct taste and enhances its storage properties. *Lactococcus lactis* or mixed cultures that contain*Lactococcus lactis*, plus a *Leuconostoc* species are used. In the making of cultured butter, fat (cream) is separated from skim milk by centrifugation of milk. The cream is pasteurized and inoculated with selected starter cultures. The ripened cream is then churned. The cream separates again into cream butter and its byproduct, sour buttermilk.

### Nisin

Nisin is an antibiotic-like substance, called a bacteriocin, produced by the "food grade" starter strain, *Lactococcus lactis* ssp. *lactis*. It is a natural antimicrobial agent with activity against a wide variety of Gram-positive bacteria, including food-borne

pathogens such as *Listeria, Staphylococcus* and *Clostridium*. The primary target o nisin is believed to be the cell membrane. Unlike some other antimicrobial peptides nisin does not need a receptor for its interaction with the cell membrane; however the presence of a membrane potential is required. Nisin is a natural preservative present in cheese made with *Lactococcus lactis* ssp.*lactis*, but it is also used as a preservative in heat processed and low pH foods. Since nisin cannot be synthesized chemically, the nisin-producing *Lactococcus lactis* strains are used for its industria synthesis.

The first established use of nisin was as a preservative in processed chees products, but numerous other applications in preservation of foods and beverage have been identified. It is currently recognized as a safe food preservative i approximately 50 countries. Nisin has been used as a preservative in variou pasteurized dairy products and canned vegetables, baked, high-moisture flou products, and pasteurized liquid eggs. There is interest in the use of nisin in natura cheese production. Considerable research has been carried out on the anti-listeria properties of nisin in foods and a number of applications have been proposed. Use of nisin to control spoilage lactic acid bacteria have been identified in beer, wine



***Lactococcus lactis* and the molecule, Nisin**

alcohol production, and high acid foods such as salad dressings. Production of highly purified nisin preparations has led to interest in the use of nisin for human ulcer therapy and mastitis control in cattle.

### Starter Cultures

Starter cultures have crucial roles to play during all phases of the cheese making and maturation process. As the culture grows in the milk, it converts lactose to lactic acid. This ensures the correct pH for coagulation and influences the final moisture content of the product. The rate of acid production is critical in the manufacture of certain products, e.g. Cheddar cheese. In mechanized operations, starters are often required to produce acid at a consistently fast rate through the manufacturing period each and every day. During ripening, culture, lipolytic and proteolytic enzymes are released from the bacteria that add a balanced aroma, taste, texture, and surface appearance to the product. The negative redox potential created by starter growth in cheese also aids in preservation and the development of flavor in Cheddar and similar cheeses. Additionally, antibiotic-like substances produced by starters (e.g. nisin) may also have a role in preservation.

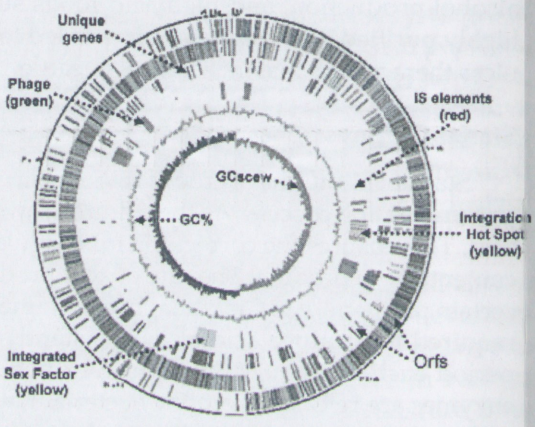
### *Lactococcus* and Vaccine Delivery

A recently discovered application of *Lactococcus lactis* is in the development of vaccine delivery systems. The bacterium can be genetically engineered to produce proteins from pathogenic species on their cell surfaces. Intra nasal inoculation of an animal with the modified strain will elicit an immune response to the cloned protein and provide immunity to the pathogen. For example, if one wished to provide immunity to *Streptococcus pyogenes*, the causative agent of strep throat, *Lactococcus* could be engineered to present the conserved portion of the streptococcal M protein required for streptococcal adherence and colonization to the nasopharyngeal mucosa. The resulting local immune response could protect the individual from strep throat caused by the streptococcus that exhibits that form of the M protein. This approach theoretically can be adapted to any pathogen that colonizes and or/enters via a mucosal surface in humans or animals. This includes human pathogens such as *Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae, Mycobacterium tuberculosis, Bordetella pertussis* and *Neisseria meningitidis*, among others.

More than 4 million deaths per year are due to respiratory diseases. Economical and effective vaccines against respiratory pathogens are needed for implementation in poorer countries where the disease burden is highest. Following respiratory tract infection, some pathogens may also invade the epithelial tissue, achieving systemic circulation and spread to other organs. Nasal administration of different antigen formulations using *Lactococcus lactis* as a delivery vehicle has shown promising results in the induction of immune responses that defeat of the pathogens at the site of infection.

*Lactococcus lactis* has been shown to deliver antigens that stimulate mucosal immunity to nonrespiratory pathogens, as well, including HIV, Human papilloma virus and the malarial parasite.

## The *Lactococcus* Genome




**Genome atlas of the chromosome of *L. lactis***

Partly due to their industrial relevance, both *Lactococcus lactis* subspecies (*lactis*and *cremoris*) are widely used as generic LAB models for research. *L. lactis* ssp.*cremoris*, used in the production of hard cheeses, is represented by the laboratory strains LM0230 and MG1363. Similarly, *L. lactis* ssp. *lactis* is employed in soft cheese fermentations, with the workhorse strain IL1403, ubiquitous in LAB research laboratories. In 2001, the genome of strain IL1403 was sequenced leading to increased understanding of LAB genomics and related applications. Currently, there are two *L. lactis* ssp. *cremoris* that have been sequenced for public release.

A French group sequenced the genome of *Lactococcus lactis* ssp. *lactis*. The genome sequence reveals 12 enzymes called aminotransferases, some of which are used to break down complex, branched, ring-shaped, and sulfur-containing amino acids. The molecules produced when the amino acids are degraded are very important for cheese flavor. Understanding which amino acids are broken down by which enzymes could give cheese makers greater control over flavor and fragrance of their fromage.

The sequence also led to the identification of 29 genes that are required to build the mesh-like peptidoglycan component of the bacterium's cell wall. Inducing some of these enzymes can accelerate the slow, expensive process of cheese ripening, during which the cheese ages and develops its characteristic flavor. Learning how to selectively activate some of these enzymes could revolutionize the cheese manufacturing process.

The *Lactococcus lactis* ssp. *lactis* genome has 2,365,589 units (bp) of DNA, which contain 2,310 predicted genes. About 64 percent of the genes have assigned roles in the cell, while 20 percent match other hypothetical genes with unknown function. Almost 16 percent of the genes bear no resemblance to genes from other species and are considered to be unique to this bacterium.

The *L. lactis* genome contains six prophages (carrying nearly 300 genes or about 14 percent of the total coding capacity) and 43 insertion elements. Sequence data also revealed new possibilities for fermentation pathways and confirmed the total lack of genes and enzymes involved in the TCA cycle although, unexpectedly, certain genes necessary for aerobic respiration were found encoded in the genome.

It is anticipated that understanding the physiology and genetic make-up of this bacterium will prove invaluable for food manufacturers as well as the

pharmaceutical industry, which is exploring the capacity of *L. lactis* to serve as a vehicle for delivering drugs and vaccines.

For example, genomic analysis of *Lactococcus lactis* subsp *lactis* revealed the presence of several genes that encode enzymes involved in the reduction of pyruvate to various end-product other than homolactic acid, including ethanol, acetic acid, formic acid, diacetyl, acetoin and butanediol. Manipulation and understanding of the regulation of these genes could defeat or enhance the synthesis of these end products leading to strain improvement in starter cultures for cheeses, buttermilk, and other dairy products.

# 19

# Fouling and Sanitizing

**Topics**

*Fouling Process • Raw Milk Bacteria Counts • Fouling • Mechanisms of milk fouling • Factors Affecting Milk Fouling • Milk Film Characteristics • Milk Proteins • Acid Rinsing • Future Farms • Trouble Shooting Cleaning Problems*

## THE FOULING PROCESS

### Denaturation and Aggregation

Fouling caused by β-lactoglobulin is classified as a series of consecutive reaction kinetics that involve the denaturation and aggregation of this protein. At temperatures above 70°C, the β-lactoglobulin molecule undergoes a second order denaturation reaction where the molecule unfolds itself and loses its secondary

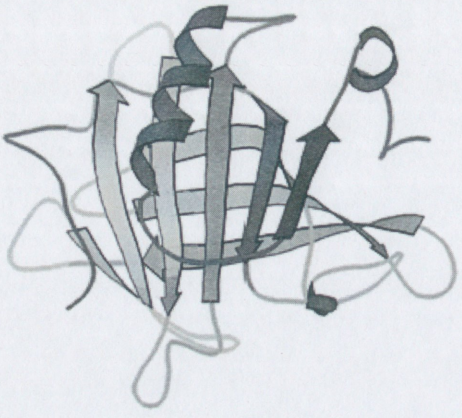


Fig. 19.1: Secondary structure of β-Lactoglobulin (2.3)

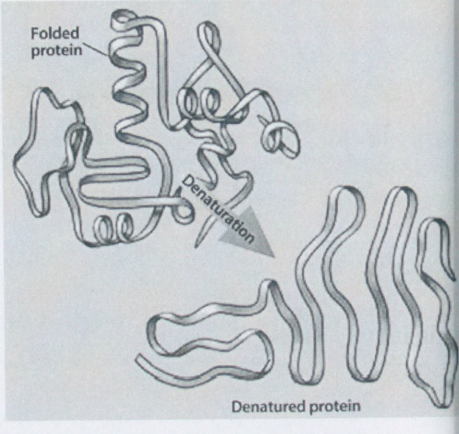



Fig. 19.2: Protein Denaturation

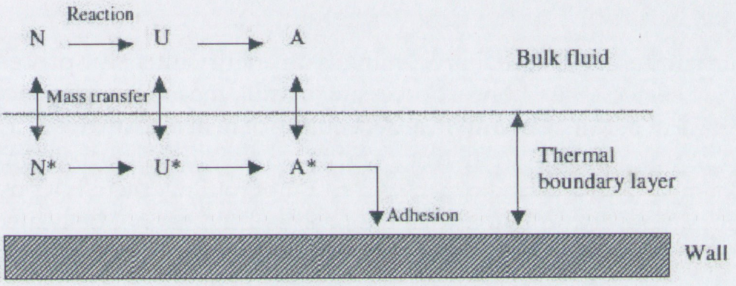



**Fig. 19.3: Outline of the four phase model**

structure due to applied external stresses to the body of the molecule. In this particular case with β-lactoglobulin, due to its sensitivity of heat, the external stress would be caused by the high temperature of the operating heat exchanger. This process is reversible.

In the molecule's denatured state, its hydrophobic core is exposed as well as the reactive disulfide and sulfhydryl (-SH) bonds. This configuration is highly unstable and requires intermolecular interactions with other denatured lactoglobulin molecules where each molecule continuously adhere to one another to form large masses which eventually can adsorb to the surfaces of heat exchangers. Contrary to denaturation, this process is not reversible.

## RAW MILK BACTERIA COUNTS

The number of bacteria present in the raw milk sample taken from the farm bulk tank represents contributions from several sources including:

1. Cows with mastitis but whose milk appears acceptable and is put into the tank.
2. Bacteria associated with soil and manure on the teats that is not thoroughly removed prior to unit attachment.
3. Bacteria sucked into the system when units fall off or are rinsed off with water that contains a high bacteria count.
4. Bacteria that accumulate and grow in various locations in the milking system during milking and between milkings and end up being transferred to the bulk tank.
5. Bacteria that grow in bulk tank milk even when well cooled.

Problems in any of these areas can influence bacteria counts. The objective of cleaning and sanitizing milking systems is to eliminate milk soils that may build up and provide opportunities for bacterial growth. Doing so minimizes system cleanliness as a risk factor contributing to high bacteria counts.

## FOULING

In the dairy industry, thermal processing is an energy intensive process since every product is heated at least once. Processing of milk means the efficiency of the heating process is of paramount importance. Fouling of heat exchangers is a serious issue as it reduces heat transfer efficiency and increases pressure drop and hence affects the economy of processing plant. As a result of fouling, there is a possibility of deterioration in product quality since the process fluid cannot be heated up to the required temperature (say for pasteurisation or sterilization). The deposits dislodged by the flowing fluid can also cause contamination.

Milk is a complicated biological fluid and contains a number of species. Its average composition in weight % is: water – 87.5, total solids - 13.0, fat – 3.9, lactose – 4.8, proteins – 3.4 (casein – 2.6, β-lactoglobulin (β-Lg) – 0.32, α-lactalbumin (α-La) – 0.12), minerals – 0.8, and small quantities of other miscellaneous species. Thermal response of these constituents generally differs from each other. β-Lg has high heat sensitivity and figures prominently in the fouling process. Milk fouling can be classified into two categories: i) Type A (protein) fouling takes place for temperatures between 75°C and 110°C. The deposits are white, soft, and spongy (milk film) and their composition is 50-70% proteins (mainly β-Lg), 30-40% minerals, and 4-8% fat, ii) Type B (mineral) fouling takes place at temperatures above 110°C. The deposits are hard, compact, granular in structure, and grey in colour (milk stone) and their composition is 70-80% minerals (mainly calcium phosphate), 15-20% proteins, and 4-8% fat.

## MECHANISMS OF MILK FOULING

Whey proteins constitute just around 5% of the milk solids but they account for more than 50% of the fouling deposits in type A fouling. β-Lg and α-La are the two major whey proteins in the milk. Both are of globular nature and heat sensitive, however, β-Lg is the dominant protein in heat induced fouling. The caseins are resistant to thermal processing but do precipitate upon acidification. Although the exact mechanisms and reactions between different milk components are not yet fully understood, a relationship between the denaturation of native β-Lg and fouling of heat exchangers has been established. Upon heating of milk, the native proteins (β-Lg) first denature (unfold) and expose the core containing reactive sulphydryl groups. The denatured protein molecules then react with the similar or other types of protein molecules like casein, α-lactalbumin (α-La) and form aggregates. The rate of fouling may be different for the denatured and aggregated proteins. Being larger in size, the transport of the aggregated proteins from the bulk to the heat transfer surfaces may be more difficult compared with the denatured proteins. According to researchers, fouling occurs when the aggregation takes place next to the heated surfaces. Researchers experimentally observed that only 3.6% of denatured β-Lg was involved in deposit formation. They found the figure to be around 5%. However, it is not clear whether fouling was primarily caused by the aggregated proteins or the denatured proteins deposited first on the heat transfer surfaces and the aggregation took place subsequently. Researchers modelled the

milk fouling process based on the assumption that only aggregated proteins resulted in fouling. According to the researchers, fouling is controlled by the aggregation reaction of protein molecules. Researchers found that the formation of protein aggregates reduces fouling. Researchers believe that β-Lg aggregates are not involved in fouling reactions. Researchers in their mathematical modelling considered that along with aggregated proteins, denatured proteins also took part in deposit formation.

## FACTORS AFFECTING MILK FOULING

Milk fouling may be unavoidable practically and continuous efforts are required to minimise it. Fouling in a heat exchanger depends on various parameters like heat transfer method, hydraulic and thermal conditions, heat transfer surface characteristics, type and quality of milk along with its processing history etc. The factors affecting milk fouling in heat exchangers can be broadly classified into five major categories:

- Milk composition
- Operating conditions in heat exchangers
- Type and characteristics of heat exchangers
- Presence of micro-organisms
- Location of fouling

### Milk Composition

The composition of milk depends on its source and hence may not be possible to change. A seasonal variation in milk fouling depends on differences in its composition. Increasing the protein concentration results in higher fouling. The heat stability of milk proteins decreases with a reduction in pH.

The calcium ions present in the milk decrease the denaturation temperature of β-Lg, promote aggregation by attaching to β-Lg, and enhance the deposition by forming bridges between the proteins adsorbed on the heat transfer surface and aggregates formed in the bulk. It has also been reported that increasing or decreasing the calcium content of milk compared with normal milk lowers the heat stability and caused more fouling.

The fat present in milk has little effect on fouling. Additives may reduce fouling by enhancing heat stability of milk but they may not be permitted in many countries. Holding milk for 24 hours before processing results in less fouling although further ageing increases fouling. Prolonged storage of milk for a few days at 5°C may enhance fouling due to the action of proteolytic enzymes. Preheating of milk in holding tubes causes denaturation and aggregation of proteins before the heating section, which then leads to lower fouling in the heat exchangers. Reconstituted milk gives much less fouling since around 25% of β-Lg is denatured during the production of milk powders.

The concentration of calcium is reported to be 9% less in the reconstituted milk, which would result in less fouling. In contrast, researchers found that UHT fouling rates of the recombined milk increased with increasing preheat treatment (preheating temperature × preheating time). The fouling deposits also had high levels of fat (up to 60% or more) compared with the deposits formed during fresh milk processing (10% or less). The difference is attributed to changes in fat-globule membranes. Researchers studied the effect of damage to milk fat globule membrane by a cavitating pump on fouling of whole milk. The fouling rate was enhanced and the argument was that the damage to the membranes results in the fat globules to coalesce, which then tend to migrate faster towards the heated wall.

### Operating Conditions in Heat Exchangers

Important operating parameters that can be varied in a heat exchanger are: air content, velocity/turbulence, and temperature. The presence of air in milk enhances fouling. However, fouling is enhanced only when air bubbles are formed on heat transfer surfaces, which then act as nuclei for deposit formation. The solubility of air in milk decreases with heating as well as a reduction in the pressure. Also the formation of air bubbles is enhanced by mechanical forces induced by valves, expansion vessels, free-falling streams etc. Fouling decreased with increasing turbulence.

According to researchers the thickness and subsequently the volume of laminar sublayer decrease with increasing velocity and as a result the amount of foulant depositing on the heat transfer surface decreases. Higher flow velocities also promote deposit re-entrainment through increased fluid shear stresses. Higher turbulence and different flow characteristics are in fact found to result in smaller induction period in plate heat exchangers compared with tubular heat exchangers. The reason for this may be the presence of low velocity zones near the contact points between the adjacent plates. The use of pulsatile flow was found to mitigate fouling when only the wall region near the heat transfer surface was hot enough to cause the protein denaturation and aggregation reactions. The reason was that the fluid spent less time near the wall due to higher mixing.

The pulsations however, enhanced fouling when the bulk fluid was also hot enough for the protein reactions to take place. Temperature of milk in a heat exchanger is probably the single most important factor controlling fouling. Increasing the temperature results in higher fouling. Beyond 110°C, the nature of fouling will change from type A to type B. It is worth mentioning that both the absolute temperature and temperature difference are important for fouling.

This means that it is feasible to have fouling in coolers where the wall temperature is lower than the bulk temperature. Researchers investigated the effect of surface and bulk temperatures on the fouling of whole milk, skim milk, and whey protein and found that the surface temperature was the most important factor in initiating fouling. When the surface temperature was less than 68°C, no fouling was observed even though the bulk temperature was up to 84°C.

**Type and Characteristics of Heat Exchangers**

Plate heat exchangers are commonly used in dairy industry as they offer advantages of superior heat transfer performance, lower temperature gradient, higher turbulence, ease of maintenance, and compactness over tubular heat exchangers. However, plate heat exchangers are prone to fouling due to their narrow flow channels.

Also milk fouling in a heat exchanger is difficult to completely eliminate, simply due to the fact that the temperature of the heat transfer surface needs to be considerably higher than the bulk temperature in order to have efficient heat transfer. Complex hydraulic and thermal characteristics in plate heat exchangers make it very difficult to analyze milk fouling. The use of co-current and counter-current flow passages within the same heat exchanger further complicates the problem. The heat transfer surface to which the deposits stick also affects fouling. However, the surface characteristics are important only until the surface gets covered with the deposits. The surface treatment may be of great benefit for fouling removal as fouling would occur after a time delay and the strength of the adhesion of the deposits onto the metal surfaces may be weaker, giving way to an easier cleaning process. Stainless steel is the standard material used in the dairy industry.

Factors that may affect fouling of a stainless steel surface are: presence of a chromium oxide or passive layer, surface charge, surface energy, surface microstructure (roughness and other irregularities), presence of active sites, residual materials from previous processing conditions, and type of stainless steel used. The modifications of the heat transfer surface characteristics through electro-polishing, surface coatings etc. may help reduce fouling by altering the surface roughness and wettability (changing the polarisation from a hydrophilic into a hydrophobic state). Increasing the surface roughness provides a larger effective surface area and results in a higher effective surface energy than a smooth surface.

As a result, the adhesion of deposits with a rough surface would be comparatively stronger. The affect of different surface coatings tend to be less on the deposit formation but more on their adhesion strength. Magnetic field treatment was observed to have no effect on the milk fouling rate. There has been an increasing use of heat exchangers that foul comparatively less e.g. fluidised bed heat exchangers, Helixchanger heat exchangers, heat exchangers equipped with turbulence promoters etc., however there is little information available about their use in thermal processing of dairy fluids. The use of pulsatile flow exchanger results in higher mass transfer that may enhance fouling in case the deposition process is mass transfer controlled.

The use of fluid bed heat exchanger was found to reduce the amount of fouling and increase the rate of heat transfer as well. Direct heating methods like steam injection and steam infusion allow to have an optimal selectivity between desired (nutritional value) and undesired (surviving microorganisms) product transformations. These methods result in low fouling rate because the desired temperatures are achieved within a very short time due to high heating rates. Also high viscous fluids can be handled more easily. The absence of heat transfer surface

in such a case is also an advantage. The resulting dilution however may not be desirable. The direct injection of hot air/nitrogen was found to give satisfactory performance in concentrating milk through evaporation of water.

Microwave heating has been used in numerous industrial applications for several years due to its advantages like faster throughput, better quality, energy saving, less space requirement etc. over conventional heating methods. However, limited lifespan of a microwave system can raise doubts over its economic viability. A number of studies have been reported on heat treatment of milk using microwaves, however these are based on general quality issues like nutrients, micro-organisms etc. instead of fouling.

In induction heating, heat is generated by placing food material inside electric coils. These coils generate oscillating magnetic fields, send electric current through the food material, and thus cause heating. Induction heating produces high local temperatures very quickly but its use has been limited to materials industry only. Ohmic heating or direct resistance heating is a novel heat treatment process where an electrical current is passed through the milk and heat is generated within the milk to achieve pasteurization or sterilization. This technique offers the potential of thermal processing of materials without relying on an inefficient mechanism like conduction of heat from a surface into the fluid. The resistance heating technique was used for milk pasteurization in the early 20th century. In recent years this technology has been in use again, after being abandoned for a major part of the 20th century.

Researchers have investigated the performance of a plate type ohmic heater for the thermal treatment of dairy products. The major advantages of ohmic heating are: absence of moving parts, non-dependency on thermal conductivity of materials, fast and uniform heating, and ability to start/stop thermal processing instantaneously. The disadvantages are: deposition and corrosion of electrode surfaces and additional safety requirements. Ohmic heating has an advantage over microwave processing where processing can be limited by the depth to which energy can penetrate the food material. Fouling cannot be completely eliminated in an ohmic heating process because when milk is heated for the pasteurization/sterilization, it results in protein denaturation, aggregation and deposition. However, since surface temperature is lower due to the generation of heat in the bulk, less fouling should take place. However, the temperature profile changes dramatically when the deposits start attaching to the electrode surfaces.

In conventional indirect heating methods like shell and tube or plate heat exchangers the deposit formation lowers the deposit/fluid interface temperature. In an ohmic heating process some heat is generated in the deposit layer as well due to its own electrical resistance. Also the deposits prevent the outward flow of heat from the bulk fluid. As a result, the deposit/fluid interface temperature increases and promotes fouling. There are two other issues that may be important in an ohmic heating process. Better mixing of the fluid may be required to overcome the wall effect and result in uniform heating but it may also promote fouling as the foulant is transferred easily from the bulk to the surface. Cooling may be employed to reduce the temperatures of electrode surfaces that would help control fouling.

Presence of Micro-organisms The formation of deposits promotes the adhesion of micro-organisms to the surface resulting in bio-fouling. Furthermore, the deposits provide nutrients to microorganisms ensuring their growth. It is worth mentioning here that most of the processes in industry are carried out at temperatures below 100°C. For example, pasteurisation is generally achieved by heating of milk at 72°C for 15 seconds in a continuous flow system. At this temperature only the pathogenic bacteria along with some vegetative cells are killed. A higher temperature of 85°C is required to kill the remaining vegetative cells. Spores are much more heat-resistant and remain active well beyond this temperature. However, their inactivation is important for the products with longer shelf life. Bio-fouling, either micro-organisms deposition or biofilm formation, in a heat exchanger raises serious quality concerns. Researchers-- have investigated the effect of bio-fouling in dairy manufacturing plants.

According to researchers, bio-fouling takes place through two different mechanisms: deposition of micro-organisms directly on the heat transfer surfaces of the heat exchanger, and deposition/attachment/entrapment of micro-organisms on/in the deposit layer forming on the heat transfer surfaces. With the supply of nutrients by the deposits, bacteria multiply. The presence of micro-organisms in the process stream and/or deposit layer does not only affect the product quality, it influences the fouling process as well. Also bacteria can get released into the process fluid due to the hydrodynamic forces and result in increased concentration of bacteria downstream. This may also result in the bacterial growth in areas which otherwise are not conducive to bio-fouling. The release pattern of thermophilic bacteria Bacillus stearothermophilus into the process stream was studied in detail.

### Location of Fouling

Protein denaturation and aggregation reactions take place as soon as milk is processed thermally. The relative amounts of the denatured and aggregated proteins depend on a number of factors like operating conditions, type and design of heat exchanger, properties of the heat transfer surface etc. The use of an efficient technology may help to mitigate fouling within a heat exchanger; however the processed milk at the exit of the heat exchanger would still have large amounts of the denatured and aggregated proteins. This would result in severe fouling at various locations further downstream. Hence controlling fouling within the heat exchanger may not yield effective results and an overall strategy may need to be developed to study the fouling process over the entire setup.

## MILK FILM CHARACTERISTICS

Whole milk contains protein, fat, lactose sugar and small amounts of various minerals, all of which are dissolved or suspended in water. Milk adheres strongly to milking equipment surfaces if allowed to dry and such films can be difficult to remove. Understanding the characteristics of milk films is important when it comes to cleaning milking and cooling equipment surfaces.

*Step 1-Rinsing Milking Equipment Surfaces*

When milking is complete and all the milk has been pumped over to the cooling tank, the remaining milk in contact with equipment surfaces is still in a liquid form. It can be easily removed by rinsing all system components with appropriate amounts of warm water (100-110° F) and dumping it down the drain after one pass through the system. The mechanical action of rinsing removes most of the milk residue from surfaces and carries it away. Remaining milk residues must be removed using appropriate cleaning compounds in hot water with proper physical cleaning action.

*Step 2-Removing Raw Milk Film Components-Milk Fat*

Milk contains 3.2-5% milk fat depending primarily on the breed and diet of the cow. Milk fat is a liquid above 100°F but below this temperature it tends to solidify and form a film on surfaces. It is not soluble in plain, cold water but in hot water it will melt and form a liquid layer. Proper temperature is an important consideration when cleaning milk fat residues from milking equipment surfaces.

Milk fat residue in milk films is removed by contact with hot, concentrated chlorinated alkaline solutions. In most milking systems, vacuum is used to pull the hot cleaning solution from the wash vat through the system. Small diameter hoses etc. are flooded with cleaning solutions moving through at relatively high speeds and this provides a physical scouring action. The combination of chemical and physical cleaning action helps remove milk residues from these components.

In large diameter milk pipelines (3-4 inches) cleaning is done differently. Generally, cleaning solutions are pulled into the pipeline by vacuum until partially full. Then, by admitting timed bursts of air into the lines solid slugs of cleaning solution are repeatedly formed and pushed through the line into the receiver at a high rate of speed (20-30 feet/sec). The slug provides scouring action in the large pipeline and the chemical solution provides chemical cleaning. The cleaning solution is recirculated for 8-10 minutes and then dumped down the drain. Wash solution starting temperatures should be approximately 165°F and the final temperature, before dumping, should be 105-110°F. Lower than this and the milk fat may start to redeposit on components.

The alkali in the cleaner specifically reacts with milk fat breaking it down into components that are suspended in the cleaning solution and dumped down the drain. This chemical action of alkali on milk fat is termed saponification and it is the backbone of milking system chemical cleaning programs. The alkaline components are typically combined with surfactants, water hardness suspending agents and chlorine to produce a chlorinated alkaline cleaner. The surfactants assist the cleaning solution to penetrate and remove soils and milk film components from equipment surfaces. Water hardness control agents (phosphates and chelating compounds) keep minerals and soils in solution and prevent their re-deposition.

## MILK PROTEINS

Raw milk contains approximately 3.2% protein. Chlorinated alkaline products are also used to remove protein. The chlorine component helps break proteins into smaller pieces, which can then be suspended in the wash solution and rinsed out. It is critical that residual milk is not allowed to dry or become cooked on by rinsing with hot water before it is cleaned. When this occurs the protein denatures or coagulates and sticks to surfaces with such force that it becomes very difficult to remove.

## ACID RINSING

After cleaning the system with hot chlorinated alkaline solutions, alkali residues are left along with some of the minerals that may be in the water. These need to be removed or neutralized to prevent film buildups. Removal is done by rinsing the system with an acid solution, which accomplishes three functions. It neutralizes alkali residues, solubilizes mineral deposits so they can be rinsed out and leaves an acid film on equipment surfaces, which helps suppress bacteria growth. Such products are not however considered sanitizers based on the EPA definition of sanitizers and are not labelled as such. Phosphoric acid solutions are widely used for acid rinses.

Nitric or sulfuric acids are also used, generally in a blend with phosphoric acid and are termed blended acids. Once these three procedures are completed; rinsing, washing with a hot chloralkali solution and acid rinsing, the system should be clean. The interval between end of washing and the next milking may be several hours or only a few minutes. The PMO requires that just prior to each milking the system shall be sanitized. This is done to eliminate bacteria that may grow on equipment surfaces between milkings even when well cleaned and acid rinsed. Flushing an EPA registered sanitizer through the system, so that all milk contact surfaces are contacted, minimizes bacteria counts at start up. Sanitizers are allowed to be used without a follow-up water rinse. Typically chlorine or iodine sanitizers are used. Alternatives to acid rinse products are acid sanitizers. These are based on organic acids that provide acid for removing mineral deposits and they are also classified by EPA as sanitizing agents.

They allow both steps to be accomplished in one operation. The main task is effectively cleaning and sanitizing milking equipment at appropriate intervals so that raw milk bacteria counts are acceptable and meet whatever bonus standards may be in place. Cleaning programs must also be cost effective, non-damaging to system components and nonthreatening to the environment within the framework of current and/or future regulations.

## FUTURE FARMS

More dairy farms in the future will milk hundreds or thousands of cows daily so it will be critical that the cleaning systems are capable of cleaning these large facilities in the short intervals available between milkings. On some dairy farms

robotic milking systems will be in use in the future. Even though they will milk cows automatically they will also have to be cleaned thoroughly and automatically after each cow has been milked. This will pose challenges because of the unpredictable pattern of use by the cows.

## TROUBLE SHOOTING CLEANING PROBLEMS

Cleaning problems may occur on any farm. The end result often is an elevated bacteria count. Some of the problems are relatively easy to locate and remedy while others require more effort. Did the water heater quit or partially fail? Is the water flow rate into the sink or cleaning vat adequate to minimize filling time and reduce heat loss? Sufficient hot water is critical for system cleaning and without it problems will arise. Checking the hot water supply for adequate temperature and volume is usually an early step in trouble shooting.

Checking it with an accurately calibrated thermometer is the simplest way to determine the actual cleaning solution temperature. Water hardness can vary a good deal and can limit cleaner effectiveness. Water hardness chemicals can link with cleaning chemicals and reduce their effectiveness. A water hardness evaluation is always a necessary first step in determining the proper cleaner to use and in what concentrations. The main water hardness chemicals are salts of calcium and magnesium.

They can accumulate in water heating equipment and reduce the capacity to below adequate. Buildups in water pipes can restrict the flow of hot water to cleaning tanks or sinks. High levels of these minerals may leave dull gray films on cleaned surfaces. They are removed by treating with acid products. Water borne minerals can combine with milk components to form a buildup of material called milkstone. This can build up in successive layers and form a tough deposit that provides a good location for bacteria to grow and possibly cause problems. Milkstone can be removed by repeated cleaning with alternating caustic and acid solutions.

Fat deposits or combinations of fat and other materials may accumulate in locations where cleaning action is insufficient. Frequently a hot, double-strength solution of alkaline cleaner is used to "shock" the system and remove the material. Eventually however changes have to be made to prevent this from re-occurring. Cleaning solution distribution throughout the system must provide adequate volume to components and be properly distributed so that all surfaces are thoroughly contacted. In large diameter pipes, such as the milk line, mechanical cleaning action is primarily controlled by the air injector. It is an air inlet valve that can be adjusted several ways to control how much air enters each time it opens, how long each opening is and how frequently each opening occurs and the interval between openings.

It must be adjusted to open when enough solution has filled the pipeline so that it pushes the solution into a slug that completely fills the diameter of the pipe. Once a slug is formed it then needs to be forced intact through the milk line at about 20-

30 feet/second and into the receiver group. Solution slugs need to be formed repeatedly during washing.

This is critical in large diameter pipelines so that the upper portion is completely cleaned. Milk meters and takeoff sensors often are tough to clean because of the resistance to fluid flow through them as well as numerous small components that are tough to reach. Water distribution to these components must be adequate and properly balanced or else certain units may be starved of cleaning solutions and become dirty as they develop buildups.

**Other Problems**

In addition to these issues other cleaning problems are often less obvious. Worn rubber tubing and short milk tubes develop cracks in the surface that hold milk residues, which promote bacterial growth. Once in this condition they cannot be properly cleaned or sanitized. Tubing, liners, gaskets should be inspected regularly and changed when they appear worn. Bacteria that attach to milk deposits in these cracks and crevices have the ability to firmly anchor themselves in place. Over time, bacterial film buildup can become significant and they are then very tough to remove.

A smooth surface, well cleaned and sanitized is the best way of limiting bacterial bio-film problems. Check for split liners. When this occurs, milk can pass into the pulsation chamber and create problems. It can also be sucked up the pulsation hose into the header tank and occasionally this will have some drainage back to the trap near the receiver. Long milking times can lead to bacterial growth and buildup in milk filters. The warm conditions and fresh milk promote rapid bacterial growth and this can cause problems.

Changing filters every 4 hours will help reduce this problem. Look closely at large receiver vessels that may have milk levels that occasionally rise high up on the walls producing a milk film that remains for hours before it is cleaned. This can promote bacterial growth. Make certain with large capacity receivers that during operation the maximum height of the liquid level is consistent.

**Cleaning Problems and Bacteria Count Issues**

The result of ineffective cleaning often is bacteria count problems. A typical well-cleaned and sanitized system should be able to produce milk with less than 10,000 bacteria/ml. When the count is greater cleanliness of the system needs to be inspected. Soiled and dirty milking systems provide food and shelter for bacteria.

The milk films protect the bacteria from cleaning and sanitizing solutions allowing them to build in numbers. It may not happen immediately but in time the number of bacteria present will increase if system cleaning is not effective. A major increase in the standard plate count may be first indication of problems simply because that is the most commonly used test.

It measures bacteria that grow in warm conditions in a 48- hour period. If a system is dirty it will allow more bacteria to grow and end up in the bulk milk. This means the starting number of bacteria for the SPC test is increased so the final count will be higher as well. The PI count is another measure of bacteria in raw milk and is an attempt to identify the presence of bacteria that may grow preferentially in cold milk.

It only measures bacteria that survive pasteurization conditions. This excludes mastitis causing bacteria and most of the normal soil organisms.

There are certain soil bacteria however that can survive in the system and cause problems later on. These are frequently associated with a dirty system. Finally don't forget the bulk tank. It represents a very large surface area that can be difficult to clean. Typically the cleaning system for tanks involves a spray ball(s), which distributes rinse water and cleaning solutions to all areas of the interior surface when working properly. The same basic program and chemicals are used as are used to clean and sanitize the pipeline and milking equipment. Typical problems include failure of the spray ball system. They may

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# Cleaning, Sanitising and Sterilising Dairy Equipment

***Topics***

Dairy water supplies • Chemicals used for cleaning • Cleaning procedure

## DAIRY WATER SUPPLIES

Water is used for all cleaning purposes in a milk processing plant. An adequate supply of water of satisfactory bacteriological and chemical quality is therefore required. Water of good bacteriological quality is important to protect public health and to avoid contamination and possible deterioration in the quality of milk and milk products.

### Bacteriological Quality

The bacterial flora of most water supplies consists mainly of Gram negative rods. Many of these may be proteolytic and lipolytic and will cause spoilage of milk and milk products if processing and storage conditions are not correct. Coliform bacteria, and in particular Escherichia coli type 1 which is largely of human and animal intestinal origin, are particularly undesirable in water. Their presence may indicate pollution of water by sewage and the possible presence of pathogenic bacteria. The bacteriological quality of water varies with its source and it may also vary with the season of the year and variations in rainfall. Water of satisfactory bacteriological quality should meet the following requirements:

- total bacterial count at 37°C should not exceed 200 per ml
- coli-aerogenes (coliforms) bacteria should be absent in 1 ml
- nutrient gelatine count (proteolytic bacteria) at 21°C for three days should not exceed 500 per ml.

surfaces to very low levels. The active sterilising ingredient is usually iodine, chlorine, nitric acid or quaternary ammonium compounds. Organic sterilisers such as chloramine-T, halane and isocyanuric acids are also used.

## CLEANING PROCEDURE

Before using any detergent or steriliser, remove as much of the dairy or food product as possible from the surface of the equipment.

1. Prewash the equipment with clean, cold water. This removes much of the dirt and should be carried out immediately after the product has been removed. Wash the equipment with warm water (50°C) to remove fatty material. If the equipment is washed thoroughly with water, much less detergent is required in later stages.
2. Wash the equipment with a detergent solution, following the manufacturer's instructions. The equipment should be cleaned thoroughly at this stage to ensure that it is chemically clean. The equipment should be scrubbed thoroughly using the detergent solution. Detergent cleaning also reduces bacterial numbers on the equipment.
3. Drain the detergent solution. It may be retained for washing other items of equipment, provided its strength is maintained. Rinse the equipment at least three times with clean cold water to remove all traces of the detergent. If not removed, traces of detergent may be incorporated in subsequent batches of dairy product. Rinsing three times with small volumes of water removes detergent residues much more effectively than rinsing once with a large volume of water.
4. Sanitise the equipment using one of the compounds mentioned above. Chlorine compounds are particularly corrosive and should only be used in accordance with the manufacturer's instructions. Rinse the equipment again with clean water to remove all residues of the sanitising agent. In the absence of a suitable chemical steriliser, the equipment can be scalded with water at 80°C.
5. Once washed and rinsed the equipment should be stored in a clean, dry, dust-free area.

**Notes:**

- Detergents and sterilisers are normally chemically active compounds and great care is required in their use and handling to avoid injury to personnel. If detergents or sterilisers come in contact with the eyes or sensitive parts of the body, rinse the affected parts thoroughly with clean water.
- Detergent sterilisers are compounds formulated to clean and sterilise equipment at the same time. They are generally expensive, but reduce the overall time required for cleaning and sterilising equipment and also reduce the amount of water needed for rinsing as only one set of rinsings are required.

# Glossary

**Acid**: synonym for sour, when lactose has been converted into lactic acid by the action of bacteria.

**Acid curd**: curd formed by the action of bacteria or by adding an acid, e.g. lemon juice or vinegar, to milk.

**Age Gelation:** Age gelation is an aggregation phenomenon that affects shelf-stable, sterilized dairy products, such as concentrated milk and UHT milk products. After weeks to months storage of these products, there is a sudden sharp increase in viscosity accompanied by visible gelation and irreversible aggregation of the micelles into long chains forming a three-dimensional network. The actual cause and mechanism is not yet clear, however, some theories exist:

1. Proteolytic breakdown of the casein: bacterial or native plasmin enzymes that are resistant to heat treatment may lead to the formation of a gel
2. Chemical reactions: polymerization of casein and whey proteins due to Maillard type or other chemical reactions
3. Formation of kappa-casein-ß -lactoglobulin complexes

**Albumin**: a water-soluble protein, a component of whey. It coagulates on heating.

**Ampere:** The measurement of the flow of an electric current through a conductor.

**Annatto**: orange-red dye used to colour cheese.

**Bacteria** are single celled organisms that can only be seen with a microscope ("microorganisms"). All processes needed for life occur within a single cell. Bacteria are considered prokaryotes. Their basic cell structure differs from cells of plants and animals (eukaryotes); for example they lack a true nucleus and have a unique cell wall. Bacteria can be found wherever life exists; some are considered useful, such as those responsible for nutrient conversion (e.g., decomposition) and food fermentations (e.g., cheese), while others are harmful, such as those responsible for food spoilage and disease. Individual bacteria are named by Genus and species (e.g., Bacillus cereus, Pseudomonas fluorescens),

water-in-oil emulsions are butter or margarine. Emulsions are inherently unstable because free energy is associated with the interface between the two phases. As the interfacial area increases, either through a decrease in particle size or the addition of more dispersed phase material, i.e. higher fat, more energy is needed to keep the emulsion from coalescing. Some molecules act as surface active agents (called surfactants or emulsifiers) and can reduce this energy needed to keep these phases apart.

**Evaporated Milk** - is made by removing about 60 percent of milk's water. It contains not less than 6.5 percent milkfat, not less than 16.5 percent milk solids-not-fat, and not less than 23 percent by weight of total milk solids. Evaporated milk is a heat-sterilized product with an extended shelf life.

**Fluffy Texture**: A spongy/marshmallowy characteristic caused by:

- Incorporation of large amount of air.
- Low total solids.
- Low stabilizer content.

**Foam**: a gas dispersed in a liquid where the gas bubbles are the discrete phase. There are many food foams including whipped creams, ice cream, carbonated soft drinks, mousses, meringues, and the head of a beer. A foam is likewise unstable and needs a stabilizing agent to form the gas bubble membrane.

**Foot-candle:** A foot-candle (fc) is a unit of measure of the intensity of light falling on a surface, equal to one lumen per square foot of surface.

**Freezing point depression**: of a solution is a colligative property associated with the number of dissolved molecules. The lower the molecular weight, the greater the ability of a molecule to depress the freezing point for any given concentration. For example, in ice cream manufacturing, monosaccharides such as fructose or glucose produce a much softer ice cream than disaccharides such as sucrose, if the concentration of both is the same.**Homogenise**: to break down the fat globules in whole milk and distribute them evenly so that the cream and milk do not separate.

**Frozen Custard** or **French Ice Cream** must also contain a minimum of 10 percent milkfat, as well as at least 1.4 percent egg yolk solids.

**Frozen Yogurt** consists of a mixture of dairy ingredients such as milk and nonfat milk which have been cultured, as well as ingredients for sweetening and flavoring.

**Gelato** is characterized by an intense flavor and is served in a semi-frozen state. Gelato contains sweeteners, milk, cream, egg yolks and flavoring.

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**Gummy Body**: This defect is the opposite of Crumbly in that it imparts a pasty or putty-like body. It is caused by:

- Too low an overrun.
- Too much stabilizer.
- Poor stabilizer.

**Half-and-Half** - Consists of a mixture of milk and cream containing not less than 10.5 percent milkfat, but less than 18 percent milkfat.

**Heavy Cream** - contains not less than 36 percent milkfat. Heavy cream may also be called "heavy whipping cream."

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**Kilowatt-hour:** The kilowatt-hour (or kWh) is a unit of energy equivalent to one kilowatt (one thousand watts) of power used continuously for one hour of time.

**Lactation**: period during which milk is secreted.

### Chemical Quality

Rain water is relatively pure and contains only traces of dissolved chemicals. The chemical impurities of a water supply are related to the topography and geology of the area from which it is obtained. Water may contain inorganic salts of calcium, magnesium and sodium as well as iron, copper, lead and zinc nitrate. The hardness of water is due mainly to the presence of inorganic salts of calcium and magnesium. There are two kinds of water hardness, temporary and permanent. Temporary hardness is due to the carbonates and bicarbonates of calcium and magnesium. These salts are easily precipitated or removed by heating; a typical example is the scale formation on the inside of a kettle. Permanent hardness is mainly due to the sulphates, chlorides and nitrates of calcium and magnesium. In the presence of certain alkalis these salts are converted into insoluble deposits and for this reason specific contituents, e.g. polyphosphates and salts of gluconic acid are incorporated into a detergent to minimise the precipitation.

It is usual to express water hardness in terms of equivalent calcium carbonate in ppm. Water supplies are classified as soft, moderately hard, hard and very hard if the total hardness (expressed as calcium carbonate) is 0--60, 60--120, 120--180 and over 180 ppm, respectively.

Deposits of calcium and magnesium salts on the surfaces of milk processing equipment reduces heat transfer efficiency and provides a nucleus for deposits of other materials present in milk. Hardness is also objectionable as it leads to waste of soaps and detergents.

Suggested chemical standards for water supplies are:

- total hardness (as $CaCO_3$) -- less than 50 ppm
- chloride (as NaCl) -- less than 50 ppm
- pH -- 6.5 to 7.5
- lead -- 0.1 ppm maximum
- iron (as Fe) -- 1 ppm maximum
- cyanide -- 0.01 ppm maximum.

The presence of heavy metals such as copper and iron in a dairy water supply is particularly objectionable as they can act as catalysts in the oxidation of milk fat leading to off-flavours in products such as butter and cream.

### Water Softeners

Chemicals and zeolites or resins are used to soften water. Temporary hardness may be removed by the addition of lime or sodium hydroxide to the water while permanent hardness is removed by the addition of sodium carbonate and sodium hydroxide.

Thorough cleaning and sanitising of all dairy equipment is an essential part of

milk processing. Water alone is inadequate for cleaning and sanitising and, therefore, chemical agents must be used.

Cleaning and sanitising dairy equipment is necessary to prevent:

- accumulation of undesirable micro-organisms in the equipment
- development of bad smells in the equipment which pass on to the product
- loss of efficiency in a dirty separator
- possible corrosion of metal parts due to lactic acid
- mould growth on wooden surfaces leading to mould contamination of the product and discolouration of the churn surface
- contamination of the product with pathogens.

Cleaning buttermaking equipment is dealt with in the section on buttermaking. This section discusses the principles of cleaning, sanitation and sterilisation of dairy equipment and the various treatments or chemicals used.

Processing equipment should be clean and the processing room should be well lit and ventilated, clean and tidy. However, sanitation of processing equipment means more than having the equipment looking clean and tidy.

The cleanliness of equipment can be classified at four levels:

1. Physical cleanliness, where all visible dirt has been removed.
2. Chemical cleanliness, where, in addition to all visible dirt, microscopically small residues have been removed.
3. Sanitation, where, in addition to being chemically clean, the equipment has been treated to inactivate or remove micro-organisms present on its surface.
4. Sterilisation, where, in addition to being sanitised, the equipment has been treated to destroy all micro-organisms present on the equipment.

Sanitation and sterilisation are easier to achieve if the equipment is initially at least physically clean. Therefore, the equipment is normally cleaned before sanitation or sterilisation.

## CHEMICALS USED FOR CLEANING

Detergents are chemical agents that assist in the cleaning process by dissolving the deposited dirt, thereby making its removal easier. Sodium salts are the commonest and cheapest detergents. Sodium hydroxide, sodium carbonate and sodium tripolyphosphate are commonly used. Synthetic detergents, such as alkyl benzyl sulphate, and biological detergents are also used.

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surfaces to very low levels. The active sterilising ingredient is usually iodine, chlorine, nitric acid or quaternary ammonium compounds. Organic sterilisers such as chloramine-T, halane and isocyanuric acids are also used.

## CLEANING PROCEDURE

Before using any detergent or steriliser, remove as much of the dairy or food product as possible from the surface of the equipment.

1. Prewash the equipment with clean, cold water. This removes much of the dirt and should be carried out immediately after the product has been removed. Wash the equipment with warm water (50°C) to remove fatty material. If the equipment is washed thoroughly with water, much less detergent is required in later stages.
2. Wash the equipment with a detergent solution, following the manufacturer's instructions. The equipment should be cleaned thoroughly at this stage to ensure that it is chemically clean. The equipment should be scrubbed thoroughly using the detergent solution. Detergent cleaning also reduces bacterial numbers on the equipment.
3. Drain the detergent solution. It may be retained for washing other items of equipment, provided its strength is maintained. Rinse the equipment at least three times with clean cold water to remove all traces of the detergent. If not removed, traces of detergent may be incorporated in subsequent batches of dairy product. Rinsing three times with small volumes of water removes detergent residues much more effectively than rinsing once with a large volume of water.
4. Sanitise the equipment using one of the compounds mentioned above. Chlorine compounds are particularly corrosive and should only be used in accordance with the manufacturer's instructions. Rinse the equipment again with clean water to remove all residues of the sanitising agent. In the absence of a suitable chemical steriliser, the equipment can be scalded with water at 80°C.
5. Once washed and rinsed the equipment should be stored in a clean, dry, dust-free area.

**Notes:**

- Detergents and sterilisers are normally chemically active compounds and great care is required in their use and handling to avoid injury to personnel. If detergents or sterilisers come in contact with the eyes or sensitive parts of the body, rinse the affected parts thoroughly with clean water.
- Detergent sterilisers are compounds formulated to clean and sterilise equipment at the same time. They are generally expensive, but reduce the overall time required for cleaning and sterilising equipment and also reduce the amount of water needed for rinsing as only one set of rinsings are required.

# Glossary

**Acid**: synonym for sour, when lactose has been converted into lactic acid by the action of bacteria.

**Acid curd**: curd formed by the action of bacteria or by adding an acid, e.g. lemon juice or vinegar, to milk.

**Age Gelation:** Age gelation is an aggregation phenomenon that affects shelf-stable, sterilized dairy products, such as concentrated milk and UHT milk products. After weeks to months storage of these products, there is a sudden sharp increase in viscosity accompanied by visible gelation and irreversible aggregation of the micelles into long chains forming a three-dimensional network. The actual cause and mechanism is not yet clear, however, some theories exist:

1. Proteolytic breakdown of the casein: bacterial or native plasmin enzymes that are resistant to heat treatment may lead to the formation of a gel
2. Chemical reactions: polymerization of casein and whey proteins due to Maillard type or other chemical reactions
3. Formation of kappa-casein-ß -lactoglobulin complexes

**Albumin**: a water-soluble protein, a component of whey. It coagulates on heating.

**Ampere:** The measurement of the flow of an electric current through a conductor.

**Annatto**: orange-red dye used to colour cheese.

**Bacteria** are single celled organisms that can only be seen with a microscope ("microorganisms"). All processes needed for life occur within a single cell. Bacteria are considered prokaryotes. Their basic cell structure differs from cells of plants and animals (eukaryotes); for example they lack a true nucleus and have a unique cell wall. Bacteria can be found wherever life exists; some are considered useful, such as those responsible for nutrient conversion (e.g., decomposition) and food fermentations (e.g., cheese), while others are harmful, such as those responsible for food spoilage and disease. Individual bacteria are named by Genus and species (e.g., Bacillus cereus, Pseudomonas fluorescens),

as are all living organisms. They are classified according to their appearance and structure and by specific characteristics of their metabolism and growth, including nutrient requirements, growth temperatures, oxygen requirements, their ability to use specific substrates (e.g., certain sugars), and by specific by-products of their metabolism. Currently, genetic profiling techniques have become standard tools in the identification/classification of bacteria, often beyond species level (e.g., sub-species, subtypes, allelic types). There are literally thousands of species of bacteria, but only select groups are of concern to the dairy industry. The following will describe the general characteristics important for characterizing bacteria that are common in milk and dairy products. To see bacteria, a microscope is required, generally one with a magnification of 1000X. Bacteria are measured in microns (1 micron = 1/1000 mm = 1/25,000 inch). When a standard light microscope is used, bacterial cells are normally stained to make them easier to see. Bacteria can be observed in milk by staining a dried milk smear on a microscope slide with a specific "milk-stain" (e.g., Levowitz-Weber Stain). Bacteria grown in a petri dish (e.g., on a semi-solid nutrient "agar" media) or in a nutrient broth can be smeared and dried on a slide and stained with a simple stain (e.g., methylene blue) or complex stain for observation. Bacteria exist in a variety of shapes, sizes and arrangements, which can be defining characteristics.

**Bacteriophage**: a virus that relies on a bacterial host for reproduction. Bacteriophages can prevent acid production in cheesemaking by destroying starter culture bacteria.

**Boiling Point Elevation**: One of the colligative properties. The boiling point of a solution is increased over that of water by the presence of solutes, and the extent of the increase is a function of both concentration and molecular weight.

**Brine**: a solution of salt and water.

**Btu:** The British Thermal Unit (Btu) is a precise measurement of energy. It is the amount of energy required to raise the temperature of one pound of water one degree Fahrenheit when the water is near 39.2 degrees Fahrenheit.

**Casein**: major protein of milk. Coagulated by the action of rennet or acids.

**Circuit:** A complete path over which an electric current can flow.

**Colligative Properties**: Properties which depend on the number of molecules in solution, a function of concentration and molecular weight, rather than just on the total percent concentration. Such properties include boiling point elevation, freezing point depression, and osmotic concentration.

**Color Rendition Index:** A measure of light quality. The maximum Color Rendition Index (CRI) value of 100 is given to natural daylight and incandescent lighting. The closer a lamp's CRI rating is to 100, the better its ability to show true colors to the human eye.

**Colostrum**: the first milk secreted after giving birth.

**Colony**: a compact mass of individual cells which has usually resulted from the multiplication of a single cell.

**Conductor:** An electrical path that offers comparatively little resistance. A wire or combination of wires not insulated from one another, suitable for carrying a single electric current. Bus bars are also conductors.

**Correlated Color Temperature – or CCT (K):** A specification of the color appearance of a lamp relating its color to that of a reference source heated to a particular temperature, measured in degrees Kelvin (K). Generally, CCT measures the "warmth" or "coolness" of the light source appearance.

**Cottage Cheese** - is the product resulting from the addition of a creaming mixture (dressing) to dry curd cottage cheese. Cottage cheese contains not less than 4 percent milkfat and not more than 80 percent moisture.

**Crumbly Body**: A flaky or snowy characteristic caused by:

- High overrun together with large air cells.
- Low stabilizer or emulsifier.
- Low total solids.
- Low protein.

**Cultured Milk** - Contains not less than 3.25 percent milkfat and not less than 8.25 percent milk solids-non-fat. It is produced by culturing any of the following milk products alone or in combination: cream, milk, partially skimmed milk or skim milk with appropriate characterizing bacteria. The addition of certain characterizing ingredients and lactic-acid producing bacteria may permit, for example, the product to be labeled "kefir cultured milk," "acidophilus cultured milk," or "cultured buttermilk."

**Current:** The movement of electrons through a conductor (measured in amperes, milliamperes, and microamperes).

**Disaccharide**: e.g. lactose, maltose. A sugar composed of two monosaccharide units.

**Dry Curd Cottage Cheese** - is a soft, unripened cheese made from skim milk and/or reconstituted nonfat dry milk. The cheese curd is formed by the addition of either lactic acid producing bacteria or acidifiers. The latter process is called direct acidification. Rennet and/or other suitable enzymes may be used to assist curd formation. Dry curd cottage cheese contains less than 0.5 percent milkfat and not more than 80 percent moisture. The product may also be called "cottage cheese dry curd."

**Emulsion**: liquid droplets dispersed in another immiscible liquid. The dispersed phase droplet size ranges from 0.1 - 10 μ m. Important oil-in-water food emulsions, ones in which oil or fat is the dispersed phase and water is the continuous phase, include milk, cream, ice cream, salad dressings, cake batters, flavour emulsions, meat emulsions, and cream liquers. Examples of food

water-in-oil emulsions are butter or margarine. Emulsions are inherently unstable because free energy is associated with the interface between the two phases. As the interfacial area increases, either through a decrease in particle size or the addition of more dispersed phase material, i.e. higher fat, more energy is needed to keep the emulsion from coalescing. Some molecules act as surface active agents (called surfactants or emulsifiers) and can reduce this energy needed to keep these phases apart.

**Evaporated Milk** - is made by removing about 60 percent of milk's water. It contains not less than 6.5 percent milkfat, not less than 16.5 percent milk solids-not-fat, and not less than 23 percent by weight of total milk solids. Evaporated milk is a heat-sterilized product with an extended shelf life.

**Fluffy Texture**: A spongy/marshmallowy characteristic caused by:

- Incorporation of large amount of air.
- Low total solids.
- Low stabilizer content.

**Foam**: a gas dispersed in a liquid where the gas bubbles are the discrete phase. There are many food foams including whipped creams, ice cream, carbonated soft drinks, mousses, meringues, and the head of a beer. A foam is likewise unstable and needs a stabilizing agent to form the gas bubble membrane.

**Foot-candle:** A foot-candle (fc) is a unit of measure of the intensity of light falling on a surface, equal to one lumen per square foot of surface.

**Freezing point depression**: of a solution is a colligative property associated with the number of dissolved molecules. The lower the molecular weight, the greater the ability of a molecule to depress the freezing point for any given concentration. For example, in ice cream manufacturing, monosaccharides such as fructose or glucose produce a much softer ice cream than disaccharides such as sucrose, if the concentration of both is the same.**Homogenise**: to break down the fat globules in whole milk and distribute them evenly so that the cream and milk do not separate.

**Frozen Custard** or **French Ice Cream** must also contain a minimum of 10 percent milkfat, as well as at least 1.4 percent egg yolk solids.

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**Gelato** is characterized by an intense flavor and is served in a semi-frozen state. Gelato contains sweeteners, milk, cream, egg yolks and flavoring.

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**Heavy Cream** - contains not less than 36 percent milkfat. Heavy cream may also be called "heavy whipping cream."

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**Ice Cream** - consists of a mixture of dairy ingredients such as milk and nonfat milk, and ingredients for sweetening and flavoring, such as fruits, nuts and chocolate chips. Functional ingredients, such as stabilizers and emulsifiers, are often included in the product to promote proper texture and enhance the eating experience. By federal law, ice cream must contain at least 10 percent milkfat, before the addition of bulky ingredients, and must weigh a minimum of 4.5 pounds to the gallon.

**Kilowatt-hour:** The kilowatt-hour (or kWh) is a unit of energy equivalent to one kilowatt (one thousand watts) of power used continuously for one hour of time.

**Lactation**: period during which milk is secreted.

**Lactose**: milk-sugar. The action of bacteria on lactose produces lactic acid.

**Lactic acid fermentation**: The production of lactic acid from lactose by the action of micro-organisms.

**Light Cream** - contains not less than 18 percent milkfat, but less than 30 percent. Light cream may also be called "coffee cream" or "table cream."

**Light Whipping Cream** - contains not less than 30 percent milkfat, but less than 36 percent milkfat. Light whipping cream may also be called "whipping cream."

**Lipolytic**: the property of splitting up or hydrolysing fat. Lipases are lipolytic enzymes; lipolytic bacteria are those that break down fat.

**Lumen:** A lumen is a measurement of light output: One lumen is equal to the amount of light emitted by one candle that falls on one square foot of surface located one foot away from one candle.

**Mastitis** : Inflammatory condition of the udder. Milk may become contaminated with bacteria during or after milking. Mastitis is an inflammation of the udder, typically caused by a microbiological infection. Mastitis can occur in all mammals, including humans. These infections can be caused by many types of microbes which can be transmitted from both environmental sources (for example, contaminated water, soil, and bedding) and other infected dairy animals). These microbes enter the udder and multiply. The microbes can enter milk as it passes through the udder during the milking process. Mastitis in dairy animals can be a source of disease causing bacteria (pathogens) and spoilage organisms in milk.

**Nonfat Dry Milk** - is made by removing water from pasteurized skim (nonfat or fat free) milk. The product contains not more than 5 percent by weight of moisture, and not more than 1.5 percent by weight of milkfat unless otherwise indicated.

**Mesophiles**: micro-organisms that have optimum growth temperatures between 25 and 45°C.

**Ohm:** The unit of electrical resistance. Resistance is one ohm when a direct current (DC) voltage of one volt will send a current of one ampere through.

**Organoleptic**: testing the effects of a substance on the senses, especially of taste and smell.

**Osmotic pressure**: A chemical force caused by a concentration gradient. It is a colligative property and the principle behind membrane processing.

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**Pasteurisation**: heating milk to 73°C for 15 seconds and cooling rapidly to less that 10°C.

**Pathogenic bacteria**: bacteria that cause disease or illness.

**pH**: is a measure of the activity of the hydronium ion ($H_3O^+$) which, according to the Debye-Huckel expression, is a function of the concentration of the hydronium ion [$H_3O^+$], the effective diameter of the hydrated ion and the ionic strength (μ m) of the solvent. For solutions of low ionic strength (μ m < 0.1) hydronium ion activity is nearly equivalent to [$H_3O^+$] which is normally abbreviated to [$H^+$]. Then, for a weak acid (HA) dissociating to H+ and A- with a dissociation constant, Ka and pKa equal to -log10 Ka, the most important relationships are defined the following two equations:

$$K_a = [H+] [A-] / [HA]$$

$$pH = \log 1 / [H+] = pKa + \log [A-] / [HA]$$

**Polysaccharide**: e.g. starch, cellulose. A complex carbohydrate of high molecular weight composed of many molecules of monosaccharides.

**Proteolytic**: protein splitting. Proteases are proteolytic enzymes; proteolytic bacteria are those that break down proteins.

**Psychrotrophs**: micro-organisms capable of growth at 5°C or below but their optimum growth temperature may be similar to mesophiles, i.e. 25–45°C.

**Relative humidity:** The ratio of water vapor contained in the air compared to the maximum amount of moisture that the air can hold at that particular temperature and pressure.

**Rennet**: extract of rennin in brine.

**Rennin**: enzyme of a digestive juice of mammals that has the property of coagulating milk.

**Starter**: bacterial culture comprising selected strains and species of lactic acid bacteria used to produce the required acid and flavour development during the manufacture of fermented dairy products.

**Sandy texture**: One of the most objectionable texture defects but easiest to detect. It is caused by Lactose crystals, which do not dissolve readily and produce a rough or gritty sensation in the mouth. This can be distinguished from "iciness" because the lactose crystals do not melt in your mouth.

**Sherbets** have a milkfat content of between 1 percent and 2 percent, and a slightly higher sweetener content than ice cream. Sherbet weighs a minimum of 6 pounds to the gallon and is flavored either with fruit or other characterizing ingredients.

**Somatic:** cells are cells from the dairy animal (predominantly white blood cells, otherwise known as leukocytes) that are normally present in milk. During most mastitis infections, the number of somatic cells present in the udder increases to help the animal fight the infection. There are several types of somatic cells that have different functions in fighting infection. Somatic cells can contain lipolytic and proteolytic enzymes, which degrade fats and proteins,

respectively. An increase in somatic cells count during a mastitis infection increases the amount of destructive enzymes present in the milk, which increases the rate of deterioration of the milk fat and protein. This effect can cause reduced yield as well as flavor and/or odor defects.

**Sorbet** and **Water Ices** are similar to sherbets, but contain no dairy ingredients.

**Sour Cream** - is the product resulting from the addition of lactic acid-producing bacteria to pasteurized cream containing not less than 18 percent milkfat. Sour cream may also be called "cultured sour cream."

**Starter**: bacterial culture comprising selected strains and species of lactic acid bacteria used to produce the required acid and flavour development during the manufacture of fermented dairy products.

**Strippings**: the last drawn milk from the udder at each milking.

**Sweetened Condensed Milk** - results from the removal of about 60 percent of the water from a mixture of milk (whole and nonfat pasteurized, homogenized milks) and safe and suitable nutritive carbohydrate sweeteners such as sucrose. This product contains not less than 8 percent milk fat and not less than 28 percent total milk solids.

**Thermoduric bacteria**: bacteria capable of surviving pasteurisation.

**Thermophiles**: micro-organisms with an optimum growing temperature of 45–75°C.

**Titratable acidity**: Ameasure of titaratable hydrogen ions. Includes H+ ions free in solution and those associated with acids and proteins.

**Volt:** A basic measurement of electrical pressure or force in a circuit. Volts x amps = watts.

**Watt:** The standard unit of measurement of electrical power. One watt is one ampere of current flowing at one volt.

**Weak Body**: Ice cream lacks "chewiness" and melts quickly into a watery liquid. Gives impression of lacking richness. May be caused by:

- Low total solids.
- High overrun.
- Insufficient stabilizer.
- **Milk** - Contains not less than 3.25 percent milkfat and 8.25 percent solids-not-fat. Addition of vitamins A and D is optional, but if added, vitamin A must be present at a level of not less than 2,000 International Units (I.U.) Per quart; vitamin D is optional, but must be present at a level of 400 I.U., if added. Characterizing flavoring ingredients may also be added.

**Whey**: greenish liquid obtained during cheesemaking.

**Wheying**-off: free whey escaping from a curd as may occur during yoghurt making.

**Yeast**: living cell which ferments sugar. It is larger than a bacteria cell.

**Yogurt** - is the product resulting from the culturing of a mixture of milk and cream products with the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yogurt contains not less than 3.25 percent milkfat and 8.25 percent solids-not-fat.

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# Advances in Dairy Science Technology

## About the Book

Dairy Technology deals with all methods of handling milk from production and consumption and includes processing, packaging, storage, transport and physical distribution. Based on the sciences of biochemistry, bacteriology, and nutrition. Its objectives are to prevent spoilage, improve quality, increase shelf-life, and make milk palatable and safe for human consumption.

The primary theme of this book is the efficient transformation of milk into high quality products. This needs a thorough understanding of the composition and properties of milk, and of the changes occurring in milk and its products during processing and storage.

The book discusses the chemistry, physics, and microbiology of milk. Besides providing knowledge of the properties of milk itself, it forms the basis for understanding what happens during processing, handling and storage, and treats the main unit operations applied in the manufacture of milk products. These are discussed in some detail, especially the influence of product and process variables on the (intermediate) product resulting. A few highly specific processes, such as churning, are discussed in product chapters.

There is no doubt that the book will have recognition by dairy scientists, students, researchers and dairy operatives and technologists.

## About the Author



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Educationist Press
*an imprint of*
**WRITE AND PRINT PUBLICATIONS**
A-2, Mittal Tower, Nimri Commercial Complex,
Ashok Vihar, Phase-4, New Delhi - 110 052
Ph: 011-45635684
Email : info@WriteAndPrint.com
Website : www.WriteAndPrint.com

WRITE& PRINT

₹2399

ISBN:- 978-93-86283-47-4

9 789386 283474