

# Milk composition and microbiology

E-learning course from ESA

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# Milk composition and microbiology

## Learning objectives :

The objective of this distance learning course is to understand the fundamentals of milk composition and microbiology of the milk. This subject is very important to a dairy manager because production of high-quality raw milk is paramount importance for successful manufacture and marketing. Milk provides essential nutrients and is a good 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 B12 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.

Fundamental knowledge in milk composition and microbiology of the milk will aim the development the students' capacity to dialogue with farmers, veterinarian and others professionals of dairy sector.

The content of this brick is:

- Milk composition;
- Milk microbiology;
- Factors affecting milk composition.

The contents of this distance learning course were adapted from: *“Principles of dairy science”* (Schmidt, Van Vleck and Hutjens, 1988), *“Dairy Microbiology”* (Robinson, 1981).

## 1. Milk composition

Milk contains more water than any other element, around 87% for dairy cows. The other elements are dissolved, colloiddally dispersed, and emulsified in water.

The quantities of the main milk constituents can vary considerably depending on the individual animal, its breed, stage of lactation, age and health status. Herd management practices and environmental conditions also influence milk composition. The average composition of cows milk is shown in Table 1.

Main constituent	Range (%)	Mean (%)
Water	85.5 – 89.5	87.0
Total solids	10.5 – 14.5	13.0
Fat	2.5 – 6.0	4.0
Proteins	2.9 – 5.0	3.4
Lactose	3.6 – 5.5	4.8
Minerals	0.6 – 0.9	0.8

Table 1. Composition of cows milk.

## 1.1. Milk fat

If milk is left to stand, a layer of cream forms on the surface. The cream differs considerably in appearance from the lower layer of skim milk. Under the microscope cream can be seen to consist of a large number of spheres of varying sizes floating in the milk. Each sphere is surrounded by a thin skin (the fat globule membrane) which acts as the emulsifying agent for the fat suspended in milk. The membrane protects the fat against enzymes and prevents the globules coalescing into butter grains. The fat is present as an oil-in-water emulsion: this emulsion can be broken by mechanical action such as shaking.

About 98% of milk fat is a mixture of triacylglycerols, with much smaller amounts of free fatty acids, mono- and diacylglycerols, phospholipids, sterols, and hydrocarbons. Milk fat also contains pigments (e.g. carotene, which gives butter its yellow colour), and waxes. Milk fat acts as a solvent for the fat-soluble vitamins A, D, E and K and also supplies essential fatty acids (linoleic, linolenic and arachidonic).

## 1.2. Milk proteins

Proteins perform a variety of functions in living organisms ranging from providing structure to reproduction. Milk proteins represent one of the greatest contributions of milk to human nutrition. Proteins are polymers of amino acids. Only 20 different amino acids occur, regularly in proteins.

The content and sequence of amino acids in a protein therefore affect its properties. Some proteins contain substances other than amino acids, e.g. lipoproteins contain fat and protein. Such proteins are called conjugated proteins as phosphoproteins, lipoproteins and chromoproteins. The phosphate phosphate is linked chemically to phosphoproteins, the casein in milk is an example. A combination of lipid and protein forms the lipoprotein and are excellent emulsifying agents. Chromoproteins are proteins with a coloured prosthetic group and include haemoglobin and myoglobin.

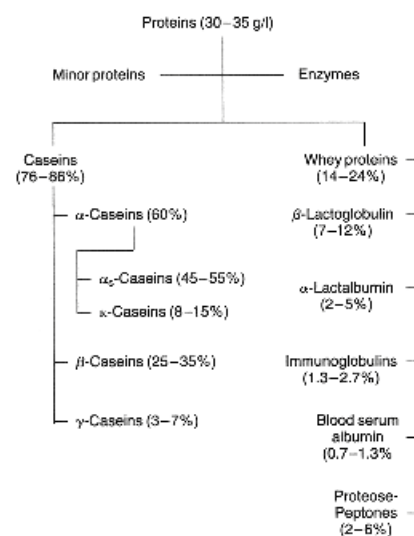


Figure 1. Milk protein fractions.

### The casein

**The casein** is a group name for the dominant class of protein in milk. Normal bovine milk contains about 3.5% protein, of which casein constitutes about 80%. Casein is easily separated from milk, either by acid precipitation or by adding rennin. In cheesemaking most of the casein is recovered with the milk fat. Casein can also be recovered from skim milk as a separate product.

Casein is dispersed in milk in the form of micelles. The micelles are stabilised by the K-casein. Caseins are hydrophobic but K-casein contains a hydrophilic portion known as the glycomacropeptide and it is this that stabilises the micelles. The structure of the micelles is not fully understood.

When the pH of milk is changed, the acidic or basic groups of the proteins will be neutralised. At the pH at which the positive charge on a protein equals exactly the negative charge, the net total charge of the protein is zero. This pH is called the isoelectric point of the protein (pH 4.6 for casein). If an acid is added to milk, or if acid-producing bacteria are allowed to grow in milk, the pH falls. As the pH falls the charge on casein falls and it precipitates. Hence milk curdles as it sours, or the casein precipitates more completely at low pH.

### The whey proteins

**The whey proteins** are also made up of a number of distinct proteins as shown in the scheme in Figure 1. Whey protein comprises the group of proteins in whey during the cheesemaking process. Whey protein also contains fragments of casein molecules.

After the fat and casein have been removed from milk, one is left with whey, which contains the soluble

milk salts, milk sugar and the remainder of the milk proteins. Like the proteins in eggs, whey proteins can be coagulated by heat. When coagulated, they can be recovered with caseins in the manufacture of acid-type cheeses. The whey proteins are made up of a number of distinct proteins, the most important of which are  $\beta$ -lactoglobulin and lactoglobulin.  $\beta$ -lactoglobulin accounts for about 50% of the whey proteins, and has a high content of essential amino acids. It forms a complex with K-casein when milk is heated to more than 75°C, and this complex affects the functional properties of milk. Denaturation of  $\beta$ -lactoglobulin causes the cooked flavour of heated milk.

### Other milk proteins

In addition to the major protein fractions outlined, milk contains a number of enzymes. The main enzymes present are lipases, which cause rancidity, particularly in homogenized milk, and phosphatase enzymes, which catalyze the hydrolysis of organic phosphates. Measuring the inactivation of alkaline phosphatase is a method of testing the effectiveness of pasteurization of milk.

Peroxidase enzymes, which catalyze the breakdown of hydrogen peroxide to water and oxygen, are also present. Lactoperoxidase can be activated and use is made of this for milk preservation.

Milk also contains protease enzymes, which catalyze the hydrolysis of proteins, and lactalbumin, bovine serum albumin, the immune globulins and lactoferrin, which protect the young calf against infection.

## 1.3. Milk carbohydrates

Lactose is the major carbohydrate fraction in milk. It is made up of two sugars, glucose and galactose (Figure 2). The average lactose content of milk varies between 4.7 and 4.9%, though milk from individual cows may vary more. Mastitis reduces lactose secretion.

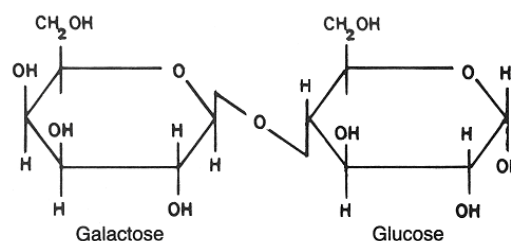


Figure 2. Structure of a lactose molecule.

Lactose is a source of energy for the young calf, and provides 4 calories/g of lactose metabolized. It is less soluble in water than sucrose and is also less sweet. It can be broken down to glucose and galactose by bacteria that have the enzyme  $\beta$ -galactosidase. The glucose and galactose can then be fermented to lactic acid. This occurs when milk goes sour. Under controlled conditions they can also be fermented to other acids to give a desired flavour, such as propionic acid fermentation in Swiss-cheese manufacture.

Lactose is present in milk in molecular solution. In cheesemaking lactose remains in the whey fraction. It has been recovered from whey for use in the pharmaceutical industry, where its low solubility in water makes it suitable for coating tablets. It is used to fortify baby-food formula. Lactose can be sprayed on silage to increase the rate of acid development in silage fermentation. It can be converted into ethanol using certain strains of yeast, and the yeast biomass recovered and used as animal feed. However, these processes are expensive and a large throughput is necessary for them to be profitable. For smallholders, whey is best used as a food without any further processing.

Heating milk to above 100°C causes lactose to combine irreversibly with the milk proteins. This reduces the nutritional value of the milk and also turns it brown.

Because lactose is not as soluble in water as sucrose, adding sucrose to milk forces lactose out of solution and it crystallizes. This causes sandiness in such products as ice cream. Special processing is required to crystallize lactose when manufacturing products such as instant skim milk powders.

Some people are unable to metabolize lactose and suffer from an allergy as a result. Pre-treatment of milk with lactase enzyme breaks down the lactose and helps overcome this difficulty.

In addition to lactose, milk contains traces of glucose and galactose. Carbohydrates are also present in association with protein. K-casein, which stabilizes the casein system, is a carbohydrate-containing protein.

## 1.4. Milk salts

Milk salts are mainly chlorides, phosphates and citrates of sodium, calcium and magnesium. Although salts comprise less than 1 % of the milk they influence its rate of coagulation and other functional properties. Some salts are present in true solution. The physical state of other salts is not fully understood. Calcium, magnesium, phosphorous and citrate are distributed between the soluble and colloidal phases (Table 2). Their equilibria are altered by heating, cooling and by a change in pH.

In addition to the major salts, milk also contains trace elements. Some elements come to the milk from feeds, but milking utensils and equipment are important sources of such elements as copper, iron, nickel and zinc.

	Total	Dissolved	Colloidal
	(mg/100 ml of milk)		
Calcium	1320.1	51.8	80.3
Magnesium	10.8	7.9	2.9
Total phosphorus	95.8	36.3	59.6
Citrate	156.6	141.6	15.0

Table 2. Distribution of milk salts between the soluble and colloidal phases.

## 1.5. Milk vitamins

Milk contains the fat-soluble vitamins A, D, E and K in association with the fat fraction and water-soluble vitamins B complex and C in association with the water phase. Vitamins are unstable and processing can therefore reduce the effective vitamin content of milk.

## 2. Milk microbiology

In addition to being a nutritious food for humans, milk provides a favorable 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 microorganisms 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 3.

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

Table 3. Bacterial types commonly associated with milk.

## 2.1. Microbial growth

Microbial growth can be controlled by cooling the milk. Most microorganisms 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.

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 molds.

Naturally soured milk is used to make many products, e.g. 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 10<sup>6</sup>/ml. High counts (more than 10<sup>5</sup>/ml) are evidence of poor production hygiene. Rapid tests are available for estimating the bacterial quality of milk.

## 2.2. Milk pasteurisation

Pasteurisation is the 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 cheesemaking, and cream is pasteurised prior to tempering for buttermaking 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 at 72°C.

## 2.3. 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. Microorganisms that survive pasteurisation are putrefactive. Although pasteurised milk has a storage stability of 2 to 3 days, subsequent deterioration is caused 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.

## 2.4. 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.

## 2.5. Microbiology of butter

Butter is made as a means of extracting and preserving milk fat. It can be made directly from milk or by separation of milk and subsequent churning of the cream. In addition to bacteria present in the milk other sources of bacteria in butter are: a) equipment, b) wash water, c) air contamination, d) packing materials, and e) personnel.



## Equipment

In smallholder buttermaking, bacterial contamination can come from unclean surfaces, the butter maker and wash water. Packaging materials, cups and leaves are also sources of contaminants. Washing and smoking the churn reduces bacterial numbers. But traditional equipment is often porous and is therefore a reservoir for many organisms.

When butter is made on a larger processing scale, bacterial contamination can come from holding-tank surfaces, the churn and butter-handling equipment.

A wooden churn can be a source of serious bacterial, yeast and mould contamination since these organisms can penetrate the wood, where they can be destroyed only by extreme heat. If a wooden churn has loose bands, cream can enter the crevices between the staves, where it provides a growth medium for bacteria which contaminate subsequent batches of butter. However, if care is taken in cleaning a wooden churn this source of contamination can be controlled. Similar care is required with scotch hands and butterworking equipment.

## Wash water

Wash water can be a source of contamination with both coliform bacteria and bacteria associated with defects in butter. Polluted water supplies can also be a source of pathogens.

## Air

Contamination from the air can introduce spoilage organisms: mould spores, bacteria and yeasts can fall on the butter if it is left exposed to the air. Moulds grow rapidly on butter exposed to air.

## Packaging

Care is required in the storage and preparation of packaging material. Careless handling of packaging material can be a source of mould contamination.

## Personnel

A high standard of personal hygiene is required from people engaged in buttermaking. Personnel pass organisms to butter via the hands, mouth, nasal passage and clothing. Suitable arrangements for disinfecting hands should be provided, and clean working garments should not have contact with other clothes.

## 2.6. Control of microorganisms in butter

Salting effectively controls bacterial growth in butter. The salt must be evenly dispersed and worked in well. Salt concentration of 2% adequately dispersed in butter of 16% moisture will result in a 12.5% salt solution throughout the water-in-oil emulsion.

Washing butter does little to reduce microbiological counts. It may be desirable not to wash butter, since washing reduces yield. The acid pH of serum in butter made from ripened cream or sour milk may control the growth of acid-sensitive organisms.

Microbiological analysis of butter usually includes some of the following tests: total bacterial count, yeasts and moulds, coliform estimation and estimation of lipolytic bacteria.

Yeast, mould and coliform estimations are useful for evaluating sanitary practices. The presence of defect producing types can be indicated by estimating the presence of lipolytic organisms.

All butter contains some micro-organisms. However, proper control at every stage of the process can minimise the harmful effects of these organisms.

## 2.7. Standardisation of milk and cream

An adjustment of the fat content of cream is required, or if the fat content of whole milk must be reduced to a given level, skim milk must be added. This process is known as standardisation.

## 2.8. Microbial tests for raw and pasteurised milk

Tests are available to know the milk microbiological quality. Bacteria, coliform and somatic cell counts

are frequently used.

### Bacteria Count

The total bacteria count is the number of bacteria in a sample that can grow and form countable colonies on *Standard Methods Agar* after being held at 32°C for 48 hours.

### Coliform Count

The coliform count is the number of colonies in a sample that grow and form distinctive countable colonies on *Violet Red Bile Agar* after being held at 32°C for 24 hours. Coliforms are generally only present in food that has been fecally or environmentally contaminated.

### Somatic Cell Count

Somatic cells are blood cells that fight infection and occur naturally in milk. The presence of mastitis (an infection of the mammary gland) in the cow will increase the somatic cell count. The somatic cell count can be determined by direct microscopic examination or by electronic instruments designed to count somatic cells.

## 2.9. Antibiotics in milk

Antibiotics are used to treat mastitis infections. Cows under antibiotic treatment for mastitis infections may have antibiotic residues in their milk, therefore, milk from treated cows is either discarded or collected into a separate tank. Milk containing antibiotic residues is not used for human consumption. The legal standard, as defined by the Food and Drug Administration (FDA), requires that milk contain no detectable antibiotics when analysed using approved test methods. Regulatory action is taken against the farm with the positive antibiotic test.

## 3. Factors affecting milk composition

### 3.1. Genetic

Milk composition varies considerably among breeds of dairy cattle: Jersey and Guernsey breeds give milk of higher fat and protein content than Shorthorns and Friesians. Zebu cows can give milk containing up to 7% fat.

The potential fat content of milk from an individual cow is determined genetically, as are protein and lactose levels. Thus, selective breeding can be used to upgrade milk quality. Heredity also determines the potential milk production of the animal. However, environment and various physiological factors greatly influence the amount and composition of milk that is actually produced. Herd recording of total milk yields and fat and SNF percentages will indicate the most productive cows, and replacement stock should be bred from these.

### 3.2. Interval between milkings

The fat content of milk varies considerably between the morning and evening milking because there is usually a much shorter interval between the morning and evening milking than between the evening and morning milking. If cows were milked at 12-hour intervals the variation in fat content between milkings would be negligible, but this is not practicable on most farms. Normally, solids-not-fat content varies little even if the intervals between milkings vary.

### 3.3. Stage of lactation

The fat, lactose and protein contents of milk vary according to stage of lactation. Solids-not-fat content is usually highest during the first 2 to 3 weeks, after which it decreases slightly. Fat content is high immediately after calving but soon begins to fall, and continues to do so for 10 to 12 weeks, after which it tends to rise again until the end of the lactation.

### 3.4. Age

As cows grow older the fat content of their milk decreases by about 0.02 percentage units per lactation. The fall in solids-not-fat content is much greater.

### 3.5. Feeding regime

Underfeeding reduces both the fat and the solids-not-fat content of milk produced, although solids-not-fat content is more sensitive to feeding level than fat content. Fat content and fat composition are influenced more by roughage (fibre) intake.

The solids-not-fat content can fall if the cow is fed a low-energy diet, but is not greatly influenced by protein deficiency, unless the deficiency is acute.

### 3.6. Disease

Both fat and solids-not-fat contents can be reduced by disease, particularly mastitis.

### 3.7. Completeness of milking

The first milk drawn from the udder is low in fat while the last milk (or strippings) is always quite high in fat. Thus it is essential to mix thoroughly all the milk removed, before taking a sample for analysis. The fat left in the udder at the end of a milking is usually picked up during subsequent milkings, so there is no net loss of fat.

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Fundamental knowledge in milk composition, microbiology and factors affecting milk composition is essential to dialogue with farmers, veterinarian and others professionals of dairy sector. This distance learning course would help you to understand and to improve the milk quality in dairy industry.