

Chapter 1. Introduction

1.1 Definition of terms

Livestock: - Animals raised/kept for sale, food or other products, or kept for use, especially farm animals such as meat and dairy cattle, pigs, poultry, and shoats.

Livestock/ animal product:- Is any material derived from the body of an animal. Examples are fat, meat/flesh, blood, milk, eggs, hide & skin, wool/feather, rennet and the like.

Processing:-Is performing a series of mechanical or chemical operations on something in order to change its state or preserve it. It is changing the chemical and physical properties of a product/substance to the desired state or changing of structure or forms of a product.

Processing animal product:-Is the manufacturing of animal products for human consumption (meat, dairy products, egg, and honey)

Technology:- Merriam-Webster says "Technology is application of knowledge to the practical aims of human life or to changing and manipulating the human environment". Technology includes the use of materials, techniques, and sources of power to make life easier or more pleasant and work more productively. It focuses on making things.

Microorganisms:- An organism that is microscopic or sub-microscopic, which means it is too small to be seen by the unaided human eye. Supplement. Microorganisms were first observed by Anton van Leeuwenhoek in 1675 using a microscope of his own design. Examples of microorganisms include bacteria, fungi, archaea, and protists.

A **microorganism** is a living thing that is too small to be seen with the naked eye. Examples of microorganisms include bacteria, archaea, algae, protozoa, and microscopic animals such as the dust mite.

Bacteria:- Bacteria are single-celled microorganism found nearly in all natural environments.

Virus: -they are extremely small organisms. They require living cell of plants, animals, or bacteria for growth.

Yeast and mould:- Those are fungi which do not have chlorophylls. The range in size is from single-celled organism to large mushroom. The true fungi produce masses of filamentous hyphae which form mycelium.

Carcass - the dressed body of a meat animal.

Enrichment - addition of specified amounts of selected nutrients in accordance with a standard identity as defined by the Food and Drugs Act.

Food system - the production, processing, transportation, distribution and preparation of food.

Fortification - addition of nutrients to a food to render it a good to superior source of the added nutrients. May include nutrients not normally associated with the food.

Packing plants - a meat processing factory. Cattle, sheep and hogs are shipped to a packing plant where they are slaughtered, inspected by government inspectors and prepared for wholesale or retail distribution.

Mastitis - inflammation of the mammary gland.

Graded - to assign, through an inspection process, grades which indicate the standard of food quality.

Palatability - refers to the avidity with which an animal selects a component from among several different feed choices.

Pasteurization - a heat process designed to destroy food pathogens and most food spoilage organisms but not to sterilize the product.

Pathogenic - able to cause disease

Pathological - pertaining to pathology

Pathology- structural and functional manifestation of disease

Post mortem - after death.

Probiotic- living organisms used to manipulate fermentation in the rumen.

Processor- the person or company that buys the farm-fresh products and prepares them for retail sale so that flavor and nutritional values are preserved. Canning, freezing, pickling and drying are a few processing methods.

Rancidity - the oxidation of fats.

rennet - extracted from the fourth stomach of the calf, enzyme component used to coagulate milk.

Salmonellosis - illness resulting from infection by salmonella species of bacteria.

Table eggs - eggs which are sold directly to the consumer, either through retail stores or in restaurants.

UHT- Ultra High Temperature heat processing results in a sterile product (140°C for 3-4 seconds).

Vacuum packaging - the packaging of a product in the absence of oxygen.

Virulence - the relative ability of a pathogenic microorganism to cause disease

White veal - the meat from a young calf fed primarily a milk based diet.

Withdrawal period -the time when a drug must not be administered prior to marketing to insure that no residues remain in the meat or milk.

Zoonosis - diseases of animals that can be transmitted to humans.

1.2 Significance of milk

Milk may be defined as the whole, fresh, clean lacteal secretion obtained by the complete milking of one or more healthy milch animals, excluding that obtained within 15 days before and 3 days after calving. Milk in technical aspect is defined as the whole, normal, clean and fresh lacteal secretion obtained by milking a healthy animal 72 hours after calving. Milk provides nine essential nutrients, including Calcium, Potassium, Phosphorus, Protein, Vitamins A, D, B12, Riboflavin and Niacin. Protein provides energy, and calcium and Vitamin D help build strong bones. Milk is considered as a nearly complete food since it is a good source of protein, fat and major minerals. Also, milk and milk products are main constituents of the daily diet, especially for vulnerable groups such as infants. In fact, consumption of dairy products has recently been linked to health benefits that are the direct antitheses of diseases and complexity that related to overweight and obesity. For example, individuals that consume dairy products are more likely to have lower weight, lower blood pressure, and decreased risk of stroke, colon cancer and osteoporosis. There is a wide range of functional foods that were developed recently and many of them are being produced in all over the world including probiotic, prebiotic and symbiotic foods as well as foods enriched with fat-reduced, salt-reduced foods or sugar-reduced foods, antioxidants and phytosterols. Among these foods, probiotic functional food has exerted positive effects on the overall health. The market of probiotic dairy foods is increasing annually. An increased demand for dairy probiotic products comes from health promotion effects of probiotic bacteria which are originally initiated from milk products, bioactive compounds of fermented dairy products and prevention of lactose intolerance.

Milk and its health benefits

The health benefits of milk and dairy products are known to humanity and may be attributed to the biologically active compounds that are existing in milk. It is a composite physiological fluid that facilitates postnatal adaptation of baby through digestive maturation simultaneously by providing the bioactive components and nutrients. It supports lymphoid tissues development and establishment of symbiotic micro flora. The importance, potency and the quantity of milk bioactive compounds are possibly more than old consideration. They comprise certain specific organic acids, vitamin A, B12, D, riboflavin calcium, carbohydrates, phosphorous, selenium, magnesium, zinc, proteins, bioactive peptides and oligosaccharides. They mostly emerge during fermentation or digestive processes while in some cases these are components of fresh milk. The possible mechanisms for cholesterol decreasing or removal by probiotic bacteria and fermented dairy products include inhibition of intestinal cholesterol absorption. Fatty acids having medium chain whey proteins and other minerals may add positive result of dairy products on body mass.

The dairy proteins play a vital role in food intake regulation, satiety and metabolic distracts relating to obesity. Blood pressure may be affected by lactic acid bacteria, milk proteins, peptides and calcium.

Antimicrobial effects are exerted by sphingolipids and their active metabolites either directly or upon their digestion. Whey was studied as a medicine as well as an aphrodisiac and skin balm during the Middle Age. Whey proteins, i.e. α -lactalbumin, lactoferrin, lactoperoxidase, serum albumin and β -lacto globulin acquire important biological and nutritional properties particularly regarding disease prevention. Immuno-stimulatory, anti-carcinogenic and anti-microbial are other whey protein activities that promote health. Milk products and their components take part in regulating the body mass through satiety signals. Therefore, whey proteins include physiological milk components for individuals with metabolic syndrome and obesity. Whey protein in high protein milk products may improve insulin sensitivity and reduce fat deposition. The bioavailability of trace elements and minerals i.e. manganese, calcium, magnesium, iron, selenium and zinc is also improved by milk proteins and peptides.

So far, more than 60 different enzymes are recognized in milk and during the heat treatment most of those enzymes will destroy and become inactive. The heat processing at high level of temperatures causes not only digestion enzymes denaturation (amylases, proteinases, phosphatases, lipases) but also digestion those enzymes having antioxidant and antimicrobial characteristics. These special characteristics are essential in milk stability as well as in the defense against pathogens; catalase, oxidoreductase, myeloperoxidase and etc. Milk antimicrobial agents have been shown bactericidal and even bacteriostatic behaviour. They are transmitted to progeny where they protect the progeny from highly contagious disorders. Lactoperoxidase, xanthine, oxidoreductase and lysozyme are the other best protecting factors in additions to immunoglobulin. Lactoperoxidase assists in milk storage as well as it inhibits the propagation of psychrotropic bacteria.

Sheep and goats were domesticated early during the Agricultural Revolution, 8000-10000 years ago. Cattle were domesticated later but have become the principal dairying species in the most intense dairying areas, although sheep and goats are very important in arid regions, especially around the Mediterranean. Buffalo are important in some regions, especially in India and Egypt. Mare's milk is used extensively in central Asia and is receiving attention in Europe for special dietary purposes since its composition is closer to that of human milk than is bovine milk. Some milk and dairy products are consumed in probably all regions of the world but they are major dietary items in Europe, North and South America, Australia, New Zealand and some Middle Eastern countries.

The functional and nutritional properties of milk proteins are superior to those of soy protein, and since cattle, and especially sheep and goats, can thrive under farming conditions not suitable for growing cereals or soybeans, dairy animals need not be competitors with humans for use of land, although high-yielding dairy cows are fed products that could be used for human foods. In any case, dairy products improve the 'quality of life', which is a desirable objective. One of the limitations of milk as a raw material is its perishability - it is an excellent source of nutrients for micro-organisms as well as for humans. However, this perishability is readily overcome by a well organized, efficient dairy industry. Milk is probably the most adaptable and flexible of all food materials.

Chapter 2. Chemical and Physical Properties of Milk

2.1 Physical status of milk

Milk is a dilute emulsion consisting of an oil/fat dispersed phase and an aqueous colloidal continuous phase. The physical properties of milk are similar to those of water but are modified by the presence of various solutes (proteins, lactose and salts) in the continuous phase and by the degree of dispersion of the emulsified and colloidal components. Data on the physical properties of milk are important since such parameters can influence the design and operation of dairy processing equipment (e.g., thermal conductivity or viscosity) or can be used to determine the concentration of specific components in milk (e.g., use of the elevation in freezing point to estimate added water or specific gravity to estimate solids-not-fat), or to assess the extent of biochemical changes in the milk during processing (e.g., acidification by starter or the development of a rennet coagulum). About 87% of milk is water, in which the other constituents are distributed in various forms. Several kinds of distribution are distinguished according to the type and size of particle present in the liquid. Some important physical properties of milk are Presented in the following sections.

2.1.1 Density

The density (ρ) of a substance is its mass per unit volume. Because temperature influences density of a substance, it is necessary to specify temperature when discussing density or specific gravity. The density of milk is of consequence since fluid milk is normally retailed by volume rather than by mass. Measurement of the density of milk using a hydrometer (lactometer) has also been used to estimate its total solids content.

The density of bulk milk (4 % fat and 8.95 % solids-not-fat) at 20 °C is approximately 1.030 kgm⁻³ and its specific gravity is 1.0321. Milk fat has a density of ~902 kgm⁻³ at 40 °C. The density of a given milk sample is influenced by its storage history since it is somewhat dependent on the liquid to solid fat ratio and the degree of hydration of proteins. To minimise effects of thermal history on its density, milk is usually pre-warmed to 40–45 °C to liquefy the milk fat and then cooled to the assay temperature (often 20 °C). The density and specific gravity of milk vary somewhat with breed. Milk from Ayrshire cows has a mean specific gravity of 1.0317 while that of Jersey and Holstein milks is 1.0330. Density varies with the composition of the milk and its measurement has been used to estimate the total solids content of milk.

2.1.2 Titratable

Titrateable acidity is pointed out the amount of alkali required to bring the pH to neutrality (phenolphthalein). This property is used in several fields. These are to determine bacterial growth during fermentations, such as during cheese making. Fresh bovine milk has no lactic acid. Mostly, the titrateable acidity is because of the casein and phosphates. Lactic acid can be produced by bacterial contamination.

Acidity, one of the most important parameters, controls the quality and processing of milk. Milk acts as a buffer. This buffer is a chemical system. It resists changes in the concentration of hydrogen ions under internal and external influences. The solids-not-fat in the milk contains the more phosphates, proteins and other weak acids. pH value is largely a reflection of these. The titrateable acidity of fresh normal milk should be about 0.14% lactic acid. If due to the microorganism' activity the milk is soured and the acidity is raised, an extra amount of alkali is required. If the increase in titrateable acidity is about 0.07% above the normal value, milk begins to taste sour. In a contrary manner, mastitis milk

which may have an initial pH beyond 7, shows a titratable acidity of 0.1% lactic acid or less. The hydrogen-ion concentration of milk or dairy products can be determined colorimetrically or electrometrically. Any one or combination of them is chosen depends upon the range of hydrogen-ion concentration to be measured. The electrometric method gives good results than the colorimetric method.

pH and acidity

An acid is a substance which dissociates to produce hydrogen ions in solution. A base (alkaline) is a substance which produces hydroxyl ions in solution. It can equally be stated that an acid is a substance which donates a proton and a base is a substance which accepts a proton. The symbol pH is used to denote acidity; it is inversely related to hydrogen ion concentration. On a scale of 0–14:

- ◆ Neutrality = pH 7
- ◆ Acidity is < pH 7
- ◆ Alkalinity is > pH 7

Fresh milk has a pH of 6.7 and is therefore slightly acidic. When an acid is mixed with a base, neutralisation takes place; similarly a base will be neutralised by an acid.

2.1.3 Electrical Conductivity

Electrical conductivity is a measure of the resistance of a particular material to an electric current. Milk has conductive properties due to the existence of charged compounds, especially mineral salts. The distribution of salt fractions between the soluble and colloidal phases has an important effect on milk conductivity value. The salts in milk contain mainly of chlorides, phosphates, citrates, carbonates and bicarbonates of potassium, sodium, calcium, and magnesium, although the salt content of milk is less. This composition is affected by factors such as animal breed, season of the year, feed, and stage of lactation.

2.1.4 Buffer solutions

Buffers are defined as materials that resist change in pH on addition of acid or alkali. Characteristically they consist of a weak acid or a weak base and its salt. Milk contains a large number of these substances and consequently behaves as a buffer solution. Fresh cow milk has a pH of between 6.5 and 6.7. Values higher than 6.7 indicate mastitic milk and values below pH 6.5 indicate the presence of colostrum or bacterial deterioration. Because milk is a buffer solution, considerable acid development may occur before the pH changes. A pH lower than 6.5 therefore indicates that considerable acid development has taken place. This is normally due to bacterial activity. Litmus test papers, which indicate pH, are used to test milk acidity; pH measurements are often used as acceptance tests for milk.

Milk acidity is an important indicator of milk quality. Acidity measurements are also used to monitor processes such as making cheese and yoghurt. The titratable acidity of milk is expressed in terms of percentage lactic acid – the principal acid produced by fermentation after milk is drawn from the udder. Fresh milk contains only traces of lactic acid. However, due to the buffering capacity of the proteins and milk salts fresh milk, in which no lactic acid has been produced, normally exhibits an initial acidity of 0.14 to 0.16% when titrated using sodium hydroxide to a phenolphthalein end-point.

2.2 Milk constituents

- **Water: 87.3%** (85.5 - 88.7%)
- **Milk fat: 3.9 %** (2.4 - 5.5%)

- **Proteins: 3.25%** (2.3-4.4%)
 - Casein: 2.6% (1.7-3.5%)
 - Serum proteins
 - Minor proteins
- **Carbohydrates (Lactose): 4.6%** (3.8-5.3%)
- **Minerals: 0.65%** (0.53-0.80%)
 - Cationic: K, Ca, Mg, K, ...
 - Anionic: chloride, phosphate, citrate, carbonate
- **Organic acids: 0.18%** (0.13-0.22%)
 - Citric, lactic, formic, acetic, oxalic
- **Enzymes** - peroxidase, catalase, phosphatase, lipase
- **Vitamins** - A, C, D, thiamine, riboflavin
- **Gases** – CO₂, N₂, O₂ (CO₂ lost after drawing).

Water is the main constituent of milk and milk processing is usually designed to remove water from milk or reduce the moisture content of the product. Milk contains more water than any other element, around 87% for dairy cows.

2.2.1 Milk Fat: 3.9%

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. **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 (Figure 2.1). 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.

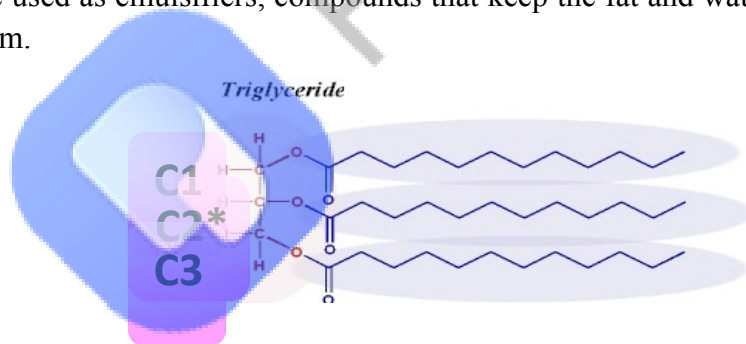


Fig. 2.1: Structure of Triglycerides

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.

Milk fat melts over a wide temperature range, from approximately -40°F (-40°C) 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 fat are a result of the melting points of the individual fatty acids that make up milk fat and their arrangement on the triglyceride molecule.

Cream consists of a large number of spherical microscopic globules of varying sizes floating in the milk. Each globule is surrounded by a thin skin - the fat globule membrane which acts as the emulsifying agent for the fat suspended in milk (Figure 2.2). The membrane protects the fat from enzymes and prevents the globules coalescing into butter grains. The fat is present as an oil-in-water emulsion that can be broken by mechanical action such as shaking.

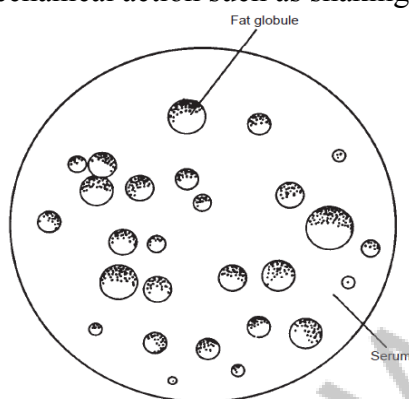


Figure 2.2: Fat globules

Fats are partly solid at room temperature. The term oil is reserved for fats that are completely liquid at room temperature. Fats and oils are soluble in non-polar solvents, e.g. ether. The lipid content of milk is usually defined as the fraction which is extracted by organic solvents. Table 2.1 gives the main lipid classes of milk fat.

Table 2.1 Composition of lipids in whole bovine milk.

Lipid	Weight (%)
Carotenoids + vitamin A	trace
Cholesterol esters	0.02
Triglycerides	98.3
Diglycerides	0.3
Monoglycerides	0.03
Free fatty acids	0.1
Cholesterol	0.20–0.40
Phospholipids	0.20–1.0

About 98% of milk fat is triacylglycerides. The partial glycerides (diglycerides and monoglycerides) and free fatty acids are probably partly left over from the biosynthesis process. Fat soluble vitamins (A, D, E and K) and pigments, e.g. carotene (gives butter its natural yellow colour) are present. Milk fat also supplies essential fatty acids (linoleic, linolenic and arachidonic).

Main variables in milk fat are as following;

1. Chain length: Fatty acids vary in chain length from 4 carbon atoms, as in butyric acid, to 20 carbon atoms, as in arachidic acid. Nearly all the fatty acids in milk contain an even number of carbon atoms.

Milk fat contains significant levels of short and medium chain fatty acids. Butyric acid (C4) is specific for milk fat of ruminant species.

2. Number of double bonds: A fatty-acid molecule comprises a hydrocarbon chain and a carboxyl group (-COOH). In saturated fatty acids the carbon atoms are linked in a chain by single bonds (e.g. stearic acid, C18:0). Unsaturated fatty acids have one double bond, e.g. oleic acid, C18:1, while polyunsaturated fatty acids have more than one double bond, e.g. linoleic acid, C18:2 (two double bonds), and linolenic acid, C18:3 (three double bonds). It is the double bonds in the carbon chain that make the fatty acid unsaturated. Two hydrogens can be added per double bond at high temperature with a suitable catalyst. This process is called hydrogenation and has the effect of converting a soft fat to a hard fat at room temperature.

3. Position of double bond: The double bond can occur in many positions (called isomers). Oleic acid has the double bond at the 9th position which may be indicated as follows: C18:1 9. Linoleic acid has two double bonds at the 9th and 12th positions which may be indicated as follows: C18:2 9, 12.

4. The proportion of saturated fatty acids present in milk fat is about 63%.

5. Oleic acid is the most abundant of the unsaturated fatty acids.

The melting point and hardness of the fatty acid is affected by the length of the carbon chain and the degree of unsaturation. As chain length increase, melting point increases. As the degree of unsaturation increase, the melting point decreases. Fats composed of short-chain or unsaturated fatty acids have low melting points and are liquid at room temperature, i.e. oils. Fats high in long-chain saturated fatty acids have high melting points and are solid at room temperature. Butterfat is a mixture of fatty acids with different melting points and therefore does not have a distinct melting point. Since butterfat melts gradually over a temperature range of 0–40°C, some of the fat is liquid and some solid at temperatures between 16 and 25°C. The ratio of solid to liquid fat at the time of churning influences the rate of churning and the yield and quality of butter. Fats readily absorb flavours, e.g. butter made in a smoked gourd has a smoky flavour. Lipids in foods are subject to two forms of deterioration that affect the flavour of food products;

i. Hydrolytic rancidity

Lipolysis, which is the breaking down of milk fat into component fatty acids, increases the concentration of free fatty acids. Lipolysis is induced by the action of naturally occurring lipase in milk which hydrolyses the triacylglycerides. The C4 to C12 fatty acids in milk are the major contributors to detectable rancidity since they are relatively water soluble and volatile at room temperature.

Susceptibility of milk to lipolysis varies widely among cows. Lipolysis increases with stage of lactation and varies inversely with milk yield. Feeding rations of low quality may also increase lipolysis in the milk. The susceptibility of milk to lipolysis is increased by homogenisation, pumping, foaming and temperature manipulation. For example when milk is cooled to 5°C, rewarmed by adding hot milk and cooled again to 5°C, the extent of lipolysis is increased; pasteurisation (72.8°C x 15 seconds) inactivates the enzyme responsible. The flavour caused by free fatty acids is not always undesirable. Free fatty acids contribute to the desirable flavour of several cheese varieties, e.g. Cheddar, Camembert and Roquefort.

ii. Oxidative rancidity

The oxidative deterioration of lipids is caused by oxidation (involving oxygen) of unsaturated fatty acids mainly oleic, linoleic and linolenic acids resulting in the production of volatile aldehydes, ketones

and alcohols. A variety of factors influence the rate of oxidation. Undoubtedly the most important factor is the composition of the fat, i.e. the nature and proportion of unsaturated fatty acids present. For example linoleate oxidises 10 to 15 times as fast as oleate. The main factors accelerating the rate of lipid oxidation are high temperature, light and trace elements (copper, iron etc). Oxidation is inhibited by exclusion of oxygen, refrigeration and packaging in opaque or coloured containers.

Biosynthesis of Milk fat

The predominant fat in milk is triacylglycerol, which contains fatty acids of short- (C_4 - C_{10}), intermediate- (C_{12} - C_{16}), or long-chain (C_{18}) length. The short-chain acids are synthesized within the mammary gland from acetate and beta-hydroxybutyrate; long-chain acids are almost exclusively derived from blood plasma fatty acids of dietary origin; and intermediate-chain acids arise from both sources. In broad terms, about 50 percent of the fatty acids in milk are synthesized in the mammary gland and the other 50 percent are derived directly from blood.

2.2.2 Milk Protein: 3.9%

Proteins are chains of amino acid molecules connected by peptide bonds. They are an extremely important class of naturally occurring compounds that are essential to all life processes. They 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. They have the following general structure:

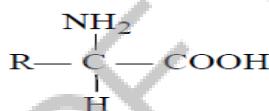


Figure 2.3: Structure of Protein

R represents the organic radical. Each amino acid has a different radical and this affects the properties of the acid. The content and sequence of amino acids in a protein therefore affect its properties. Some proteins contain substances other than amino acids and are called conjugated proteins. These include :

- **Phosphoproteins** in which phosphate is linked chemically to the protein, e.g. casein in milk and phosphoproteins in egg yolk.
- **Lipoproteins** are combinations of lipid and protein. They are excellent emulsifying agents. Found in milk and egg yolk.
- **Chromoproteins** have a coloured prosthetic group and include haemoglobin and myoglobin.

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.

Protein Biosynthesis

Most of the proteins present in milk are synthesized in the mammary gland, although some immunoglobulins and albumins are transferred from the blood. Blood leukocytes can also cross mammary barriers either by passing between secretory cells or by pushing secretory cells directly into the lumen. The synthesis of milk protein requires that both essential and nonessential amino acids be supplied to the mammary gland. Mephram (1982) has classified essential and nonessential amino acids into three groups according to uptake by the mammary gland. Group I essential amino acids

(methionine, histidine, phenylalanine, tyrosine, and tryptophan) are taken up in amounts just sufficient to meet milk protein synthesis needs. Group II essential amino acids (valine, leucine, isoleucine, arginine, lysine, and threonine) are taken up in excess. Group III is the nonessential amino acids. The amounts taken up vary with animal, time, and availability. In addition to free amino acid uptake from blood, there is evidence that red blood cells and the recycling of amino acids also contribute to the cellular amino acid pool.

Casein (Milk protein) and Whey Protein

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 milk solution 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.

i. Casein Protein

Casein was first separated from milk in 1830 by adding acid to milk, thus establishing its existence as a distinct protein. Casein is made up of a number of fractions and is therefore heterogeneous (Figure 2.4). About four kinds of polypeptide chains designated α_s (which can be broken down during cheese ripening to α_{s1} , α_{s2} and α_{s3}), β - and κ -caseins, together with some derivatives, e.g. γ -casein, formed by proteolysis of these chains, are included in the casein category. In general caseins are high in phosphorus, low in sulphur and are not significantly affected by moderate heat.

All the major caseins associate with themselves and with each other. 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 (α_{s1} , α_{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. Thus about 95% of the casein in milk exists as particles of colloidal dimensions known as *micelles*. 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.

Casein is easily separated from milk, either by acid precipitation or by adding rennin at about pH 4.6. In cheese making most of the casein is recovered with the milk fat. Casein can also be recovered from skim milk as a separate product. Caseins are hydrophobic but κ -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.

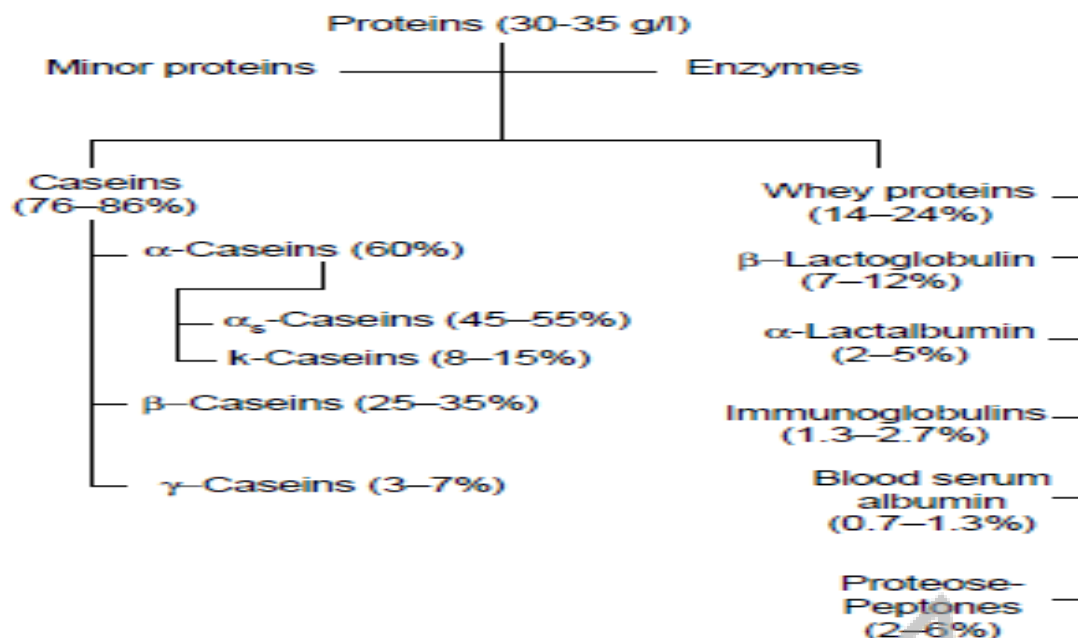


Figure 2.4: Casein Protein fractions

Industrially, hydrochloric acid (HCl) is the principal acid used; sulphuric acid (H₂SO₄) is used occasionally, but the resulting whey cannot be used for animal feed as it may cause intestinal disorders. When milk is treated with rennin the κ-casein fraction is hydrolysed to give para-κ-casein and macropeptides. In the presence of Ca⁺⁺ casein coagulates as follows:

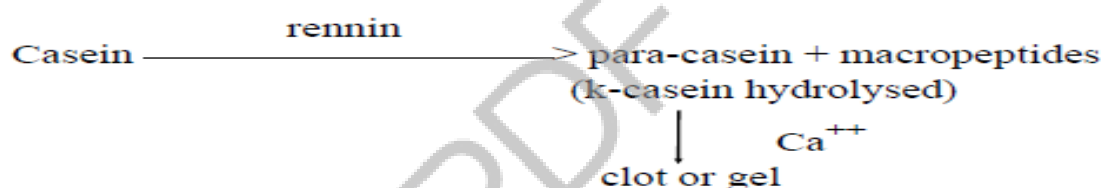
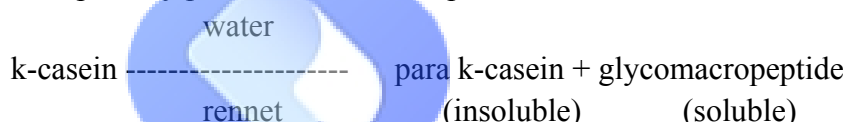


Figure 2.5 Casein coagulation

Since κ-casein stabilises the other caseins and its hydrolysis leads to the coagulation of the casein fraction, the primary phase can also be expressed as:



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.

The proportion of different casein proteins were— α_{s1}-casein 38%, α_{s2}-casein 10%, β-casein 36% and κ-casein 13%. Casein is not soluble at its isoelectric pH. Casein is not a globular protein; it associates extensively and is present in milk in large aggregates, the casein micelles, which also contain the colloidal calcium phosphate (CCP). On acidification, the CCP dissolves. Most of the κ-casein molecules are glycosylated to various extents. Part of the β-casein is split by proteolytic enzymes into γ-casein and proteose peptone. The α_s- and β-caseins are phosphoproteins that have a number of phosphate groups esterified to serine residues; they precipitate with Ca²⁺ ions, but κ-casein protects them from precipitation. However, κ-casein is easily attacked by the **rennet enzyme chymosin**, which

splits off a portion of the κ -casein molecule;

- it thereby loses its protective ability.
- As a result, the casein precipitates in the presence of Ca ions.
- These reactions are the basis of the clotting of milk by rennet and, thus, of cheese making.
- Casein altered in this way is called *para-casein* and can be obtained by means of renneting.
- Casein does not show denaturation. However, heating at temperatures above approximately 120°C causes the casein to slowly become insoluble due to chemical changes.

❖ **Casein is unique to milk**

Determine properties of milk, e.g.,

- its white color,
- stability to heat or ethanol and
- coagulation by rennet
- ◆ **Micelle** is a submicroscopic aggregation of molecules, as a droplet in a colloidal system

Properties of casein micelles

- ◆ It is composed of millions of casein sub-micelles that are clumped together
 - ◆ - almost visible under microscope
- ◆ Its shape is almost spherical
- ◆ Its size range 50-500 nm in diameter (ave. 120 nm) and
- ◆ Its molecular mass range from 10⁶-10⁹ Da
- ◆ Caseins are destabilized mainly by
 - ◆ Proteolytic enzymes – cleave of the casein-macropptides
 - ◆ Acidification

This process influences the technological properties of milk. The following figure 2.6 shows the structures of casein micelles.

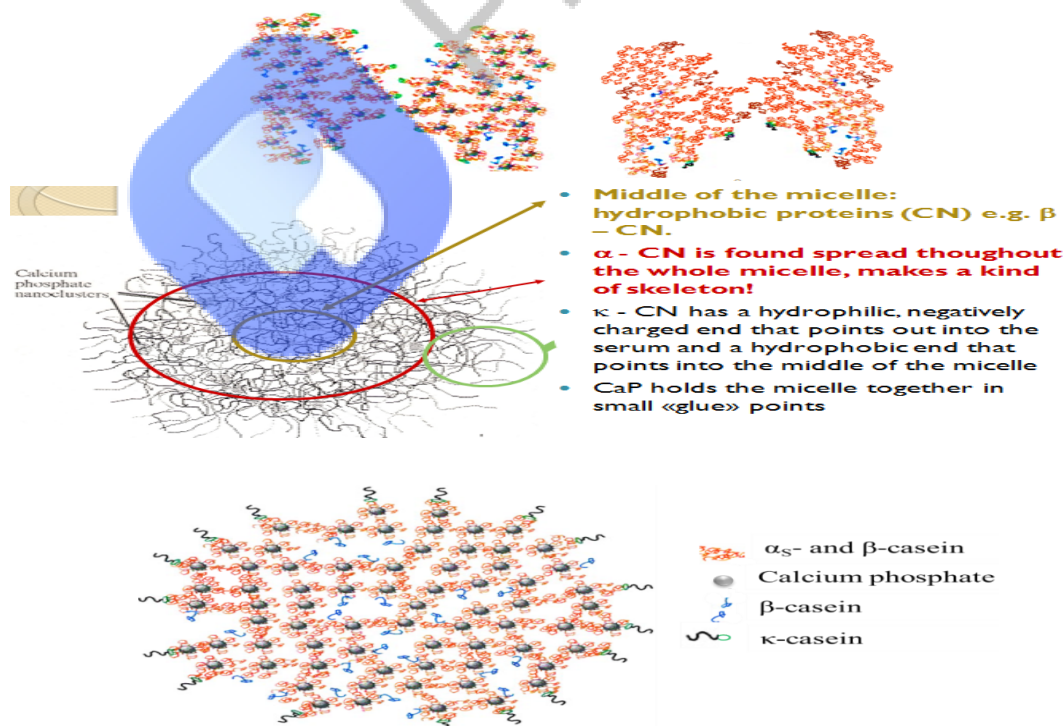


Figure 2.6 Structure of Casein Micelles

ii. Serum protein (Whey protein)

The supernatant after the caseins precipitate, contains four principal proteins in the whey fraction, β -lactoglobulin, α -lactalbumin, blood serum albumin, immunoglobulins and a number of minor proteins, e.g. lactoferrin and enzymes. Most of the whey proteins are denatured by heat, i.e. they become less soluble if milk is heated. β -lactoglobulin is the principal whey protein of the cow, goat and sheep, although there are slight interspecies differences. β -lactoglobulin accounts for about 50% of the total whey proteins or about 11% of the total protein in milk. Related but substantially different proteins occur in porcine milk. No β -lactoglobulin has been identified in human, camel or horse milk in which α -lactalbumin is the principal whey protein. Denaturation of whey proteins and β -lactoglobulin, in particular, is of major technological significance. β -lactoglobulin interacts with k-casein during heating and this reduces the heat stability of milk, slows down rennet clotting during cheese manufacture and gives a soft curd which tends to retain water. Denaturation of β -lactoglobulin causes the cooked flavour of heated milk.

α -lactalbumin represents about 20% of the protein of bovine whey (3.5% of the total milk protein) and is a relatively minor protein in terms of quantity. It functions as part of the enzyme system involved in lactose synthesis. The immunoglobulins are antibodies which are present in high concentrations in colostrum. Infants and mammals are born without circulating antibodies and the main way in which they acquire these is by ingestion of colostrum.

Whey 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. 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.

The immunoglobulin in milk vary widely in concentration and composition (colostrum has a high immunoglobulin content). Except proteose peptone, all serum proteins are globular proteins. At their IEP they remain in solution, but they are heat sensitive. They become insoluble at pH values < 6.5 if milk is heated. No doubt this change is related to the denaturation of the proteins involved. The denaturation does not result in aggregation, but the proteins precipitate onto the casein micelles and remain dispersed.

✚ **α -lactalbumin** acts as coenzyme in the synthesis of lactose.

- ✚ The protein is a small, compactly folded, more or less spherical molecule.
- ✚ It does not associate, except at low ionic strength.

Ca is strongly bound and stabilizes the protein conformation. Removal of the Ca, or lowering the pH to about 4, which also loosens the Ca^{++} , causes partial unfolding into a melt globule state. In this state the protein is subject to irreversible heat denaturation at relatively low temperatures. Native α -lactalbumin shows complete renaturation after heat treatment if no other proteins were present during heating.

β -lactoglobulin is the major serum protein, and its properties tend to dominate the properties of whey protein preparations, especially the reactions occurring upon heat treatment. Its solubility strongly depends on: pH and ionic strength, but it does not precipitate on acidification of milk; the same holds true for the other serum proteins. Below pH 5.5, β -lactoglobulin associates to form an octamer, although smaller aggregates also occur. Also at pH values above 7.5, only monomers occur.

■ **β -lactoglobulin** tends to bind some polar molecules, which may not be strange because of its high hydrophobicity

- The binding concern, for example, retinol (vitamin A) and some fatty acids.

- (Blood) serum albumin is a minor protein that presumably gains entrance to milk by 'leakage' from blood serum.
- Immunoglobulin (Ig) are antibodies synthesized in response to stimulation by specific antigens.
 - They specifically occur in blood.
 - They are large glycoprotein molecules of heterogeneous composition, even within one subclass.
- The main natural function of the Ig is to immunize the calf.
- During the first few days after parturition, the calf can absorb intact Ig from colostrum into the blood through its Gastro Intestinal Tract (GIT).

Lactoferrin is an inhibitor of some bacteria including *Bacillus* spp. The inhibition is caused by removal of iron, more precisely Fe^{3+} ions, from the serum. To be sure, the **lactoferrin** concentration in cows' milk is low; in human milk it is far higher. Generally, Milk protein plays a great role in technological processing of dairy products such as different cheese varieties.

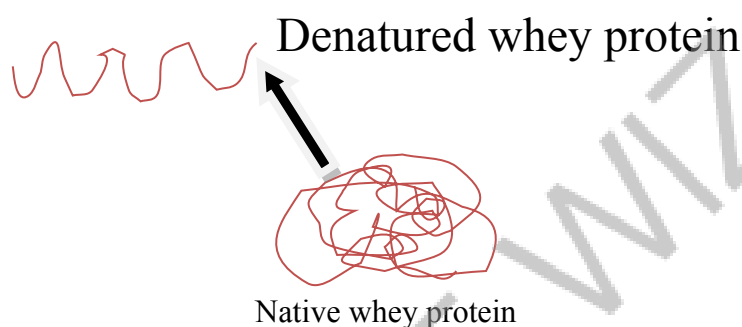


Figure 2.7 Denaturation of whey protein

Denaturation of Protein

Denaturation is the alteration of a protein shape through some form of external stress (for example, by applying heat, acid or alkali), in such a way that it will no longer be able to carry out its cellular function. Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to communal aggregation. Once this post-translational modification process has been completed, the protein begins to fold (spontaneously, and sometimes with enzymatic assistance), curling up on itself so that hydrophobic elements of the protein are buried deep inside the structure and hydrophilic elements end up on the outside. The final shape of a protein determines how it interacts with its environment.

- Denaturation of proteins results in change of function and ability to interact with other compounds, including proteins.
- Denaturation of protein enzymes results in inactivation of activity.

The conformation of a globular protein is thus stabilized by a great number of weak bonds. If the peptide chain unfolds, its conformational entropy greatly increases, and several groups, especially the peptide bonds, become hydrated. These factors make up a large amount of free energy, and the total free energy of the bonds causing a compact conformation is only slightly larger. In other words, the conformational stability is relatively small.

Slight changes in conditions can therefore lead to unfolding. Such an unfolding is called *denaturation*. It transforms a native globular protein into a more or less disordered protein. Several agents can cause denaturation of globular proteins, and some will be briefly mentioned:

- **High temperature** always leads to denaturation (increased effect of the change in conformational entropy), although the temperature needed varies; a common value is 70°C .

Low temperature can cause some proteins to denature (hydrophobic bonds become quite weak or even

repulsive); cooling is nearly always needed to at least -20°C . Somewhere between the two temperatures mentioned-say, at 25°C the stability of the conformation is at maximum.

- **High pressure**, i.e., a hydrostatic pressure above, say, 200 MPa (2 kbar) can also cause denaturation (by breaking H-bonds).

High pH, e.g., above 8 or 9, may cause denaturation (due to mutual repulsion between negatively charged groups); such pH values are rarely applied in practice. Also at very low pH denaturation may occur.

- Several *reagents* added to a protein solution in high concentrations cause denaturation, often by breaking H-bonds, but these reagents are not applied in dairy manufacture. In the laboratory, urea is often used.

- Often, a combination of two agents can cause ready denaturation, for example, a moderate temperature increase and a moderate pH increase.

2.2.3 Carbohydrates

Milk contains approximately 4.9% carbohydrate that is predominately lactose with trace amounts of monosaccharides and oligosaccharides. 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. 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 (20°C) the equilibrium ratio is approximately 37% α - and 63% β -lactose. At temperatures above 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 has been found in the milk of nearly all mammals and is unique to milk. Both glucose and galactose are abundant in the mammalian metabolism; lactose is only synthesized in the Golgi vesicles of the lactating cells. This occurs due to the presence of α -lactalbumin, a protein unique to milk. This protein modifies the action of the common enzyme galactosyl-transferase to catalyze the formation of lactose from uridine-diphosphate-galactose and glucose. Thus, **lactose** is a disaccharide of glucose and galactose. Lactose can be separated from milk or, in industrial practice, from whey, by letting it crystallize. Crystalline lactose is produced in large amounts, and it is mainly used in foods and in pharmaceuticals; nearly all pills contain lactose as a filling material. Lactose is also used as raw material for a range of chemical or enzymatic derivatives, such as lactitol, lactulose, and oligosaccharides.

Suitable reagents or enzymes can cause mild *oxidation* of lactose, whereby the aldehyde group is converted to a carboxyl group. Somewhat more vigorous oxidation ruptures the glycosidic linkage and produces carboxyl groups in the remaining sugars. Gentle *reduction* of lactose converts the aldehyde group to an alcohol group. More intense reduction cleaves the glycosidic linkage and results in the formation of alcohol groups in the remaining sugars. *Hydrolysis* of lactose by acid does not occur easily. If it occurs (high temperature and low pH), many other reactions take place as well. Several reactions of lactose occur when milk is heated. Lactose may isomerize into *lactulose*. That means the glucose moiety converts to a fructose moiety. The quantity of *lactulose* in heated milk products can be used as an indicator of the intensity of the heat treatment. Other reactions occurring during heat treatment are *caramelization* and *Maillard reactions*, which are to some extent related. The latter occur in the presence of amino groups, especially the ϵ -amino group of lysine residues in proteins. These reactions can lead to formation of flavor compounds and brown pigments, and to a decrease in the nutritionally available lysine.

Sweetness: a lactose solution is approximately 0.3 times as sweet as a sucrose solution of the same concentration. In milk, the sweetness is, to some extent masked by the protein, primarily casein. Consequently, unsoured whey has a sweeter taste than milk. If the lactose in milk is hydrolyzed into glucose and galactose, the sweetness is considerably enhanced.

Lactose is a disaccharide composed of two sugars, glucose and galactose (Figure 2.8). The average lactose content of milk varies between 4.7 and 4.9%, although milk from individual cows may vary more. Mastitis reduces lactose secretion. Lactose is a source of energy for the young calf and provides 4 calories/g of lactose metabolised. It is less soluble in water than sucrose. It can be broken down to glucose and galactose by bacteria that have the enzyme β -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.

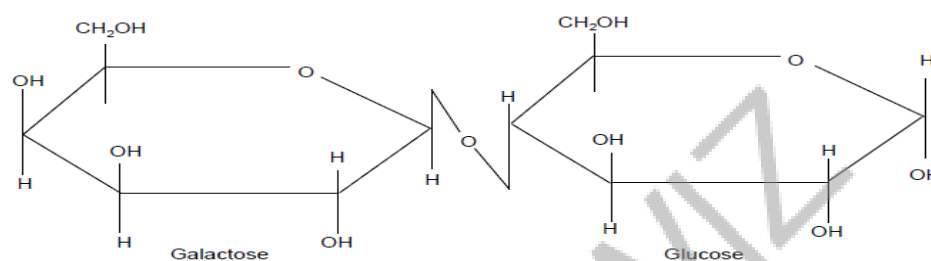


Figure 2.8: Structure of a lactose molecule

In cheese making, almost all of the 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 also used to fortify baby-food formulations. Lactose can be sprayed on silage to increase the rate of acid development in silage fermentation. Heating milk 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 crystallises. This causes sandiness in such products as ice cream. Special processing is required to crystallise lactose when manufacturing products such as instant skim milk powders. In addition to lactose, milk contains traces of glucose and galactose. Carbohydrates are also present in association with protein. κ -casein, which stabilises the casein system, is a carbohydrate-containing protein.

Nutritional Aspects of lactose

Lactose primarily provides the young animal with energy, but it has other functions, such as giving a sweetish flavor. Lactose cannot be taken up into the blood; it must first be hydrolyzed into glucose and galactose. This occurs slowly, which prevents a sudden large increase of the glucose level of the blood after ingesting a substantial amount of milk. High blood glucose levels are considered to be detrimental. Moreover, some sugar (galactose and lactose) can reach the large intestine (colon), where it serves as a carbon source for several benign colon bacteria.

Lactose is hydrolyzed by the enzyme *lactase*, more precisely, β -galactosidase, which is secreted in the small intestine. At least 60% of people over 4 years old, the activity of *lactase* enzyme is greatly reduced (to 5 to 10%), and they thus poorly metabolize lactose; these people are called *lactose mal-absorbers*. Drinking milk considerably enhances the activity of their colon flora. Roughly half of the mal-absorbers then develop significant problems, ranging from flatulence to severe diarrhoea; these are called *lactose intolerant*. They cannot drink milk in significant quantities (say, 100 ml per day). It has often been observed that fermented (sour) milk products hardly produce lactose intolerance,

although they still contain about two thirds of the original lactose; the explanation is not fully clear. Another possibility is to treat milk with lactase: the lactose then is almost fully hydrolyzed into glucose and fructose.

Physicochemical Aspects of lactose

A. Mutarotation

In solution, conversion of α - to β -lactose and vice versa occurs via the open chain form, which is very short-lived. These changes may be observed by using a *polarimeter*. The rotation of the plane of polarization then is found to change (to mutate) with time because α - and β -lactose differ in specific rotation. Hence, the term 'mutarotation.' The mutarotation rate K depends strongly on temperature. At room temperature it takes many hours before mutarotation equilibrium is reached; at 70°C a few minutes. pH has also great effect on K . Several substances may affect the mutarotation rate. Mutarotation also depends on lactose concentration.

B. Solubility

α - and β -lactose differ considerably in solubility and in the temperature dependence of solubility. If α -lactose is brought in water, much less dissolves at the onset than later. This is because of mutarotation, α -lactose being converted to β , so the α -concentration diminishes and more α can dissolve. If β -lactose is brought in water, more dissolves at the onset than later (at least below 70°C); on mutarotation more α -lactose forms then can stay dissolved, and α -lactose starts to crystallize. The solubility thus depends partly on the mutarotation equilibrium, the rate of dissolution on the mutarotation rate. The so-called final solubility is identical whether we dissolve α - or β -lactose.

C. Crystal Forms

Usually, α -lactose crystallizes as a hydrate containing one water molecule of crystallization. The crystals are very hard, slightly hygroscopic, often fairly large, and dissolve slowly. The water of crystallization is very strongly bound. Above 93.5°C, anhydrous β -lactose crystallizes from an aqueous solution. β -lactose is not very hygroscopic, and it dissolves quickly; its solubility is good. Obviously, dehydrating α -hydrate is difficult. It may cause problems when determining the dry-matter content of milk and milk products; this determination implies evaporation of water at elevated temperature. Maintaining the temperature >93.5°C during the assay is of paramount importance to prevent formation of α -lactose hydrate crystals.

Amorphous lactose is formed during rapid drying, as in a spray drier. Amorphous lactose contains at least a few percent of water and can quickly dissolve on addition of water. But then, α -lactose hydrate may start to crystallize. If the water content of the amorphous lactose is low, say, 5%, crystallization is postponed. However, the product attracts water from moist air, and when moisture content rises to about 8%, α -lactose hydrate starts to crystallize (at room temperature). The postponed crystallization is an important factor in relation to spray dried powders made from skim milk or whey because it leads to hard lumps in the powder; eventually, the whole mass of powder turns into one solid cake. In practice, it is almost impossible to obtain pure crystals of whatever form. For example, α -hydrate usually contains a little β -lactose, and vice versa.

In summation,

- ◆ Lactose is found in Milk of (almost) all mammals
- ◆ Primary source of energy for the neonate

- ◆ Highest concentration (7%) in human milk
- ◆ Principal component of cow's milk (4.5 - 5%)
- ◆ The first record of the isolation of lactose was in 1633, by Bartoletus, through the evaporation of whey
- ◆ Lactose in solid form can either be in a crystalline state or in an amorphous state.
- ◆ Crystalline lactose can exist in a number of distinct forms.
 - Most well known are α -lactose monohydrate and β -lactose.

α -Lactose monohydrate

- The most common
- Crystallize below 93.5°C
- Contain 1 molecule of water

α -Anhydrous

- Anhydrous α -lactose may be prepared by dehydrating α -hydrate *in vacuo* at a temperature between 65 and 93.5 °C;
- it is stable only in the absence of moisture.

β -lactose

- Crystallize at Temperature above 93.5°C
- Crystals of pure have a characteristic kite-like form
- Particles with crystalline β -lactose are more brittle than α -lactose monohydrate crystals
- Sweeter than α -lactose

2.2.4 Minor milk constituents

In addition to the major constituents already discussed, milk also contains a number of organic and inorganic compounds in small or trace amounts, some of which affect both the processing and nutritional properties of milk.

i. Milk salts

The salts of milk are composed mainly of the chlorides, phosphates, citrates, carbonates and bicarbonates of sodium, potassium, calcium and magnesium. Approximately 20 other elements are found in milk in trace amounts. These include copper, iron, lead, boron, manganese and iodine. Milk salts are important in human nutrition, stability of milk lipids and in the processing of milk proteins. The ash content of cow milk remains relatively constant at 0.7 to 0.8%, but the relative concentrations of the various ions vary considerably. The composition is influenced by a number of factors including breed, individuality of the cow, stage of lactation, feed, infection of the udder and season of the year.

Certain milk salts, e.g. sodium and potassium chlorides are sufficiently soluble to be present almost in the dissolved phase. The content of others, in particular calcium phosphate, is greater than can be maintained in solution at the normal pH of milk. Consequently, these exist partly in soluble form and partly in insoluble or colloidal form. Although salts comprise less than 1 % of the milk they influence its rate of coagulation and other functional properties. Calcium, magnesium, phosphorous and citrate are distributed between the soluble and colloidal phases. Their equilibria are altered by heating, cooling and by a change in pH.

ii. Milk vitamins

Milk is a good source of thiamin, riboflavin and vitamin B12 . Milk contains small amounts of niacin, pantothenic acid, vitamin B6, 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), low-fat (1% fat), and skim milk must be fortified with vitamin A and 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. Vitamins are unstable and processing can therefore reduce the effective vitamin content of milk.

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. Riboflavin is a heat stable vitamin and is not affected by severe heat treatments. At very high temperatures 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.

iii. Enzymes:

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.

- There are a large number of enzymes in milk and the functions of many are not well-defined.
- 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.
- 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.

2.3 Factor affecting composition of milk

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.

2.3.1 Genetic factor

A change in milk composition using traditional breeding techniques occurs slowly, although new techniques of genetic manipulation may allow faster progress in the future. Heritability estimates for yield are relatively low at about 0.25 but heritability estimates for milk composition are fairly high at 0.50. The priority placed on each genetic trait depends upon its economic or profit impact. Milk yield per cow tends to receive the most attention by producers. However, component yields should not be overlooked. Genetic selection should be directed toward increasing fat, protein and non-fat solids yields. But, because component percentages tend to have negative genetic associations with yield traits, a change in these percentages is not likely to be achieved through genetic selection alone. Both milk yield and composition vary considerably among breeds of dairy cattle.

Table 2.2: Average composition (%age) of milk, from different breeds of cow

Breed	Fat	Protein	Lactose	Ash
Zebu	5.6	3.1	4.6	0.71
Ayrshire	3.8	3.4	4.8	0.70
Friesian	3.4	3.2	4.6	0.74
Guernsey	4.9	3.8	4.8	0.75
Jersey	5.1	3.8	4.9	0.75
Shorthorn	3.6	3.4	4.8	0.70

Variability among cows within a breed

The potential fat content of milk from individual cows within a breed varies over a wide range both in yield and in the content of the various constituents. The potential fat content of milk from an individual cow is determined genetically, as are protein and lactose levels. Thus selection for breeding on the basis of individual performance is effective in improving milk compositional quality. Herd recording of total milk yields and fat and solids-not-fat (SNF) percentages will indicate the most productive cows, and replacement stock should be bred from these.

2.3.2 Environment factors

i. Season

The variation is related to changes in both the types of feed available and climatic conditions. Lush spring pastures low in fiber depress milk fat. Hot weather and high humidity decrease dry matter intake and increase feed sorting, resulting in lower forage and fiber intake.

ii. Feeding regime

Underfeeding reduces both the fat and the SNF content of milk, although SNF content is the more sensitive to feeding level. Fat content and fat composition are influenced more by roughage (fiber) intake. The SNF content may fall if the cow is fed a low-energy diet, but is not greatly influenced by protein deficiency, unless the deficiency is acute.

2.3.3 Physiological factors

i. Age (Parity)

Milk protein content gradually decreases with advancing age. A survey of Holstein Dairy Herd Improvement Association (DHIA) lactation records indicates that milk protein content typically decreases 0.10 to 0.15 unit over a period of five or more lactations or approximately 0.02 to 0.05 unit per lactation. As cows grow older the fat content of their milk decreases by about 0.02 percentage units per lactation while the fall in SNF content is about 0.04 percentage units.

ii. Interval between milking

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

iii. 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. The high protein content of early lactation milk is due to mainly the high globulin content. The variation in milk constituents throughout lactation are shown in figure 2:9.

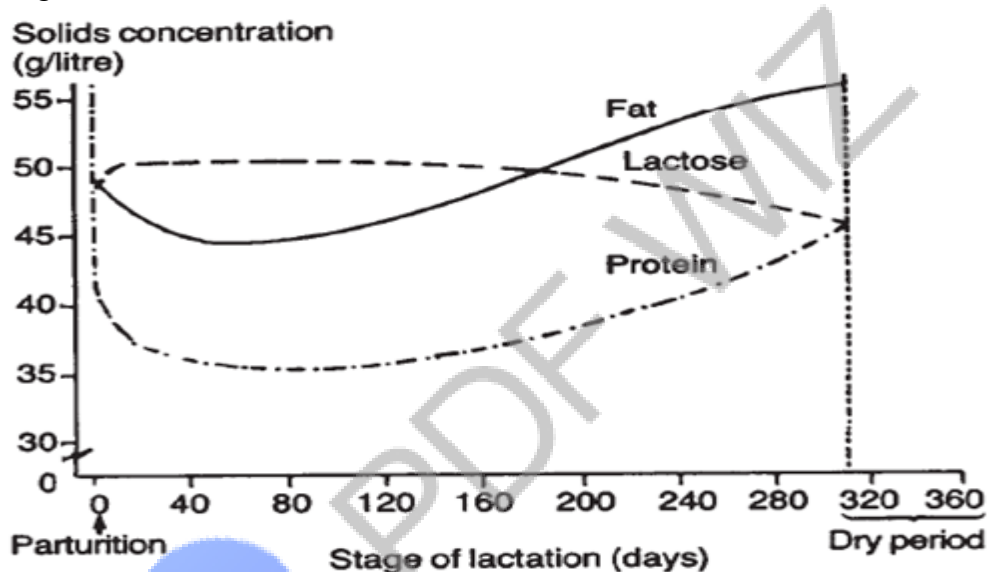


Figure 2:9 Composition of milk across lactation length

iv. Completeness of milking

The first milk drawn from the udder contains about **1.4%** fat while the last milk (or stripping) contains about **8.7%** fat. Thus, it is essential to milk the cow completely and thoroughly mix 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 milking, so there is no net loss of fat.

2.3.4 Pathological factors

Disease

Although other diseases can affect milk component, content and distribution, mastitis has been the predominant disease studied. Mastitis results in a reduction in fat and casein content and an increase in whey content of milk. These changes in the milk proteins, in conjunction with alterations in lactose, mineral content and milk pH, result in lower cheese yields and altered manufacturing properties. Milk from cows with elevated somatic cell counts (greater than 500,000 somatic cells/ml) has longer coagulation time and forms weaker curds than milk from cows with lower somatic cell counts.

Chapter 3. Milk Microbiology

Milk must be of good hygienic quality. This is essential in terms of public health, the quality of the products made from milk, and the suitability of milk for processing. Components that are foreign to milk but enter the milk via the udder or during or after milking, as well as any changes occurring in the milk, are often detrimental to its quality. Milk is a good source of nutrients and edible energy, not only for mammals but also for numerous microorganisms, which thus can grow in milk. These microorganisms are primarily bacteria, but some molds and yeasts can also grow in milk. Microorganisms are living creatures that are not visible with the naked eye. On looking into the microscope, the extended world of algae, protozoa, yeasts, bacteria, and viruses becomes brightly illuminated.

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

3.1 Bacteria

Bacteria are microscopic single-celled organisms that are present in air, water and on most solid materials. Their small size is typical and has important consequences not only for the morphology, but also for the activity and flexibility of the metabolism of bacteria. The diameter of most of bacteria is less than $1\mu\text{m}$, and their average volume is $1\mu\text{m}^3$. Many bacteria are rod-shaped, others are spherical, and some are spirally curved. Reproduction in bacteria typically occurs by binary division and is universally independent of sexual events; it is asexual. An important taxonomic characteristic for bacteria is the *Gram stain reaction*. This reaction is determined by microscopic examination of cells that have been successively stained with the basic dye crystal violet, treated with an iodine solution, and rinsed with alcohol. Gram-positive cells retain the violet stain, whereas Gram-negative cells are decolorized by alcohol. The final step in the procedure is application of a red stain (usually safranin) so that Gram-negative cells can be visualized.

Cells are either spherical or rod-shaped (Figure 3.1); spherical bacteria are called *cocci* while 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: paired cocci cells.
- Staphylococci: a number of cells clustered together.
- Streptococci: a number of cells arranged in a chain.

Some bacteria are motile. They move using flagellae—long, hair-like appendages growing out of the cell. Some rod-shaped bacteria may form spores when the cells are faced with adverse conditions such as high temperature. Once suitable conditions are re-established the spores germinate to form new cells.

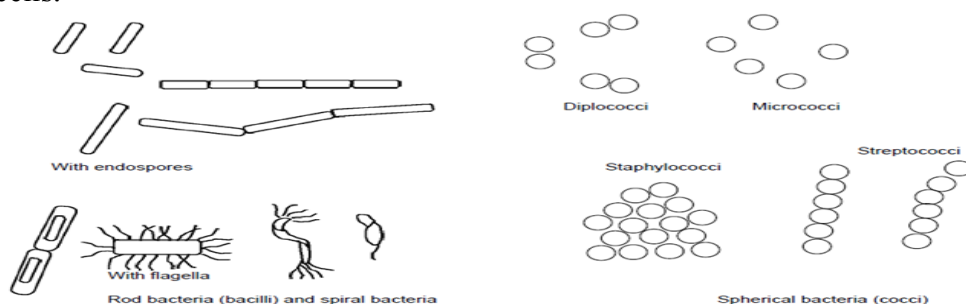


Figure 3.1: Rod-shaped (Bacilli) and spherical (cocci) bacteria

Close examination of the simple cell reveals that it is composed of the following components (Figure 3.2):

- ✚ Cell wall which gives the cell its shape and retains the constituents
- ✚ Cell membrane for filtering in food constituents and discharging waste products
- ✚ Nucleus where the genetic material of the cell is stored. The cytoplasm is a semi-liquid proteinaceous substance which contains starch, fat and enzymes.

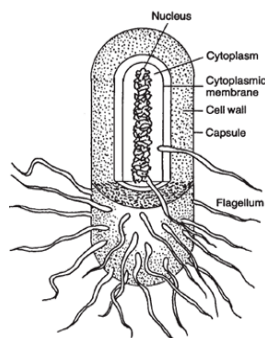


Figure 3.2: Schematic illustration of bacterial Structure

3.1.1 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* which can be defined as the time taken for the cell count to double.

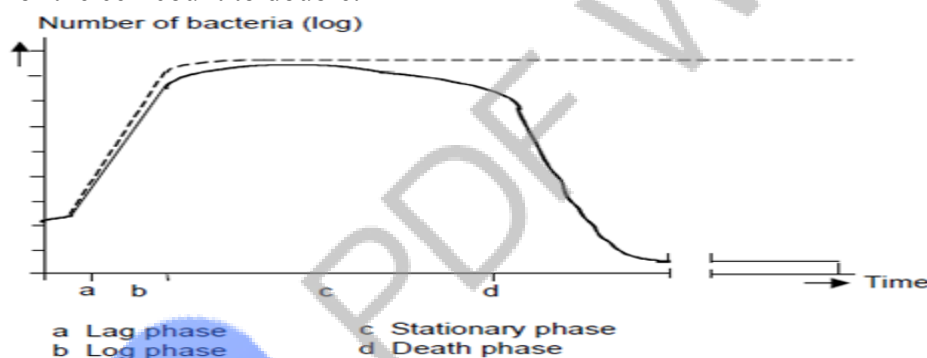


Figure 3.3: The four phases of bacterial growth

- 1. Lag phase:** There is usually some delay in growth after inoculation of bacteria into a new medium. During this time the bacteria adapt to the medium and synthesise the enzymes needed to break down the substances in it.
- 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. Plotting 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.
- 3. Stationary phase:** As the bacteria dominate the growth medium they deplete the available nutrients and 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 ones resulting in no net change in cell numbers.
- 4. Death phase:** In this phase the formation of new cells ceases and the existing cells gradually die off.

3.1.2 Factors affecting bacterial growth

Bacterial growth is affected by temperature, nutrient availability, water supply, oxygen supply, and acidity of the medium.

i. Temperature

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 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. Psychrotrophic (grow at temperatures below 16°C), mesophilic (grow best at temperatures between 16 and 40°C), and thermophilic bacteria grow best at temperatures above 40°C.

ii. 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 which contains both material for cell formation and the necessary energy. The organic matter must be soluble in water and of low molecular weight to be able to pass through the cell membrane.

iii. Water

Bacteria cannot grow without water. Many bacteria are quickly killed by dry conditions, although others can tolerate such 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 a 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*.

iv. Oxygen

Animals require oxygen to survive but bacteria differ in their requirements for and in their ability to utilise oxygen. Aerobic bacteria need oxygen for growth, however, it is toxic to anaerobic bacteria. Some bacteria can live either with or without oxygen and are known as facultative anaerobic bacteria.

v. 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. Most bacteria prefer a growth environment with a pH of about 7, i.e. neutrality. Bacteria that can tolerate low pH are referred to as aciduric. Lactic acid bacteria in milk produce acid and continue to do so until the pH of the milk falls below 4.6, at which point they gradually die off.

Fresh milk 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 (lactic acid bacteria) are useful in milk processing, causing milk to sour naturally, leading to fermented products such as *irgo*. However, milk can also contain pathogenic bacteria, such as *Salmonella*, *Mycobacterium tuberculosis*, *Listeria* and *Brucella*, and can thus transmit disease. Other bacteria can cause spoilage of the milk, and spoilage and poor yields of products.

3.1.3 Beneficial bacteria

Beneficial Microorganisms are used in foods in several ways.

These include:

- Actively growing microbial cells, e.g. conversion of milk to yogurt by lactic acid bacteria.
- Non-growing microbial cells, (increase shelf life of refrigerated raw milk or raw meat)
- Metabolic by-products (e.g. lactic acid, acetic acid, some essential amino acids, and bacteriocins produced by different microorganisms are used in many foods.
- Microbial cellular components (used in food for different single-cell proteins (SCPs), e.g. dextran, cellulose, and many enzymes purposes.

Food fermentation involves a process in which raw materials are converted to fermented foods by the growth and metabolic activities of the desirable microorganisms. The Microorganisms (MO) utilize some components present in the raw materials as substrates to generate energy and cellular components, to increase in population, and to produce many usable by-products (also called end products). The unused components of the raw materials and the microbial by-products (and sometimes microbial cells) together constitute fermented foods. Many of the fermented foods consumed currently have been produced and consumed by humans for thousands of years.

The basic principles developed by these ancient civilizations are used even today to produce many types of fermented foods by a process known as *natural fermentation*. In this method, either the desirable microbial population naturally present in the raw materials or some products containing the desirable microbes from a previous fermentation (called back slopping), are added to the raw materials. In another type of fermentation, called controlled or pure culture fermentation, the microorganisms associated with fermentation of a food are first purified from the food, identified, and maintained in the laboratory. When required for the fermentation of a specific food, the microbial species associated with this fermentation are grown in large volume in the laboratory and then added to the raw materials in very high numbers. Microbial species, when used in controlled fermentation, are also referred to as *starter cultures*.

Lactic Starter Cultures

At present, bacterial species from 12 genera are included in a group designated as lactic acid bacteria because of their ability to metabolize relatively large amounts of lactic acids from carbohydrates. The genera include:

- | | |
|--------------------------|----------------------------|
| ◆ <i>Lactococcus</i> , | ◆ <i>Aerococcus</i> , |
| ◆ <i>Leuconostoc</i> , | ◆ <i>Vagococcus</i> , |
| ◆ <i>Pediococcus</i> , | ◆ <i>Tetragenococcus</i> , |
| ◆ <i>Streptococcus</i> | ◆ <i>Carnobacterium</i> , |
| ◆ <i>Lactobacillus</i> , | ◆ <i>Weissella</i> , and |
| ◆ <i>Enterococcus</i> , | ◆ <i>Oenococcus</i> |

Major lactic starter cultures were discussed as following

A. *Lactococcus*

This genus includes several species, but only one species, *Lactococcus lactis*, has been widely used in dairy fermentation. It has three subspecies (ssp.), ssp. *lactis*, ssp. *cremoris*, and ssp. *hordniae*, but only the first two are used in dairy fermentation. The biovar *Lac. lactis* ssp. *lactis* biovar *diacetylactis* is also used in dairy fermentation. The cells are sporulating, and facultative anaerobic to micro-aerophilic. In general, they grow well between 20 and 30°C, but do not grow in 6.5% NaCl or at pH 9.6. They are generally capable of hydrolyzing lactose and casein. Natural habitats are green vegetation, silage, the dairy environment, and raw milk.

B. *Streptococcus*

Only one species, *Streptococcus thermophilus*, has been used in dairy fermentation.

C. *Leuconostoc*

The species grow well between 20 and 30°C, with a range of 1 to 37°C. Some species can survive 60°C for 30 min. *Leuconostoc* species are found in plants, vegetables, silage, milk and some milk products, and raw and processed meats. Recently, two new genera have been created from it: *Weissella* and *Oenococcus*.

D. *Pediococcus*

They are Gram-positive, non-motile, non-sporulating, facultative anaerobes. They grow well between 25 and 40°C; some species grow at 50°C. Depending on the species, they are found in plants, vegetables, silage, beer, milk, and fermented vegetables, meats, and fish. The genus has seven to eight species, of which *Ped. pentosaceus* and *Ped. acidilactici* are used in vegetables, meat, cereal, and other types of fermented foods. They have also been implicated in ripening and flavor production of some cheeses as secondary cultures.

E. *Lactobacillus*

Gram-positive, rod-shaped, usually non-motile, non-sporulating and facultative anaerobes. While growing on glucose, depending on a species, they produce either only lactic acid or a mixture of lactic acid, ethanol, acetic acid, and CO₂. Growth temperature can vary from 1 to 50°C, but most that are used as starter cultures in controlled fermentation of foods grow well from 25 to 40°C. Several species involved in natural fermentation of some foods at low temperature can grow well from 10 to 25°C. They are distributed widely and can be found in plants; vegetables; grains; seeds; raw and processed milk and milk products; raw, processed, and fermented meat products; and fermented vegetables; some are found in the digestive tract of humans, animals, and birds. Many have been associated with spoilage of foods.

The three *Lactobacillus delbrueckii* subspecies are used in the fermentation of dairy products, such as some cheeses and yogurt. They grow well at 45°C and ferment lactose to produce large amounts of lactic acid. *Lab. acidophilus* and *Lab. reuteri* are considered beneficial intestinal microbes (probiotic) and present in the small intestine. *Lab. acidophilus* is used to produce fermented dairy products and also either added to pasteurized milk or made into tablets and capsules for consumption as probiotics.

Other Starter Cultures

i. *Bifidobacterium*

They are non-spore forming, non-motile, and anaerobic, although some can tolerate O₂ in the presence of CO₂. The species grow optimally at 37 to 41°C, with a growth temperature range of 25 to 45°C. They usually do not grow at a pH above 8.0 or below 4.5. They ferment glucose to produce lactic and acetic acid in a 2:3 molar ratio without producing CO₂, and also ferment lactose, galactose, and some pentoses. They have been isolated from feces of humans, animals, and birds and are considered beneficial for the normal health of the digestive tract. They are present in large numbers in the feces of infants within 2 to 3 d after birth, and usually present in high numbers in breast-fed babies. They are usually found in the large intestine. Some of these species have been added to dairy products to supply live cells in high numbers to restore and maintain intestinal health in humans.

ii. *Propionibacterium*

The cells are Gram-positive, non-motile, non-sporulating, anaerobic (can also tolerate air) and ferment glucose to produce large amounts of propionic acid and acetic acid. Grow optimally at 30 to 37°C. They have been isolated from raw milk, some types of cheeses, dairy products, and silage. At present, four species of dairy propionibacterium are included in the genus: *Pro. freudenreichii*, *Pro. jensenii*, *Pro. thoenii*, and *Pro. acidipropionici*. All four are associated with natural fermentation of Swiss-type cheeses, but *Pro. freudenreichii* has been used as a starter culture in controlled fermentation.

iii. *Brevibacterium*

The genus contains a mixture of gram-positive, non-spore-forming rods, some of which have

important applications in cheese production and other industrial fermentations. *Brevibacterium linens* is used in cheese ripening as it has extracellular proteases. Capable of growing in high salt and wide pH ranges.

iv. *Acetobacter*

A species in this genus, *Ace. aceti*, is used to produce acetic acid from alcohol. The cells are Gram-negative; aerobic; rods, can be motile or non-motile. They are obligate aerobes and oxidize ethanol to acetic acid and lactic acid to CO₂ and H₂O. They grow well from 25 to 30°C. They are found naturally in fruits, beer, sugar cane juice and soil. Some species synthesize large amounts of cellulose.

3.1.4 Pathogenic bacteria

Raw milk may contain a very wide range of pathogens, derived from the milk animals, the environment or from farm workers and milking equipment which include *Salmonella* spp. (particularly *Salmonella typhimurium* and *Salmonella dublin*, a virulent serotype in humans), *E. coli* O157, *L. monocytogenes* and *Campylobacter* spp. etc. These pathogens may be present even in hygienically produced milk of generally good microbiological quality. Hence, raw milk can be a potentially hazardous product. Because, the microbiological safety of milk cannot be assured without the use of pasteurization or an equivalent process.

1. *Salmonella*

Salmonellae are not able to survive the typical minimum pasteurization processes generally prescribed in legislation. Therefore, their presence indicates that the process has not been carried out effectively, or that post-process contamination has occurred. For example, an outbreak of salmonellosis in Kentucky in 1984 was associated with pasteurized milk, but an investigation of the dairy concerned showed that pasteurization temperatures were inadequate, and could have been as low as 54.5°C for 30 minutes.

2. *Campylobacter* spp.

Campylobacter spp. are not capable of surviving milk pasteurization treatments although they are able to survive for long periods in milk at refrigeration temperatures. Nonetheless, outbreaks of campylobacteriosis associated with pasteurized milk have occurred. For example, a large outbreak in the UK in 1979 caused by *Campylobacter jejuni* was estimated to have affected at least 2,500 schoolchildren, and was associated with free milk provided in schools. The reason could be that raw milk may have bypassed the pasteurization process. Birds are known to be an important reservoir of *Campylobacter* infection.

3. *Listeria monocytogenes* => *Gram positive bacillus*

An outbreak of listeriosis in Massachusetts during 1983 resulted in 49 cases, 14 of whom subsequently died. Three deaths and a miscarriage in Boston, USA between 2007-8 have been linked to presence of *Listeria* in pasteurized milk. The explanation offered for both these findings was that the organisms might have been protected during heat treatment within leucocytes in the milk. *L. monocytogenes* is likely to be present in wet dairy processing environments, and post-process contamination is therefore a particular hazard. The organism has been shown to be capable of significantly more rapid growth in pasteurized milk than in raw milk at 7 °C, and is also capable of growth at 4 °C in pasteurized milk. Therefore, effective HACCP-based controls to prevent post-process contamination are critical, particularly the cleaning and sanitizing of all milk contact surfaces.

4. *Verotoxigenic Escherichia coli*

Dairy cattle are an important reservoir for *E. coli* O157:H7 and this organism may therefore be present in raw milk, usually through fecal contamination. For this reason, raw milk is a high-risk food for this serious intestinal pathogen. However, *E. coli* O157:H7 is not a heat-resistant organism and there is no evidence that it is able to survive pasteurization. Despite this, there have been outbreaks associated with pasteurized milk. In 1994, an outbreak in Scotland affected over 100 people and was associated with consumption of pasteurized milk from a local dairy and the cause was thought to be a fault in the operation of the pasteurizer.

5. *Yersinia enterocolitica*

Although there has been a question about the ability of *Y. enterocolitica* to survive milk pasteurization, the majority of the evidence indicates that it is inactivated. Therefore, the presence of the organism in pasteurized milk is likely to be the result of post-process contamination. In 1976, an outbreak affecting 36 children was associated with the consumption of contaminated chocolate milk. Another outbreak in 1982 was the largest food-borne yersiniosis outbreak ever recorded in the USA, and was also associated with pasteurized milk. Pigs are a well known reservoir for *Y. enterocolitica*. *Y. enterocolitica* is capable of psychrotrophic growth, and could therefore multiply in pasteurized milk during storage. Measures should therefore be taken to prevent post-process contamination as with *L. monocytogenes*.

6. *Staphylococcus aureus*

Staph. aureus is rarely involved in food poisoning associated with consumption of pasteurized milk. *Staph. aureus* does not generally grow at temperatures below 7°C, and enterotoxin production is inhibited at low temperatures. The organism is also known to be inhibited by the presence of competing species. Nevertheless, an outbreak in California affecting 500 school children was associated with chocolate-flavored milk. The cause was thought to be growth of *Staph. aureus* in raw milk, and the subsequent persistence of the heat-stable enterotoxin through pasteurization.

7. *Mycobacterium avium subsp. paratuberculosis*

MAP is the causative organism of Johne's disease in cattle, a chronic wasting disease, and may occasionally be present in raw milk. Concerns have been raised that MAP might be able to survive pasteurization if present at levels above 100 cells per ml, especially if clumps of cells are present, and that pasteurized milk may therefore be a vehicle for Crohn's disease. On the basis of new heat-resistance studies, many UK dairies have increased pasteurization times from 15 to 25 seconds.

3.1.5 Spoilage bacteria

Milk and dairy products are vulnerable to spoilage or contamination with pathogens or microbial toxins. Among others environmental contaminants including

- ◆ Gram negative bacteria, Gram positives including spore formers, yeasts and molds could greatly influence and contribute to the spoilage of milk and its products.

Bacillus spp. have been implicated in spoilage of raw and pasteurized milk, UHT, concentrated and canned milk products of pasteurized milk. *Clostridium* spp. have been implicated in the rancid spoilage and “late blowing” of numerous cheeses.

The thermophilic microflora of milk consists largely of Gram-positive spore formers, mainly *Bacillus* spp., *Clostridium* and organisms with heat-resistant vegetative cells, such as *Micrococcus*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, *Corynebacterium* and *Alcaligenes*. Of these, the spore-

formers are most important in spoilage, since the other species are not generally psychrotrophic and are unable to grow in refrigerated milk (<5°C).

Several *Bacillus* spp. contain *psychotropic* strains, notably *B. cereus* and *Bacillus circulans*, which may grow at temperatures as low as 2 °C could cause spoilage on refrigerated milk. However, at slightly higher temperatures (7 - 8 °C), *B. cereus* in particular may grow quite rapidly, produce a type of spoilage known as 'bitty cream' or 'sweet curdling', caused by the action of **lecithinase** on the phospholipids in fat globules.

Bacterial spoilage in butter

Surface taints may develop as a result of growth of *Shewanella putrefaciens* (formerly *Alteromonas putrefaciens*), and *Pseudomonas putrefaciens* or *Flavobacterium* spp. Such spoilage may be apparent within 7 to 10 days of chilled storage. The surface layers are initially affected, but eventually spoilage is apparent throughout the product. A putrid or cheesy flavour develops due to the breakdown of protein. Rancidity, proteolytic activity and fruity odours may be caused by the growth of *Pseudomonas fragi* and, occasionally, *Pseudomonas fluorescens*. Black discoloration of butter is also caused by *Pseudomonas nigrificans*, *Pseudomonas mephitica* is responsible for a skunk-like odour, and an organism formerly known as *Lactococcus lactis* var. *maltigenes* may be responsible for a 'malty' flavour defect linked to the formation of 3-methylbutanal. Lipolytic spoilage of butter has been associated with the presence of *Micrococcus*.

Fungal spoilage in butter

Moulds are still important spoilage organisms for butter, and mould growth may produce surface discolorations and taints. A number of genera have been associated with spoiled butter, including *Penicillium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Geotrichum*, *Alternaria*, and *Rhizopus*. Yeasts may also cause spoilage of butter. Lipolytic species such as *Rhodotorula* may grow on the surface at chill temperatures and may tolerate high salt concentrations. Other yeasts associated with spoilage include *Candida lipolytica*, *Torulopsis*, and *Cryptococcus*.

Fungal spoilage in cheese

Although the growth of molds on the surface or in the body of some cheese varieties is essential for ripening, mold growth is generally not desirable. Mould spoilage is usually **unpleasant in appearance**, and may result in **musty taints** and **odors**. Moulds are also responsible for liquefaction of the curd. There is also the possibility of mycotoxin production in some cases. Moulds commonly involved in cheese spoilage include members of the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Fusarium*, *Monilia* and *Alternaria*.

Bacterial spoilage in cheese

In fresh cheeses with a **sufficiently high pH**, such as cottage cheese, bacterial spoilage may occur. This is likely to be caused by **Gram-negative, psychrotrophic species**, such as pseudomonads and some coliforms. These organisms may contaminate the product through water used to wash the curd. *Pseudomonas* spp., *Alcaligenes* spp., *Achromobacter* spp. and *Flavobacterium* spp. are the psychrotrophic bacteria of concern. *Pseudomonas fluorescens*, *Pseudomonas fragi* and *Pseudomonas putida* cause

- bitterness, putrefaction and a rancid odour,
- liquefaction, gelatinization of curd, and
- slime and mucous formation on cheese surfaces.

Late blowing, is caused by **clostridia** that are able to produce butyric acid from lactate may occur after 10 days in varieties such as **Gouda**, or after several months in some **Swiss cheeses**, late blowing sometimes also occurs in **Cheddar**

Species commonly involved are

- ◆ *Clostridium butyricum*,
- ◆ *Clostridium tyrobutyricum* and
- ◆ *Clostridium sporogenes*, spores of which survive pasteurization and can be present in cheese milk.

3.2 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.



Figure 3.4: structure of moulds

3.3 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. In the dairy industry, specific molds and yeasts are essential for the ripening of certain cheese types. Most molds are able to grow in situations in which yeasts and bacteria cannot survive because of high osmotic pressure, acidity, or low water content. Molds are characteristically strict aerobes, whereas yeasts can grow under aerobic as well as anaerobic conditions.

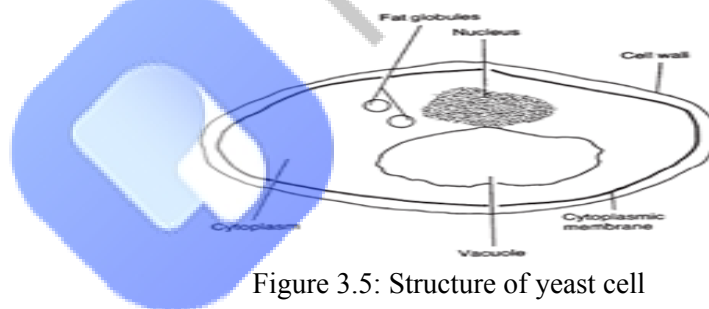


Figure 3.5: Structure of yeast cell

3.4 Viruses

Viruses are extremely small organisms comprising a spherical head containing the genetic material, and a cylindrical tail. They must invade other cells to reproduce. Viruses that attack bacterial cells are known as bacteriophages. Bacteriophages that attack acid-producing bacteria inhibit acid production in milk thereby causing problems in the manufacture of fermented milks, yoghurt and cheese.

3.5 Sources of contamination

Milk when secreted into an uninfected animal's udder is sterile and invariably, it becomes contaminated during milking, cooling, processing and/or storage. Microbial contamination of milk can be from the internal and/ or external sources that are described in the following section.

A. Interior of udder

Varying numbers of bacteria are found in aseptically drawn milk with the reported counts of <100-

10,000 CFU/ml from normal udder, but an anticipated average is 500-1000 CFU/ml in advanced countries. Microorganisms enter the udder through the duct at the teat tip that varies in length and its surface is heavily keratinized. During progress of a milking, bacteria are present in the largest numbers at the beginning and then gradually decrease. This is mainly due to the mechanical dislodging of bacteria, particularly in teat canal, where the numbers are probably highest. Because of this discarding of first few streams of milk helps in lowering the counts of microbes in milk.

If an animal is infected from mastitis, microbial contamination from within the udder of animal contributes notably to the total numbers of microbes in the bulk milk. The influence of mastitis on the total bacterial count of milk depends on the type of the infecting microbe. Most common microbial agents of mastitis in milch animals are given in the following figure. These include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli* and *Corynebacterium pyogenes*.

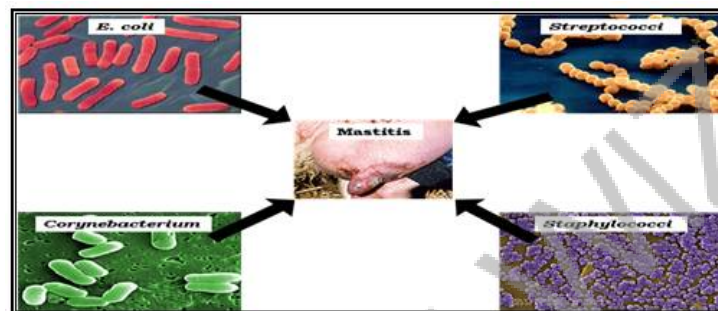


Figure 3.6: Structure of yeast cell

B. Exterior of udder

In addition, to the udder infections, unclean udder and teats of animal also contribute significantly to the total bacterial counts of milk. The microbes that are naturally associated with the skin of the animals as well as those derived from the environment, where the cow is housed and milked are predominant in the milk. The environmental conditions such as soil, manure, mud, feed or bedding; determines what kind of microbes will dominate in milk. Udder and teat become soiled with dung, mud, bedding materials. With heavily soiled udder teats the counts may be 1,000,000 cfu/ml. Economy washing with sodium hypochlorite accompanied by drying, helps in reducing the number of microbes. Different category of microbes that occurs in the exterior of udder are:

- micrococci and coagulase negative staphylococci (Predominant)
- faecal streptococci (Next), but Gram negative bacteria including coliforms are less.
- Teat surface may also contain clostridial spores that are usually found in cows fodder, bedding and faeces.
- *Psychrotrophic* and *thermoduric* bacteria predominate on the teat surfaces.

C. Coat of the cow

The coat serves as a vehicle to contribute bacteria directly to milk. The hairs around udder, flanks and tail contribute to the higher bacterial count in milk. The coat may indirectly contribute microbes into air, especially *Bacillus* spp.

D. Animal shed and surroundings

Milk produced on farms with poor hygiene practices may undergo significant spoilage and have a shorter shelf-life. Microbes associated with the bedding materials include: Coliforms, Spore-formers,

Staphylococci, Streptococci and other Gram negative bacteria.

E. Milking staff

The staffs involved at different stages of milk production plays a pivotal role in maintaining hygiene and preventing milk contamination. The hand contacts or dislodging of dust and dirt particles by milker may add varieties of microbes to milk. Risks of contamination from milker are definitely higher, when cows are hand-milked. Milker with infected wounds on hands contributes pathogenic *Streptococcus* spp. and micrococci. The common microbial pathogens from humans causing diseases such as typhoid, paratyphoid and dysentery may contaminate the milk. Microbial pathogens causing scarlet fever, septic sore throat, diphtheria, cholera etc. contaminate the milk.

F. Milking equipment (storage containers and transportation systems)

Milk residues left on the equipment contact surfaces supports the growth of a variety of microbes. Since, it is very difficult to remove all milk residues and deposits from the milk contact surfaces of milking equipments; hence equipment with smooth surfaces and minimal joints should be used. The tanker and collecting pipes are also the potential sources of contamination, if not adequately cleaned. In addition, biofilms can easily build up on the enclosed, hard to clean surfaces. Unclean or improperly cleaned milk cans and lids if they are still moist, results in multiplication of thermophilic bacteria like *Bacillus cereus*.

G. Water supplies

At dairy-farms, the water can be a predominant source of microbial contamination. Water used in production should be of good bacteriological quality. Inadequately or uncleaned, storage tanks, untreated water supplies from natural sources like bore wells, tanks and rivers, may also be contaminated with the faecal microbes (e.g. Coliforms, *Streptococci* and *Clostridia*). In addition, a wide variety of saprophytic bacteria (i.e. *Pseudomonas*, Coliforms, other Gram negative rods, *Bacillus* spores, *Coryneform* bacteria and lactic acid bacteria) may also be present in water and may contaminate the milk potentially.

H. Airborne contamination

Aerial contamination of milk by bacteria is insignificant, in comparison to microbes with those that are derived from the teat surfaces. The microbial counts of air in sheds rarely exceed 200 cfu/l. Micrococci account for >50% of the aerial microflora. Air contains dust, moisture and bacteria; hence its entry should be minimized in milk. *Micrococci*, *Coryneforms*, *Bacillus* spores, *streptococci*, and Gram negative rods are the major genera present in air. In general, more air incorporated into milk leads to the faster growth of bacteria. Following are some of the practices that increase aerial counts in milk:

- ◆ Sweeping of floors just before milking process
- ◆ Handling hay and feed shortly before milking process
- ◆ Brushing of animals prior to milking process
- ◆ Having the dusty bedding materials for animals
- ◆ Allowing dust and dirt to accumulate on the walls or ceiling of sheds

Chapter 4. Milk processing

4.1 Why processing

Historically, the most important reason why livestock products need processing is to make them last longer before spoiling. For example early civilization use techniques like salting of meat, and fermenting of milk in to different dairy products.

Therefore milk needs further processing so as:

- ❖ To control/minimize spoilage, poisonous, and pathogenic organism.
- ❖ Increase its shelf life.
- ❖ To fulfil the consumers preference on processed and value added products (which may have unique aroma , flavor, texture or color).
- ❖ To alter the constitutional/concentration level of nutrient in the animal products.
- ❖ For easy of transportation, storage, and marketing.

Advantages of milk processing include:

- Provides regular income
- Improves nutrition
- Selling processed milk products is more profitable than selling fresh milk
- Generates employment
- Improves quality and safety

The main methods of milk processing include:

- 1) Heat treatment
- 2) Increasing the acidity by fermentation
- 3) Removing water by concentration (boiling) or by evaporation
- 4) Adding sugar to make milk confectionary for cheese (butter) production.

Some types of processing involve more than one of these methods.

Generally when perishable products have longer shelf life, consumers can enjoy them for a greater part of the year, distributors can ship them over greater distances, and retailers can stock them on shelves for extended periods. Milk contains a nutritious substance and they are a good substrate for the growth of microorganism. Therefore, be it with simple or complex, traditional or modern, and easy or sophisticated, the need for milk processing is vital for the provision of safe food for human. The level of processing technology depends on weather, the technology available and affordable by the society/country. For example technologies may use from simple cooling, pasteurization to drying of products or use of new technology like high pressure processing, irradiation, etc, with the aim of producing high value end products. **Notice that**, not all dairy products undergo the same degree of pressure and temperature.

4.2 Processing techniques

4.2.1 Pasteurization

Pasteurization is the first step in milk processing. Pasteurization means heating every particle of the milk or milk product to a specific temperature for a specified period of time. In pasteurization, the milk is heated to a temperature sufficient to kill pathogenic bacteria. It makes the milk safe and healthy, and also improves the keeping quality. The process of pasteurization is named after the French chemist Louis Pasteur (1822–1895), who is regarded as the founder of the study of modern microbiology. It is intended to create only minimal chemical, physical and organoleptic changes in products to be kept in cold storage.

The Processing stages for pasteurized milk include:

- Receipt and filtration of raw milk
- Separation of all (part) of the milk fat (to standardize milk, or produce milk that has a specific fat content for making yoghurt or cheese)

- Pasteurization, followed by cooling to 10°C
- Homogenization (not done on a small scale)
- Packaging and cold storage
- Cold distribution

Types/Methods of Pasteurization

Many farmers pasteurize their milk by direct boiling. However, direct boiling is unhygienic, because it can lead to contamination from outside particles or bacteria. Indirect heating is a better way to pasteurize milk. Place the milk can inside a larger metal vessel containing water, so that the water forms a jacket around the milk can and heat the larger outside vessel using an open flame, or gas stove, or electrical hot plate.

Other methods of pasteurization

- Basically 2 methods - **batch** and **continuous flow**

Batch (or "vat") pasteurization or Holding Method

- The simplest & oldest method for pasteurizing milk.
- In batch process (batch pasteurizer), a large quantity of milk is held in a heated vat at 63°C. For 30 minutes, followed by quick cooling to about 4°C
- Milk has to be stirred constantly to make sure that each particle of milk is heated
- Used for making starter cultures in the processing of cheese, yogurt, buttermilk, for pasteurizing some ice cream mixes.
- This process can be carried out at home on the stovetop using a large pot or, for small-scale dairies, with steam-heated kettles and fancy temperature control equipment

High Temperature Short Time (HTST)/ Flash Pasteurization/ Continuous flow

- The most common methods in use today, especially for higher volume processing.
- Faster & more energy efficient than batch pasteurization
- Uses a higher temperature than conventional pasteurization, but the temperature is maintained for a shorter time.
- The product is then rapidly cooled to below 10°C, a temperature at which it can then be stored.
- Milk is forced between steel plates or through pipes heated on the outside by hot water.
- Uses stainless steel heat exchange plates where product flows on one side while the heating media flows on the opposite side to raise milk temperatures to at least 72°C for at least 15 seconds, followed by rapid cooling.
- The system is a modular unit that includes a plate-and-frame heat exchanger, stainless steel balance tank, pumps, holding tube, valves piping and controls.

Pasteurization temperature and time

The temperature/time combinations stated below are similar in effect and all have the minimum bactericidal effect required for pasteurization.

Product	Temperature and time
Pasteurized milk and skim milk	63°C / 30 minutes 72°C / 15 second
Pasteurized cream (10% fat)	75°C / 15 second
Pasteurized cream (35% fat)	80°C / 15 second
Pasteurized, concentrated milk, ice cream mix, sweetened products, etc.	80°C / 25 second

Pasteurized products

Should last for up to 48 hours without refrigeration, and should last for several days when stored refrigerated. Note: Longer keeping qualities and between 10 and 16 days at 4°C are now achievable, when produced from high-quality raw milk, under optimum technical and hygienic conditions.

Pasteurization Temperature vs. Time	
Vat (Batch) Pasteurization	
Temperature	Time
63°C (145°F)*	30 minutes
HTST Pasteurization	
Temperature	Time
72°C (161°F)*	15 seconds
89°C (191°F)	1.0 second
90°C (194°F)	0.5 seconds
94°C (201°F)	0.1 seconds
96°C (204°F)	0.05 seconds
100°C (212°F)	0.01 seconds

Temp.	Time	Pasteurization Type
63°C *	30 minutes	Vat Pasteurization
72°C *	15 seconds	High temperature short time Pasteurization (HTST)
89°C	1 second	Higher-Heat Shorter Time (HHST)
90°C	0.5 seconds	Higher-Heat Shorter Time (HHST)
94°C	0.1 seconds	Higher-Heat Shorter Time (HHST)
96°C	0.05 seconds	Higher-Heat Shorter Time (HHST)
100°C	0.01 seconds	Higher-Heat Shorter Time (HHST)
138°C	2 seconds	Ultra Pasteurization (UP)

Note:

- ◆ If the fat content of the milk product is 10 % or more, or
- ◆ if it contains added sweeteners, or
- ◆ if it is concentrated (condensed),
- ◆ the specified temperature shall be increased by 3°C.

NOTE

- Ultra High Temperature (UHT) is aseptic processing and involves heating the milk using commercially sterile equipment and filling it under aseptic conditions into hermetically sealed packaging.
- The product is termed "shelf stable" and does not need refrigeration until opened.
- Some of the diseases that pasteurization can prevent are TBC, Polio, Salmonella, Typhoid fever.
- Milk labeled "pasteurized" is usually treated with HTST method
- HTST pasteurized milk has a refrigerated shelf life of 2 -3 weeks,
- Ultra pasteurized milk can last sometimes 2-3 months when refrigerated
- When UHT treatment is combined with sterile handling and container technology (such as aseptic packaging), it can be stored unrefrigerated for 3-4 months.

Factors affecting the quality of pasteurized milk

The main control points for ensuring good quality pasteurized milk products are:

- 1) Raw milk quality,
- 2) Processing conditions
- 3) Temperature and holding time,
- 4) Post-processing contamination and
- 5) Storage temperature.

4.2.2 Cooling

Ideally, microbial contamination of raw milk and milk products should be addressed primarily through preventive measures on the farm and throughout processing. However, far too many contamination sources exist to prevent entry of all bacteria. Therefore, milk handling and processing strategies are designed to reduce and control bacterial numbers in processed products to protect milk quality and milk safety. The first of these measures involves efficient cooling of milk to 4°C immediately following milking. Reduced temperatures inhibit growth of mesophils and thermophils and reduce the activity of degradative enzymes. Modern dairy farms use refrigerated bulk storage tanks which maintain milk at 4°C or below. As bulk tank milk pick-up typically occurs daily or every other day, product from multiple milkings is frequently mixed and stored in the same tank. To prevent fresh, warm milk from the most recent milking from raising the temperature of milk already present in the bulk tank, many farms employ pre-tank cooling systems to reduce product temperature before addition to the tank. Cooling of milk causes several changes, the most important ones being:

1. The growth of most microorganisms is much slower, if not stopped, and so are the changes induced in milk by their metabolism.
2. Nearly all chemical and enzymatic reactions are retarded.
3. Autoxidation of lipids is decreased.
4. Changes in solubility and association of salts occur. The amount of micellar calcium phosphate decreases, and the pH increases.
5. The casein micelles attain a higher voluminosity and part of the casein, especially β -casein, goes into solution. This results in an increased viscosity and an enhanced susceptibility to attack by plasmin.
6. The fat globule membrane loses some components and its structure is altered.
7. Cold agglutination of fat globules occurs, e.g., enhancing creaming rate.
8. The triglycerides in the fat globules will partly crystallize

4.2.3 Homogenization

It is a process that makes a mixture the same throughout the entire substance. In milk processing, the aim is to prevent (delay) the natural separation of cream from the rest of the emulsion. Homogenization is the process of breaking up the fat into smaller sizes so that it no longer separates from the milk, allowing the sale of non-separating 2% and whole milk. This is accomplished by forcing the milk at high pressure through small orifices. Homogenization of milk causes disruption of milk fat globules into smaller ones. Milk products are usually homogenized to prevent separation during storage. Other dairy products are homogenized to improve water binding, reduce free fat etc.

Objectives Homogenization include:

- Counteracting creaming
- Improving stability toward partial coalescence
- Creating desirable rheological properties: Homogenized and subsequently soured milk (e.g., yogurt) has a higher viscosity than un-homogenized milk.

4.2.4 Souring by fermentation or acidification

Fermentation, a form of naturally-occurring acidification has long been used for food preservation. If raw milk is stored, it spoils by microbial action. At moderate temperatures, lactic acid bacteria are generally predominant, and the milk spontaneously becomes sour. Nearly all types of fermented milk products are based on such souring activity of lactic acid bacteria. Lactic acid bacteria are the prime agents in producing soured (fermented) milk and dairy products. Although they are genetically diverse, common characteristics of this group of bacteria include being Gram-positive, non-motile, and non-spore forming. Lactic acid bacteria are unable to produce iron-containing porphyrin compounds, such

as catalase and cytochrome. Thus, they grow anaerobically but are aerotolerant. They obligatorily ferment sugars with lactic acid as the major end product. They tend to be nutritionally fastidious, often requiring specific amino acids and B vitamins as growth factors.

4.2.5 Creaming

The fat fraction separates from the skim milk when milk is allowed to stand for at least 30 to 40 minutes. This is known as “creaming”. The first step in making cream, butter, ghee, etc is cream separate from the fresh milk. This can be done through gravitational or centrifugal separation.

Gravitational separation

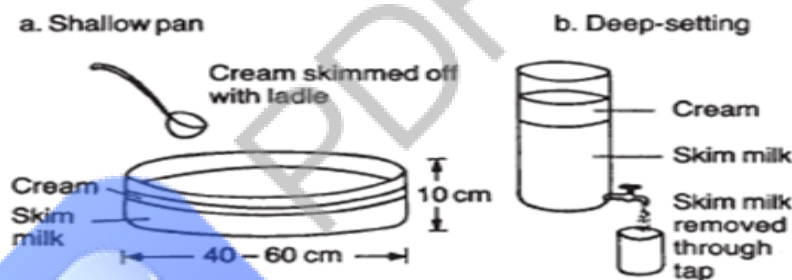
allow the milk to settle. The cream is lighter than the other milk components. It rises to the top, and can be separated.

Batch separation by gravity

Cream can be separated from milk by allowing the milk to stand in a setting pan in a cool place. This can be done in either of two ways.

Shallow pan: Milk, preferably fresh from the cow, is poured into a shallow pan 40 to 60 cm in diameter and about 10 cm deep. The pan should be in a cool place. After 36 hours practically all of the fat capable of rising by this method will have come to the surface, and the cream is skimmed off with a spoon or ladle. The skim milk usually contains about 0.5 to 0.6% butterfat.

Deep-setting: Milk, preferably fresh from the cow, is poured into a deep can of small diameter. The can is placed in cold water and kept as cool as possible. After 24 hours the separation is usually as complete as it is possible to secure by this method. Under optimum conditions, the fat content of the skim milk averages about 0.2 - 0.3%.



Centrifugal separation

This requires a simple machine called a centrifuge. The centrifugal separator was invented in 1897. Gravity separation is slow and inefficient. Centrifugal separation is quicker and more efficient leaving less than 0.1% fat in the separated milk. The centrifuge can be driven (i.e. rotated) by hand, or by an electric motor. Centrifugal separation can be used for any liquid, to separate different components. Milk is placed in a bowl, which is then rotated. When it rotates, the heavier portion (i.e. the skim milk) moves to the outside, and the lighter portion (cream) moves towards the center of the bowl. The speed of rotation can vary from 2000 rpm in small manual separators to 20,000 rpm in large electric separators.

Freshly drawn or uncooled milk is ideal for skimming, i.e. most of the cream can be easily separated. If the milk is too cold (below 22°C), some of the fat becomes solid, and skimming efficiency is greatly reduced. Milk must therefore be heated to liquefy the fat. Heating the milk to 45°C gives the best skimming efficiency. Heating to above 60°C reduces creaming; milk that is heated to above 100°C retains very little creaming ability.

Efficiency of separation is influenced mainly by four factors: the speed of the bowl, residence time in the bowl, the density differential between the fat and liquid phase and the size of the fat globules.

Speed of the separator

Reducing the speed of the separator to 12 rpm less than the recommended speed results in high fat losses with up to 12% of the fat with skim milk.

Residence time in the separator

Overloading the separator reduces the time that the milk spends in it and consequently reduces skimming efficiency.

Effect of temperature

Freshly drawn, uncooled milk is ideal for exhaustive skimming. If the temperature of the milk falls below 22°C skimming efficiency is seriously reduced.

Size of the fat globules

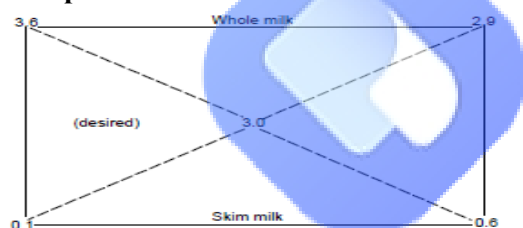
Separation of cream from homogenized milk is difficult.

Other factors: Other factors that affect the skimming efficiency are the quality of the milk and maintenance of the separator. Milk in poor physical condition or which is curdy will not separate completely and a separator in poor mechanical condition will not separate milk efficiently.

4.2.6 Standardisation of milk and cream

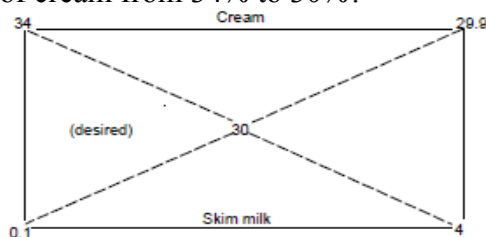
If fine adjustment of the fat content of cream is required or if the fat content of whole milk must be reduced to a given level, skim milk must be added. This process is known as standardisation. The usual method of making standardisation calculations is the Pearson's Square technique. To make this calculation, draw a square and write the desired fat percentage in the standardised product at its centre and write the fat percentage of the materials to be mixed on the upper and lower left-hand corners. Subtract diagonally across the square the smaller from the larger figure and place the remainders on the diagonally opposite corners. The figures on the right-hand corners indicate the ratio in which the materials should be mixed to obtain the desired fat percentage. The value on the top right-hand corner relates to the material on the top left-hand corner and the figure on the bottom right relates to the material at the bottom left corner.

Example 1



In this example, the fat content of whole milk is to be reduced to 3.0%, using skim milk produced from some of the whole milk. Using Pearson's Square, it can be seen that for every 2.9 litres of whole milk, 0.6 litres of skim milk must be added.

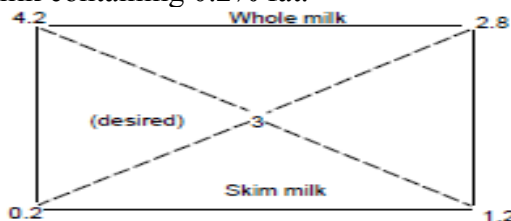
Example 2: How much skim milk containing 0.1% fat is needed to reduce the percentage fat in 200 kg of cream from 34% to 30%?



If 29.9 parts of cream require 4 parts of skim milk, 200 parts of cream require x parts of skim milk.

Weight of skim milk needed = $x = 200 \times 4/29.9 = 26.75$ kg

Example 3: The fat content of 300 kg of whole milk must be reduced from 4.2% to 3% using skim milk containing 0.2% fat.



Every 4.0 kg of the mixture will contain 2.8 kg of whole milk and 1.2 kg of skim milk. If 2.8 kg of whole milk requires 1.2 kg skim milk, 300 kg of whole milk requires $1.2 \times 300 / 2.8 = 128.6$ kg of skim milk. Thus, 128.6 kg of skim milk (0.2% fat) must be added to 300 kg of whole milk (4.2% fat) to give 428.6 kg of milk containing 3% fat.

4.3 Milk products

4.3.1 Fermented milk products

General Aspects

When the sour milk has been used, and fresh milk is put in the same vessel without rigorous cleaning of that vessel, the fresh milk is inoculated with the remaining bacterial flora. The milk now sours more quickly, generally due to a smaller number of bacterial species and strains. If this process is repeated under fairly constant conditions (especially in regard to temperature), natural selection leads to an almost pure lactic acid fermentation, although some other bacteria may be present. This then acts as a starter for the fermentation. The fermented milk thus obtained has a longer keeping quality and, often, a pleasant flavor.

Types of fermented milks

Fermented milks are classified into four different types:

- (1) products of lactic fermentation in which strains of mesophilic lactic acid bacteria are used,
- (2) products of lactic fermentation with thermophilic lactic acid bacteria,
- (3) products obtained through alcohol-lactic fermentation, involving yeasts and lactic acid bacteria,
- and (4) products where, in addition to fermentation type (1) or (2), growth of a mold occurs.

Mesophilic Fermentation

A. Cultured Buttermilk

Conventional buttermilk is actually the aqueous liquid released during the manufacture of butter by churning of soured cream. This buttermilk has distinctive characteristics due to the presence of butter aroma (mainly diacetyl) and part of the natural fat globule membrane material that is released during churning. It is fermented by mesophilic lactic acid bacteria. It has a mild acidic taste with an aromatic diacetyl flavor and a smooth viscous texture. *Lactococcus lactis* ssp. *cremoris* and *lactis* are responsible for the acid production, whereas *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* ssp. *cremoris* are the primary sources of the characteristic aromatic flavor of the product because of their ability to produce diacetyl. After pasteurization, the milk is fermented at 20°C to 22°C to ensure a balanced growth of acid- and flavor-producing species. Incubation at higher temperature would favor the growth of *Lc. lactis* ssp. *lactis*, resulting in excess acid production and diminishing the flavor production by the aroma bacteria.

B. Sour Cream

Cultured cream or sour cream is produced by the fermentation of pasteurized cream with a fat percentage of 18% to 20%.

The cream is inoculated with an aromatic starter and incubated at 20°C to 22°C until the pH has reached

a value of 4.5. The functions of the starter culture are the same as in cultured buttermilk.

Thermophilic Fermentation

A. Yogurt

Yogurt is probably the most popular fermented milk. The essential flora of yogurt consists of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. For a satisfactory flavor to develop, approximately equal numbers of both species should be present. The yogurt bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, grow in milk better when present together than each alone (protocooperation). The cocci as well as the rods contribute significantly to the properties of yogurt.

The mass ratio of the two species depends on the properties of the strains and is often approximately 1:1. The ratio is best maintained if the incubation time is 2.5 h at 45°C, and the final acidity is pH \approx 4.2. The ratio between the species keeps changing. Initially, the streptococci grow faster due to the formation of growth factors by the rods and finally the cocci are slowed down by the acid produced. Meanwhile, the rods have started to grow faster because of the growth factors (CO₂ and formic acid) formed by the cocci. As a result, the original ratio is regained. Varying the conditions during incubation changes the ratio between the two *spps* as follows:

1. *Incubation time*: A shorter incubation time, (lower acidity), will cause too high proportion of cocci.
2. *Incubation temperature*: The rods have a higher optimum temperature than the cocci. Incubation at a slightly higher temperature > 45°C will shift the ratio in favor of the rods; incubation at a lower temperature will enhance the cocci.

B. Bulgarian Buttermilk

Bulgarian buttermilk is a high-acid fermented milk, made from pasteurized whole milk, inoculated with *Lb. delbrueckii* ssp. *bulgaricus* alone and incubated at 38°C to 42°C for 10 to 12 h, until a curd forms. The product has a sharp flavor and is popular only in Bulgaria.

C. Acidophilus Milk

Acidophilus milk is cultured with *Lb. acidophilus*, whose primary function is to produce lactic acid. Moreover, *Lb. acidophilus* is considered to be a probiotic bacterium, and has been claimed to confer various health benefits. It is not a natural representative of the milk flora and grows slowly in milk.

D. Probiotic Fermented Milk

Probiotic fermented milks are made with various lactic acid bacteria, including bifidobacteria. *Lactobacillus acidophilus*, specific strains of *Lb. casei*, and *Bifidobacterium* spp. are the most commonly used probiotic bacteria in the manufacture of fermented milks. These and some other microorganisms are thought to confer health and nutritional benefits to the consumer, through their activity in the intestinal tract. The number of types of fermented milks made with probiotic microorganisms has increased markedly over the past few decades. These products may contain a probiotic microorganism in addition to *S. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*. The resulting products are commercialized under trade names like Bioghurt, Bifighurt, Biogarde, and Culture, to name a few.

Yeast–Lactic Fermentation

A. Kefir

Kefir is made of ewes', goats', or cows' milk. During the fermentation, lactic acid and alcohol are produced. Originally, it was made in Russia and south western Asia. It is now being made in various countries on an industrial scale by using cows' milk.

The microflora of kefir is variable. Lactococci (*L. lactis* spp. *lactis* and *cremoris*, and *L. lactis* ssp. *lactis* biovar *diacetylactis*), *Lb. acidophilus*, etc can form lactic acid, whereas yeasts, including

Candida, *Kluyveromyces*, and *Saccharomyces* species, produce alcohol. Kefir of a satisfactory quality is believed to contain acetic acid bacteria also.

B. Kumiss

Kumiss is a well-known milk drink in Russia and western Asia. Formerly, the cultured milk was valued because of its supposed control of tuberculosis and typhus. The product is traditionally made of mares' milk. The fermenting flora is variable as in kefir. It contains 0.7% to 1% lactic acid, 0.7% to 2.5% alcohol, 1.8% fat, and 2% protein; it has a grayish color. During its manufacture, protein is substantially degraded. Together with the fermentation compounds formed, the proteolysis is responsible for a specific flavor.

Role of Fermentation

- Acid production
- Increased shelf life
- Increased nutritional value
- Improve taste and aroma
- Improve texture and appearance
- Improve colour

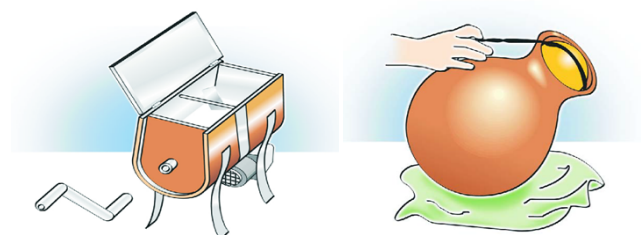
4.3.2 Butter and Ghee

A. Preparation of butter

Butter should contain 80% fat, 16% moisture, and 2% milk solids non fat (SNF). It may contain a small amount of salt (2%) to improve shelf life and taste. However, excess moisture (more than 20%) reduces the quality of butter. Butter is generally made from cream by churning and working. The churning proceeds most easily at a temperature of around 15 to 20°C. Butter is an emulsion of water in oil. Churning efficiency is measured in terms of the time required to produce butter granules and by the loss of fat in the buttermilk. Efficiency is influenced markedly by churning temperature and by the acidity of the milk or cream.

The following are the most important specific requirements for the product and its manufacture:

- 1. Flavor:** Off-flavors of the fat are to be avoided, especially those caused by lipolysis. If the cream is heated too intensely, the butter gets a cooked flavor.
- 2. Shelf life:** Spoilage by microorganisms may cause several off-flavors (putrid, yeasty, cheesy, and rancid). Autoxidation of the fat can occur, especially at prolonged storage, even at a low temperature (−20°C), leading to a fatty or even a fishy flavor.
- 3. Consistency:** Butter derives its firmness largely from fat crystals that are aggregated into a network. Butter should be sufficiently firm to retain its shape. On the other hand, the butter should be sufficiently soft.
- 4. Yield:** Some fat is lost in the skim milk and in the buttermilk. If the water content is below the legal limit (for example, 16%), this also means a loss of yield.
- 5. By-products:** Buttermilk is sometimes desirable, but often it is not, due to insufficient demand. Sour-cream buttermilk is only applicable as a beverage (or as animal feed), but it keeps poorly due to rapid development of an oxidized flavor.



Washing the butter

When the desired grain size is obtained, the buttermilk is drained off and the butter washed several times in the churn. Each washing is done by adding only as much water as is needed to float the butter and then turning the churn a few times. The water is then drained off. As a general rule two washings are enough but in very hot weather three may be necessary before the water comes away clear. In the hot season the coldest water available should be used for washing, and in the cold season water about 2 to 3°C colder than the churning temperature should be used.

Salting and working butter

The butter is spread on the worker which has been previously soaked with water of the same temperature as the washing water. If salted butter is required, it should be salted before working at a rate of 16 g salt/kg or according to taste. Salt is added to butter most commonly using the dry-salting method in which dry salt is sprinkled evenly over the butter and worked in. The salt used should be dry and evenly ground and of the best quality available. Butter must be adequately worked if it is to be stored for a long time. First, working distributes the salt uniformly in the moisture and this helps inhibit microbial growth. Secondly, it distributes the salt solution into many tiny droplets rather than fewer large ones. Surplus good-quality butter can be stored, but should contain more salt than usual---at least 30 g/kg and a low moisture content (14-15%). A salt concentration in excess of 3% gives little advantage and can adversely affect the flavour of the butter.

A number of factors influence churning time and recovery of butterfat as butter:

- Milk acidity
- Churning temperature
- Degree of agitation
- Extent of filling the churn.

Effect of acidity

Fresh milk is difficult to churn ---churning time is long and recovery of butterfat is poor---however, milk containing at least 0.6% lactic acid is easier to churn.

Effect of temperature

Sour milk is normally churned at between 15 and 26°C, depending on environmental temperature. At low temperatures churning time is long. As churning temperature increases churning time decreases. ILCA trials have shown that when churning sour whole milk using the traditional method, fat recovery values of 67% and 44% were obtained with churning temperatures of 18°C and 25°C, respectively. Controlling the temperature is therefore critical. If too cool, butter formation is delayed and the grain is small and difficult to handle. If the temperature is too high, the butter yield will be low because a large proportion of the fat will remain in the buttermilk, and the butter will be spongy and of poor quality. Cream should be churned at 10--12°C in the hot season and at 14--17°C in the cold season. If butter is slow in forming, adding a little water which is warmer than the churning temperature, but never over 25°C, usually causes it to form more quickly. The optimum churning temperature is between 15 and 17°C.

Degree of agitation

Increasing agitation reduces churning time. Fitting an agitator to a traditional churn reduces churning time and increases butter yield. The percentage of fat recovered as butter is increased, with as little as 0.2% fat remaining in the buttermilk. The advantage of using the ILCA internal agitator was demonstrated when churning sour whole milk at 18°C. Using the traditional clay pot a fat recovery of 67% was obtained compared to a 76% fat recovery when using the clay pot fitted with the internal wooden agitator.

Extent of filling the churn

Filling to more than half the volumetric capacity increases churning time considerably but does not reduce fat recovery. An airtight churn should be ventilated frequently during the first 10 minutes of churning to release gases driven out of solution by the agitation. Thus, when churning whole milk, the following conditions should be adhered to:

- ◆ Milk acidity should be greater than 0.6%
- ◆ The temperature should be adjusted to about 18°C
- ◆ Internal agitation should be used to reduce churning time and increase fat recovery
- ◆ The churn should not be filled to more than half its volumetric capacity.

B. Preparation of Ghee

- Ghee is anhydrous (dry) butterfat. It contains 99.9% butterfat. Ghee is made by removing moisture from cream or butter, by evaporation.
- Ghee is used for cooking.
- Ghee can be made from either cream or butter. Making it from butter requires less energy.
- Heat the butter over a slow fire until all the moisture has evaporated and the temperature rises to about 120-125°C. The product is almost entirely butterfat. It contains almost no moisture and milk SNF, which inhibits bacterial growth. Much of the typical flavour comes from the burned milk SNF remaining in the product. Ghee is a more convenient product than butter in the tropics because it keeps better under warm conditions.

4.3.3 Cheese

Cheese is a very profitable product. There are more than 2000 types of cheese. Nine litres of milk will give one kilo of cheese. When fresh milk is left to become sour, the casein aggregates. If souring occurs at not too low a temperature and without any stirring or shaking of the milk, a gel is formed. Some whey separation generally occurs when the gelled or clotted milk is kept for some time. This can be enhanced by heating and stirring; the mass then separates into curd grains and whey. By allowing more of the whey to drain out from the curd for instance, by hanging the curd in a cloth, a primitive fresh cheese is obtained (baker's cheese, quarg, or simply 'curds').

This may have been the origin of cheese making. However, for centuries, milk has also been clotted by the addition of specific agents, especially rennet, an extract of calf stomach. Various vegetable clotting agents were also used, for instance, from cardoon flowers, or fig tree latex. Altogether, the art of transforming milk into curd and whey is very old. To make a real cheese, other process steps are needed: shaping (often by pressing), salting, and curing. These steps could readily evolve, and further evolution has led to a great variety of cheese types. However, all cheeses have a few things in common:

- Greater part of the casein and milk fat are concentrated in the cheese; a very nutritious product.
- Cheese keeps much longer than milk, and has longer shelf life than fermented milks. During keeping there are changes in its properties: this is called *ripening* or *maturation*. Cheese generally has a distinct and characteristic flavor due to a great number of flavor compounds formed during ripening. None of the milk components is fully retained and new substances may be added, notably salt.

Essential Process Steps

The manufacture of cheese involves several different processes, some of which are essential for (nearly) all cheese varieties:

1. *Clotting of the milk*: This is accomplished by means of enzymes or acid (or both). As discussed earlier, the enzymes involved remove the caseino-macropeptide 'hairs' from κ -casein; the resulting paracasein micelles will then aggregate. Acid, formed from lactose by lactic acid bacteria, dissolves the colloidal calcium phosphate of the micelles and neutralizes the electric charge on the resulting particles, which will then aggregate.

2. *Removal of the whey*: The gel formed is prone to spontaneous syneresis, i.e., expulsion of whey. Whey expulsion is generally enhanced by cutting the gel into pieces and by stirring the curd. The drier the curd, the firmer and the more durable the cheese will become.
3. *Acid production in the cheese during its manufacture*: The reduced pH of curd and cheese due to lactic acid produced affects such parameters as syneresis, consistency, and ripening of the cheese.
4. *Salting*: Cheese contains added NaCl, generally 1 to 3%. This does not apply to some fresh-type cheeses. The salt affects durability, flavor, and consistency of the cheese, both directly and by its effect on ripening.
5. *Fusion of curd grains into a coherent loaf that is easy to handle*: The cheese may acquire a rind, which protects the interior. Pressing enhances curd fusion.
6. *Curing*: Ripening is the main factor determining the typical flavor and texture of a given cheese variety. To achieve this, the cheese is kept for a variable time under suitable conditions.

Cheese varieties

Many cheese varieties are manufactured around the world but they are all broadly classified by hardness, i.e. very hard, hard, semi-soft and soft, according to their moisture content. Cheese is usually made from cow milk, although several varieties are made from the milk of goats, sheep or horses.

Fresh

Fresh cheeses never go through an aging processes and are only good for a few weeks. Often still containing some whey, they typically are soft with a mild flavor. Examples: Feta, cottage

Soft Ripened

Soft ripened cheese retains some of its whey and can be recognized by their white “bloomy” rind. Having only slightly more flavor than fresh cheeses, they are but terry and almost runny in texture. Examples: Camembert, Brie, Chèvre Log

Hard

Hard cheeses are dense and heavy, having been cheddared early in their production. They may have been washed in brine or have an oiled or waxed rind to prevent them from drying out.

Examples: Cheddar, Parmigiano-Reggiano, Gruyere

Veined

Commonly known as blue cheese, veined cheese is recognized by its lines of blue-green mold spread throughout its interior. Its texture can vary, but veined cheese tends to have a strong flavor.

Examples: Stilton, Gorgonzola, Maytag Blue

Semi-soft Ripened

Similar to soft ripened cheeses, these are rubbery and elastic with a gray or brown rind. They also have a more pronounced flavor than softer cheeses. Examples: Edam, St. Neuchâtel, Pont L’Eveque

4.4 Milk and milk product quality measurement

Biological, chemical and physical tests/analysis of milk and milk products is very crucial activity which should perform in any dairy processing plants, even after processing for effective marketing of the milk products. Generally milk analysis is carried out to determine:-

- ◆ Freshness
- ◆ Adulteration
- ◆ Bacterial content, and
- ◆ Any spoilage
- ◆ Milk constituents for payment calculation

Milk sampling

Precise sampling is the first pre-requisite for fair and just quality control system. Liquid milk in cans and bulk tanks should be thoroughly mixed to disperse the milk fat before a milk sample is taken for any tests. Representative samples must be taken for any investigation on quality. For best results, milk or cream must be sampled when it is at a temperature between 15 and 32°C.

Common testing methods for milk and milk product quality measurement

1. **Organoleptic test:-** Judging the quality of milk by its taste and smell requires considerable skill which could only be acquired through practice. Organoleptic tests are used in all dairies and an experienced person can pick out bad samples with a high degree of accuracy.
 - Evaluation of foreign materials in the milk
 - Evaluation of odour/ flavor and aroma
 - Evaluation of appearance of milk (color, texture like clot)
2. **Determination of pH:-** The pH value gives a measure of the true acidity of milk. The pH of normal cow milk ranges from 6.6 to 6.8. The value is reduced by the development of acidity. On the other hand, the pH value of mastitic milk is alkaline, the value being over 7.0. The pH test is mainly used for the detection of abnormal mastitic milk.
3. **Sediment test:-** Sediment test on raw milk reveals the extent to which visible insoluble matter has gained entrance to the milk and the extent to which such material has not been removed from milk by single service strainer.
 - ◆ The sediment test presents a simple, rapid and a quantitative measure for indicating the cleanliness of milk with respect to visible dirt.
 - ◆ The test is carried out by allowing a measured quantity of milk to pass through a fixed area of a filter disc and comparing the sediment left with the prepared standard.
4. **Determination of specific gravity:-**

The specific gravity of milk is the ratio of density of milk to density of water at a definite temperature. Milk is heavier than water. The Specific gravity of cow milk varies from 1.018 to 1.036 and on averages 1.032. It varies with temperature, being lower at higher temperature and *vice versa*, but the rate of this variation is not uniform. The specific gravity of milk is determined to:

 - Calculate the SNF of milk and also Total solid
 - Detect the adulteration of milk with water or skimmed milk
5. **Detection of adulteration:-** There are several ways in which milk can be adulterated.

The modes of adulteration commonly encountered in market samples are:-

 - a) Removal of fat by skimming
 - b) Addition of skimmed milk to whole milk
 - c) Addition of water, and
 - d) Addition of starch and cane sugar for rising density to prevent detection of added water by lactometers.

➤ **Detection of skimming:** the following points give an indication of the removal of excess fat from milk.

 - Lower percentage of fat
 - Higher density reading of the sample at 27 °C and
 - Higher ratio of SNF: Fat

➤ **Detection of milk mixed with skim milk:** when skim milk has been added to whole milk, it could be inferred from the following facts.

 - a) Lower percentage of fat
 - b) Higher density reading of the sample at 27 °C and

- c) Higher percentage of SNF and
- d) Higher ratio of SNF : Fat
- **Detection of extraneous water:** Presence of extraneous water in milk is detected by the following facts:
 - Lower percentage of fat and SNF
 - Lower density reading of milk at 27 °C and
 - Depression of freezing point
- **Detection of starch:** Starch or cereal flours may be added to make up the density of milk to prevent detection of added water. The presence of starch or cereal flours is detected by the following test;
 - ◆ Place in a test tube about 3ml of well mixed samples. Bring it to boil by holding the tube over a flame.
 - Allow it to cool to room temperature
 - Add a drop of 1% iodine solution
 - Presence of starch is indicated by the appearance of a blue color which disappears when the sample is boiled and re-appears on cooling.
- 6. **Acidity test:** The acidity of milk is generally of two types
 - **Natural or apparent acidity**
 - a) fresh milk has trace initial acidity due to its buffering capacity
 - b) The natural acidity is due to presence of salts like citrate, phosphates, casein and dissolved CO₂ gas in the milk.
 - **Real or developed acidity:** The production of acid in milk is normally termed "souring". The percentage of acid present in dairy products at any time is a rough indication of the age of the milk and the manner in which it has been handled. The acidity of milk due to natural and developed is known as *Titrate acidity*.
- 7. **Clot- on boiling test:-**Acidity decreases the heat stability of milk. The clot-on- boiling test is used to determine whether milk is suitable for processing, as it indicates whether milk is likely to coagulate during processing (usually pasteurization). It is performed when milk is brought to the processing plant, if the milk fails the test it is rejected. It has the advantage that no chemicals are needed.

CHAPTER 5

MEAT PROCESSING

Introduction

Definition: Meat processing can be defined as any mechanical, chemical, or enzymatic treatment of meat, which alters the form from which it originally, occurs.

Principles of Meat Processing Technology

Meat processing technology comprises the steps and procedures in the *manufacture of processed meat products*. Processed meat products, which include different types and local/regional variations, are food of animal origin, which contribute valuable animal proteins to human diets. Animal tissues, in the first place *muscle meat* and *fat*, are the main ingredients, besides occasionally used other tissues such as *internal organs* and *blood* or *ingredients of plant origin*. All processed meat products have been in one way or another physically and/or chemically treated. Meat processing technologies

include:

- ☐ Cutting/chopping/comminuting (size reduction)
- ☐ Mixing/tumbling
- ☐ Salting/curing
- ☐ Utilization of spices/non-meat additives
- ☐ Stuffing/filling into casings or other containers
- ☐ Fermentation and drying
- ☐ Heat treatment
- ☐ Smoking

Processing of meat serves a number of functions, which may include any one or more of the following:

- Shelf-life extension: This is the most important process, since it is a highly perishable commodity
- **Tenderization** (mechanical, enzymatic or other means)
- Manipulation and control of composition (protein, fat, and moisture content) and portion (size, weight, and shape)
- Improvement of consumer convenience

The major factors that challenge the establishment of efficient meat processing and hence shelf life extension in the tropics includes:

- High ambient temperature accompanied by high humidity, making storage and transportation of meat expensive.
- Inadequate services such as: Water, Electricity, and Roads facilities

5.1 Meat production and consumption in Ethiopia

Meat and meat products make important nutritional contribution to the diet of the people. Significant percentage of the recommended dietary allowances for proteins, vitamins-B, Mg, Fe and Zn are contributed by red meat and poultry. Meat is composed of water, fat, protein, minerals and small proportion of carbohydrate. It is recognized as a highly nutritious food being an excellent source of high quality protein, containing a good balance of the essential amino acids. Meat also contributes significant percentage of other minerals including Na and K.

Ethiopians, like residents of other developing countries, do not consume an adequate amount of meat. The few that do, however, maintain a meat diet of beef, sheep, goat, and poultry. Most Ethiopians do not consume pork, in addition to many types of fish, due to religious beliefs and cultural taboos. The consumption of sufficient meat is a rare extremity in most developing countries. While developed countries consumed a consistent level of 77kg of meat per capita annually, developing countries struggled to maintain a diet with only 25kg of meat per capita annually. More specifically, while the United States had an average meat intake of over 120kg per capita annually, Ethiopians remained slightly below the meat intake of all low-income countries consuming 9kg per capita annually (FAOSTAT, 2004).

Meat production in Ethiopia is highly traditional similar to most of African countries. Quality of meat produced in the country is of low quality. Although the livestock population is high because the meat production is not yet modernized, the meat production is low and the cost for a kilo of meat is currently >250 ETB. The small amount of meat produced in the country is mainly from cattle, sheep, goat and poultry. Fish meat is consumed mainly in towns near lakes.

5.2 Structure of Muscle

The architecture of skeletal muscle is characterized by a very particular and well-described arrangement of muscle fibers (also referred to as myofibers or muscle cells) and associated connective tissue. At the whole muscle level, the size of a muscle is determined mostly by the number and size of individual muscle fibers.

Meat is composed of three distinct muscle types:

- *Skeletal muscle (striated and voluntary)*
- *Smooth muscle (non-striated and involuntary)*
- *Cardiac muscle (striated and involuntary)*

1. Skeletal muscles are the most important of the three types from an economic point of view. Directly or indirectly attached to bone. These muscles facilitate movement and/or give support to the body.
2. Smooth muscles are commonly referred as visceral muscles and are found throughout the digestive, reproductive tracts, and blood vessels of the animal.
3. Cardiac muscle, as the name implies, refers exclusively to heart muscle.

Fat, nerves, veins, arteries, ligaments and tendons are an integral part of a combination of muscle and must be considered as meat. The edible organs of the gland are designated as variety meats to contrast them with the skeletal meats of the carcass. It consist the tongue, liver, pancreas (sweetbreads), kidney, spleen, etc. Meat derives mainly from skeletal muscle, and this topic focuses on the structure of the skeletal muscle.

Skeletal muscles are a very contractile system made up of cylindrical, multinucleated muscle fibers (cells) of varying lengths surrounded by layer of connective tissue sheath. The outer sheath of the muscle is the **epimysium**. The **premysium** surrounds bundles of muscle cells (fiber bundles) and the **endomysium** envelops each individual muscle cell. Beneath the endomysium is the cell membrane: **sarcolemma**. Surrounding the myofibril is sarcoplasm, which contains various discrete cell components:

- ✓ The nuclei, responsible for cell morphology and differentiation.
- ✓ Mitochondria, responsible for oxidative metabolism
- ✓ The sarcoplasmic reticulum, responsible for transmitting the nerve signals
- ✓ Glucose granules, which together with lipid serve as the course of much the cell's metabolic and mechanical energy.

The presence of smaller and more numerous the muscle in a cut imply greater amount of connective tissue. The meat derived from such muscle will be less tender.

Composition of meat

- Lean muscle consists of app. 20% protein, 72% water, 7% fat, and 1 % ash.
- As the animal is fattened, these proportions change resulting in reduction in the percentage of protein and water and a proportionate increase in fat.

a) Proteins:- muscle contains different types of proteins serving different functions.

• **Actomyosin** - major protein in muscle consisting of two proteins, *actin* and *myosin* combined in a ratio of 1:3. It is the structural component that gives muscle the power of movement.

• **Collagen** - the single most abundant protein in mammalian species, being present in bone, skin, tendons, cartilage, and muscle. The primary functions are to provide strength and support. It is the major factor influencing tenderness.

• **Elastin** - much tougher than collagen, but muscle tissue contains very little elastin.

•**Myoglobin, haemoglobin, nucleoproteins** - water soluble proteins. Provide pigment (red color) to meat. *Myoglobin* (found in muscle cells) serves the function of transporting oxygen to mitochondria from the blood stream and returning CO₂ to the blood stream. *Haemoglobin* (found in red blood cells) returns these elements to the lung for exchange.

•**Creatine, creatinine, purins:-** nitrogenous water soluble substances (not true proteins). Have little nutritive value in themselves but are physical and chemical substances that excite the flow of gastric juices.

b) Fats:- Aside from its high calorific value, fat plays a role in adding palatability to meat. Supply needed fatty acids, like *linoleic acid*, carry fat soluble vitamins (*A, D, E, K*). Saturated fats aid in the keeping quality of meats because they are less subject to oxidation. However, nowadays consumers prefer high proportion of unsaturated fats in meat due to health concerns.

c) Carbohydrates:- Liver is the carbohydrate reservoir of the animal body, containing one-half of the total. Stored in the form of glycogen. Glycogen changes to lactic acid and the process is reversible in the live animal but not in the dressed meat. Because of this the lactic acid content of the carcass increases during ageing (ripening).

d) Water:- Mature beef may contain about 45% water, while veal may run up to 72% water.

5.3 Slaughtering facilities

Slaughter This could simply be described as the killing and bleeding out of animals. In the red meat industry, it is called sticking and in the poultry industry, it is called neck cutting. The scientific term for slaughtering is called exsanguination (Gregory, 1987). It is generally agreed that the two essentials in slaughter of food animals are that, they shall be dispatched without unnecessary suffering and that the bleeding of the animal shall be as complete as possible. The slaughter house shall have the essential facilities for the following activities:

- Receiving the animal (Reception)
- Ante-mortem inspection area and equipments
- Isolation of sick/diseased animals
- Resting place for animals before slaughter
- Carrying out humane slaughter – stunning box
- Flaying, dressing and washing of the carcasses
- Hanging carcasses and edible offal
- Handling by-products
- Handling solid and liquid wastes
- Inspection of meat
- Chilling and Freezing facilities
- Emergency slaughter
- Staff welfare
- provision of hot and cold of potable water
- Toilets and changing rooms

5.4 Methods of slaughtering

Pre-slaughter stunning is required in many developed countries to ensure the animal is unconscious prior to killing. The usual methods are:

Electrical stunning electrodes are placed across the animal's brain and a current is applied for a few seconds. This is mainly used for sheep and for pigs, although can also be used for calves and cattle.

Captive bolt stunning uses a gun-like device to deliver a blow to the animal's head, which may penetrate the skull. This method is mainly used for adult cattle but can also be used for adult pigs, sheep and calves.

Water bath electrical stunning is used for poultry; conscious birds are hung by their feet from metal shackles, which form one electrode, on a conveyor belt. Their heads are then dipped into an electrified water bath.

Gas stunning or stun/killing (mainly carbon dioxide, CO₂): used for pigs, poultry and fish.

5.5 Meat inspection

Meat inspection is commonly perceived as the sanitary control of slaughter animals and meat. The aim of meat inspection is to provide safe and wholesome meat for human consumption. The responsibility for achieving this objective lies primarily with the relevant public health authorities who are represented by veterinarians and meat inspectors at the abattoir stage.

The objectives of meat inspection are twofold:

- a. To ensure that only apparently healthy, physiologically normal animals are slaughtered for human consumption and that abnormal animals are separated and dealt with accordingly.
- b. To ensure that meat from animals is free from disease, wholesome and of no risk to human health. These objectives are achieved by *ante-mortem* and *postmortem* inspection procedures and by hygienic dressing with minimum contamination.

Basic functions of meat inspection

1. Detection and destruction of diseased or contaminated meat
2. Assurance of clean and sanitary handling and preparation
3. Minimization of microbial contamination
4. Prevention of adulteration
5. Prevention of false labeling
6. Application of inspection stamps (Increase the consumers' confidence)

5.5.1 Ant-mortem inspection

The term *ante-mortem* means “before death.” It is the inspection of live animals before they are slaughtered. *Ante-mortem inspection* of live animals is a screening process to remove obviously diseased animals from the food supply prior to slaughter and to identify animals that require a more extensive *post-mortem examination*. It is the first line of defense in protecting the public from potentially harmful meat products. Those animals that exhibit abnormal signs must be withheld from normal slaughter and *segregated* for closer examination.

Ante-mortem inspection assessments of the Veterinary Inspector are based on:

- ☐ The absence or presence and extent of any clinical signs of disease.
- ☐ The presence and extent of any conditions that may result in the rejection of the carcass or its parts as a source of human food.
- ☐ The presence of excitement or disturbed activity.
- ☐ The presence of any disability.
- ☐ The treatment or exposure of the animals to drugs, chemicals, biological substances or radioactive materials. It is generally carried out to look for:
 - Zoonotic diseases
 - Animal diseases making the meat unfit
 - Injury, fatigue, stress and welfare

- It is the most desirable practice, and is of great value, for it aids in the detection of animals suffering from infectious diseases, particularly anthrax, rabies, etc. which are communicable to man.
- Many diseases of a toxic or infectious nature are difficult to detect in the carcass and organs after slaughter. These include tetanus and rabies
- The presence of ante-mortem inspection helps in identifying suspect animals. Slaughtering them separately - allowing close post-mortem examination & interpretation.
- Has value in the prevention of food-poisoning outbreaks, for many of these outbreaks can be traced to the consumption of meat from animals slaughtered while obviously ill but whose carcass and organ may show little noticeable changes on post-mortem examination.

5.5.2 Post-mortem inspection

Post-mortem inspection covers the inspection of the carcasses and parts of meat and poultry used for human food. It takes place after the animal or poultry has been slaughtered thus the term “post-mortem,” meaning “after death” in Latin. Post-mortem inspection covers the steps in the slaughter process that begin at stunning and ends at the step where the carcass is placed in the cooler. The purpose of post-mortem inspection is to protect the public health by ensuring that the carcasses and parts that enter commerce are wholesome, and properly marked, labelled, and packaged. This means that any carcasses or parts that are unwholesome, and thereby unfit for human food, do not enter commerce. In performing inspection methods, making regulatory decisions, documenting findings, and taking enforcement actions when appropriate are important. In relation to post-mortem inspection we are guided by the following regulations, directives, and notices.

Look for evidence of disease or other abnormality liable to render the carcass unfit for human consumption either in whole or in part. The information gathered also helps in the diagnosis of disease conditions for disease control strategies.

Procedure:

The inspection shall include:

- ❖ Visual examination of slaughtered carcass and organs
- ❖ Palpation and Incision of appropriate organs
- a) **Head:** examination of the outer surface and eyes, followed by inspection of the gums, lips, cheeks, and tongue for FMD, necrotic etc.
- b) **Lymph nodes:** mandibular, atlantal, supratharyngeal and parotid lymph nodes for detection of lesions of tuberculosis
- c) **Lungs:** visual examination, followed by palpation for tumor (lesions), abscess or pneumonic conditions. Incision and inspection of the tissue
- d) **Liver and associated lymph nodes:** surface observation and palpation for abnormalities, opening the bile duct for liver-flukes
- e) **Spleen palpation**
- f) **Kidney palpation and incision**

There are slight differences in procedure for different species, but the overall requirement of the inspection is to find evidence of disease or abnormality. Sanitation aspects in the abattoirs (facilities and equipment, personal hygiene, clothing, swage and waste disposal control) should also be inspected to check compliance with the sanitary laws.

5.6 Fabrication of meat

It is breaking down of a carcass of meat into consumer cuts or boned meat. Meat, poultry, and fish are the most costly part of the food budget of a foodservice operation, whatever the scale. Depending on the prevailing local market rates for food and labor, in-house fabrication may be less expensive than buying prefabricated menu cuts. As a further economic benefit, trim and bones can be used to prepare other dishes (e.g., stocks, soups, sauces, and forcemeats). In addition, the chef can offer specialty cuts that their customer cannot readily find in supermarkets or deems to be too expensive or difficult to cook at home.

The difference between butchering/slaughter and fabricating meats is subtle. In the broad view, however, butchering means cutting an entire animal into large cuts known as primal cuts. Subsequent cuts are made to fabricate the primal into a variety of smaller cuts (known as sub-primal or wholesale cuts). Fabrication, though it involves the meat-cutting techniques used in the butcher shop, is essentially the fine-tuning of an item purchased from a butcher or meat purveyor to produce the menu cuts familiar to most chefs and restaurant patrons.

5.7 Methods of Meat Preservation

Meat preservation is principally concerned with the application of measures to delay meat spoilage which are caused by microbial, chemical and physical changes. Of these, microbial spoilage (caused by bacteria) is the most common. Microbes thrive in moisture and since meat is 70% water, it spoils easily through microbial action. Meat, being a rich source of nutrients, also becomes an excellent food for bacteria. Methods of meat preservation, however different superficially, are alike in that they employ environmental conditions which discourage the growth of micro-organisms.

They may be grouped in to three broad categories based on:

- a. control by temperature,
- b. control of moisture, and
- c. by lethal agencies (bactericidal, bacteriostatic, fungicidal and fungistatic).

Animal tissues are sterile, or nearly so, except lymph nodes in the living and growing animal. But during slaughtering and preparation to human food, it becomes subject to degradation by chemical, physical and biological reactions. Meat is an ideal culture medium for microbes: high in moisture, rich in nitrogenous foods & minerals and accessory growth factors, sufficient in fermentable carbohydrates. Preservation of meat, poultry and fish is accomplished by creating an unfavorable environment for the growth of spoilage organisms (bacteria, yeasts, moulds and parasites) and the prevention of chemical oxidation of lipids which leads to rancidity, through:

1. Extreme heat or cold (temperature regulation)
2. Deprivation of water and sometimes oxygen
3. Excess of saltiness or imposition of osmotic pressure changes
4. Increased acidity

Methods of preservation used include: cooling, drying, smoking, salting, freeze-drying, canning, irradiation or a combination of these.

1. Drying

It is removal of moistures from meat. The method involves the reduction of the original water content (70% of the meat to about 15%). In general effect of drying on meat is the reduction in water content (below 30%), and water activity (below 0.90%). There are two types of drying:

- i. **Natural drying** - natural sunlight is used to reduce the moisture content of the meat.
- ii. **Artificial drying** - a chamber equipped with heating elements maintained at a temperature of 43°C-49°C and a relative humidity of 85% is used for drying. This is more expensive than sun drying but its dried products have a better quality and can be sold at a higher price.

Dried meats are an important part of the diet in many developing countries: chiefly used at small scale and individual households level (e.g. Quanta, in Ethiopia) it provides families with protein diet of durability and lightness.

2. Curing

Meat curing can be understood as the addition of salt, nitrite and/or nitrate, sugar and other ingredients for the purpose of preserving and flavoring meat.

Ingredients for curing: Common salt as the principal active agent, together with alkali metal NaNO_2 (sodium nitrite) as supplementary agent, and others like sugar, phosphates, spices, vinegar and wine will be used. The rate of curing of intact meat depends on the diffusion of curing ingredients into the tissues.

In the process of curing, salt may be applied in different ways:

Salting - a simple method of dehydration in which the salt causes the withdrawal of water from the tissue of both the meat and spoilage organisms, resulting to the shriveling and inactivation of the cells.

Dry curing - applying salt to meat products by rubbing the salt directly on the outside surface of the meat. This is the oldest practice with the slow rate of curing.

Wet curing - injecting (immersing in) salt solution (brine) into the meat. Injecting greatly enhances its distribution within the meat, and hence the rate of curing. The brine is pumped into the meat with needle and a pressure source of liquid, (commercially, mechanized pumping).

Addition of sodium nitrite enhances the colour of cured meat, and stabilizes flavour. When added, it reacts with myoglobin and haemoglobin of trapped red blood cells to form the typical cured meat colour. From purple red colour of myoglobin to the brown of meat myoglobin. Eventually to dark red of nitric oxide myoglobin.

3. Smoking

There are two methods of smoking:

a. Natural- is the exposure of the meat to wood smoke which causes the deposition of pyroligneous acid on the meat surface that acts as preservative and re flavoring agent. Hardwood, guava leaves or any kind of wood may be used.

b. Artificial - smoke flavor is incorporated in the pumping pickle for ham and bacon.

Smoking extends the shelf-life of fresh meat, and provides the product a desirable flavour. Too wet or too dry meat is not suitable for smoking. Therefore products need to be exposed to short drying period, so that the surface of the product will be slightly moist, prior to smoking. Chemical components arising from the smoke are responsible for the preservative effect and development of acceptable aroma. The chemicals include: acids, alcohols, aldehydes, ketones, phenols, carbonyl compounds.

The phenolic fraction - primarily responsible for the preservative properties, and smoky aroma and flavour

The carbonyl fraction - responsible for the desirable amber-brown colour generated during smoking

4. Cooling

a. Chilling /Refrigeration/

In tropical countries, the traditional preference is for freshly slaughtered meat. The dressing of carcass should be carried out at a hygienically and technically adequate level.

Methods of meat chilling

The interval between slaughter and initiation of chilling should not exceed two hours. The dressed carcasses have to hang in a chill room at about 0 to 3.5°C. During the chilling process the internal temperature of the meat should be reduced from about 40° C to about 0 to 4° C; this takes from 14 to 36 hours, 20 hours is often considered to be the optimum time.

Storage life of chilled meat: chilling is practically useful when meat is to be preserved for a relatively short time. By this process the flavor, appearance and nutritive value of the meat is scarcely affected. The temperatures between 0 and 3°C inhibit most organisms and render the product commercially shelf stable for two to three weeks. Under good hygienic conditions, the shelf life could be extended up to 3 to 4 weeks by holding the temperature at about -1°C. Therefore it is important to maintain the temperature as low as possible, but always above the initial freezing point of meat (-1 to -1.5° C) to get long storage life.

b. Freezing

Freezing is the most common method of meat preservation for extended periods, resulting to crystallization of the water in the tissues, thus inactivating the enzymes and the bacteria present. Frozen meat is that which is cooled to and stored at temperatures well below its freezing point. The meat so treated can be preserved for a long period of time and transported over long distances.

Freezing of meat can be achieved by

- *Freezing chamber:* Meat is pre-cooled for about 2-6 days and finally freeze to about a temperature of -18 to -10°C
- *Plate freezer:* The meat is cut into piece. The cut pieces are loaded to refrigerated plate and this will be kept inside the refrigerator. Meat freezes in 30 to 60 min. But cutting larger quantities of meat in to pieces is a problem
- *Blast freezer:* The carcass is cut into two and freeze in *blast* freezer

Physical changes that might occur in freezing of meat

1. The physical state of muscle plasma is considerably altered: Muscle plasma such as globulin and albumin, though normally soluble, become insoluble at a certain concentration. This change is irreversible when the meat is thawed.
2. Loss of moisture and formation of large ice crystals if the rate of freezing is slower. This condition stretches and ruptures muscle tissues. Upon thawing the meat presents unpleasant appearance (freezer burn).

Effects of speed freezing

The freezing of meat lies between -1 and -1.5. Ice crystals commence to form in the meat at this temperature and continue to form as the temperature is further lowered:

- At -1.5°C, 35.5% of the muscle water is ice.
- At -5°C, 82% of muscle water is ice
- At -10°C 94% of muscle water is ice.

During freezing the water present in the muscle fibres diffuses from the muscle plasma to form ice crystals, the speed with which the freezing process is conducted having an important bearing on the size of ice crystals and the further quality of the product.

- **Slow freezing** tends to yield extra-cellular ice crystals, which subsequently grow due to the collection of water both from the extra-cellular spaces and from within the cells. This growing crystals mechanically disrupt the structure tunnelling their way through the meat.
- **Rapid freezing** results in very little water separation and the crystals are therefore small in size and less expansive.

Storage life of frozen meat:- Some bacteria can be destroyed by freezing, but in others the effect of low temperature is merely to inhibit their growth and multiplication until conditions favorable to their growth appear.

Freezing has no great value as a method of rendering a carcass affected with pathogenic bacteria.

- *Anthrax bacilli* withstands -13°C
- *Salmonella* withstands -175°C
- *Tubercle bacilli* remain alive for 2 years at -10°C

Table 1: Durability of frozen and chilled meat:

Type of meat	Frozen storage life	Chilled storage life
Beef	about a year	< 3 week
Veal	slightly less than a year	1-3 week
Mutton	8 - 9 month	-
Lamb	7 - 8 month	1 - 2 week
Pork	about 6 months	1 - 2 week

Oxidative rancidity

The growth of spoilage organisms is inhibited at freezer temperatures. The principal factor limiting the storage life of frozen meats is oxidative rancidity. Rancidity develops when the carcass fat absorbs oxygen from the air. The tendency to absorb oxygen depends on the level of saturation of the fatty acid forming the fat. Any fat that has one or more double bonds in the carbon chain will be vulnerable to a cleavage caused by the oxygen taking the place of the bond and forming aldehydes and fatty acids. These products so formed are no longer pleasing in taste and odour, and it is called oxidative rancidity.

To combat oxidative rancidity

- Trimming the fat closely before freezing or freeze only those cuts that are quite lean
- Eliminating air using air-tight, moisture proof, and properly applied wrapping material
- Adding anti-oxidants like nitrite into the product.

5. Canning

It is the hermetic or air tight sealing of food in can or jars at a desired temperature and pressure for specific period of time. Commercial canning of meat represents the second most common preservation method for extended periods of time. In this method prepared meat products are sealed in a container and are subjected to heat for a definite period of time and then cooled. Heating destroys organisms and deactivates enzymes; the permanent sealing of the container prevents re-infection by further organisms.

Canned meat products are of two types:

- Sterilized products and
- Pasteurized products.

The former is shelf stable while the latter requires refrigeration to inhibit spoilage. Commercial sterility refers to the destruction of spoilage organisms and their spores such that a product will not undergo spoilage under indefinite periods. Sanitation is extremely important in meat-canning plants, since the fewer the number of organisms present, the more effective is the sterilization process. A time and temperature relationship is required for the destruction of most micro-organisms. In non acid foods such as meat the destruction of bacterial spores is slow, and these foods require temperatures of about 115°C.

Heat treatment of foods below temperatures needed for sterilization is referred to as pasteurization. With pasteurization, a substantial proportion of the microbial load is reduced, some are attenuated (sub lethal-injury) while spores may be stimulated to germinate, and enzymes are inactivated. With proper refrigeration, such product can be stored for about 6 months. The susceptibility of micro-organisms to the heat treatments depends on the pH, and water activity of the product. At lower pHs and higher water activity (content), spores can be destroyed relatively easily.

Micro-organisms of concern in meat

- *Clostridium botulinum*
- *Stap. aureus*
- *Salmonella*
- *Clostridium perferingens*
- Others include *Str. species*, *E.coli*

6. Sausages

Sausages are products of meat processing industries manufactured from raw meat and various ingredients. Each ingredient has its own function. Varieties of sausages are manufactured using different procedures and proportions of raw materials.

- *Fresh sausage* - made from selected cuts of fresh meat, must be stored in a refrigerated state prior to consumption
- *Uncooked smoked sausages* -made from cured or fresh meat. Smoked but not cooked prior to sale, require refrigeration
- *Cooked sausages* - made from fresh meats, cured, fully cooked and smoked during processing, needs refrigeration.
- *Dried and semidried sausages* - made from fresh meat, cured during processing, may or may not be smoked; controlled bacterial fermentation enables these products to have low pH (4.7 to 5.3), cool place for dry sausages; refrigeration for semi-dried.

5.8 Types of meat products

Meat is one of the staples of traditional diets in most parts of the world. Until the advent of modern refrigeration technique, meat spoils quickly without some types of treatment. As methods for preserving meat were developed, the number of meat product types increased as well. Hence from these points of view, meat product types are classified as follows:

1. **Processed meat:** processed meat products are those that are chopped and developed in to products that have additives and a different texture than the original meat. Many common types of prepackaged meat-based foods fall in to this category, including lunch meat.
2. **Cured meat:-** cured meat that has been cured usually retained the shape and texture of the original cutoff meat. Cured meat is treated for a long period of time in a liquid solution, which may contain

many ingredients but always a high concentration of salt. Most hams are cured with a combination of salt and sugar.

3. **Smoked meat:** these products are those that have been preserved through the use of heat, smoke and often salt. Typically the flavors of smoked meat comes from the type of wood used for smoking.
4. **Pickled meat:** pickling meats is a method of preparation that closely resembles curing, but with the addition of vinegar to the solution. The combination of salt and vinegar that is allowed to seep in to the meat gives it a sharp flavor; because of this, the method is mostly used in areas where it is traditional.
5. **Dried meat:** Drying meat is one of the oldest method of treating meat. The method involves cutting meat in to small strips and placing it on an outdoor surface, allowing the ambient heat of the environment to reduce the water content.
6. **Fermented meat:** Fermented meats are generally ground, spiced and made in to sausage. These meats also can be smoked, cooked, cured or uncooked. After the meat is prepared, it is generally hung and left to ferment for varying amount of time depending on the type.

5.9 Meat quality and grading

3.9.1. Meat quality

It is normally defined by the compositional quality (lean to fat ratio) and the palatability factors such as visual appearance, smell, firmness, juiciness, tenderness, and flavour. The nutritional quality of meat is objective yet "eating" quality, as perceived by the consumer, is highly subjective.

Visual Identification: The visual identification of quality meat is based on colour, marbling and water holding capacity. Marbling is small streaks of fat that are found within the muscle and can be seen in the meat cut. Marbling has a beneficial effect on juiciness and flavour of meat. Meat should have a normal colour that is uniform throughout the entire cut. Beef, lamb, and pork should also have marbling throughout the meat.

Smell: The product should have a normal smell. This will be different for each of the species (i.e. beef, pork, chicken), but should vary only slightly within the species. Any rancid or strange smelling meat should be avoided.

Firmness: Meat should appear firm rather than soft. When handling the retail package, it should be firm, but not tough.

Juiciness: Juiciness depends on the amount of water retained in a cooked meat product. Juiciness increases flavour, helps soften meat making it easier to chew, and stimulates saliva production in the mouth. Water retention and lipid content determine juiciness.

Tenderness: Has been linked to several factors, such as the animal's age, sex or the muscle location. One important way to tenderize meat is by aging. Carcasses are aged by holding them at refrigeration temperatures for extended periods of time after slaughter and initial chilling.

Flavour: Flavour and aroma are intertwined to create the sensation the consumer has during eating. These perceptions rely on the smell through the nose and on the sensations of salty, sweet, sour and bitter on the tongue. Meat flavour is affected by type of species, diet, cooking method and method of preservation (e.g. smoked or cured).

3.9.2 Meat grading

It is analyzed by the combined effect of meat quality and yield characteristics which comprises all factors such as age, sex, marbling, tenderness, and yield of meat from a specific animals and grossly

grouped in to quality and yield grading of meat; and also sub-divided in to 9 (10) quality and 5 (A-E) yield grades.

In beef, yield grades estimate the amount of boneless, closely trimmed retail cuts from the high-value parts of the carcass-the round, loin, rib, and chuck. However, they also show differences in the total yield of retail cuts. We expect a YG 1 carcass to have the highest percentage of boneless, closely trimmed retail cuts, or higher cut ability, while a YG 5 carcass would have the lowest percentage of boneless, closely trimmed retail cuts, or the lowest cut ability. The USDA Yield Grades are rated numerically and are 1, 2, 3, 4, and 5.

Yield Grade 1 - The carcass is covered with a thin layer of external fat over the loin and rib; there are slight deposits of fat in the flank, cod or udder, kidney, pelvic and heart regions.

Yield Grade 2 - The carcass is almost completely covered with external fat, but lean is very visible through the fat over the outside of the round, chuck, and neck. Usually, there is a slightly thin layer of fat over the inside round, loin, and rib, with a slightly thick layer of fat over the rump and sirloin.

Yield Grade 3 - The carcass is usually completely covered with external fat; lean is plainly visible through the fat only on the lower part of the outside of the round and neck. Usually, there is a slightly thick layer of fat over the rump and sirloin. Also, there are usually slightly larger deposits of fat in the flank, cod or udder, kidney, pelvic and heart regions.

Yield Grade 4 - The carcass is usually completely covered with external fat, except that muscle is visible in the shank, outside of the flank and plate regions. Usually, there is a moderately thick layer of external fat over the inside of the round, loin, and rib, along with a thick layer of fat over the rump and sirloin. There are usually large deposits of fat in the flank, cod or udder, kidney, pelvic and heart regions.

Yield Grade 5 - Generally, the carcass is covered with a thick layer of fat on all external surfaces. Extensive fat is found in the brisket, cod or udder, kidney, pelvic and heart regions.

Most countries do not grade on eating quality

- ❖ Notable exceptions are US and Australia
- ❖ US – Carcasses graded on yield and quality
- ❖ Quality grade is based on visual assessment of marbling (loin) and maturity
- ❖ Marbling is the amount and distribution of visible flecks of fat within the eye muscle at 12th/13th rib
- ❖ Marbling is primary factor in determining quality grade
- ❖ Maturity (physiological age) is assessed visually: Degree of ossification of cartilage on vertebrae

5.10 Fish processing

5.10.1 Over View

The term fish processing refers to the processes associated with fish and fish products. Larger fish processing companies often operate their own fishing fleets or farming operations. The products of the fish industry are usually sold to grocery chains or to intermediaries. A central concern of fish processing is to prevent fish from deteriorating. Fish processing can be subdivided into fish handling, which is the preliminary processing of raw fish, and the manufacture of fish products.

Fish is a highly perishable food, which needs proper handling and preservation if it is to have a long shelf life and retain a desirable quality and nutritional value. The most obvious method for preserving the quality of fish is to keep them alive until they are ready for cooking and eating. For thousands of years, China achieved this through the **aquaculture of carp**. Other methods used to preserve fish and

fish products include the control of temperature using, refrigeration or freezing, control of water activity by drying, salting, smoking or freeze-drying, vacuum packing and *etc.* Fish processing is also concerned with proper waste management and with adding value to fish products. There is an increasing demand for ready to eat fish products, or products that do not need much preparation.

5.10.2 Handling live fish (Live fish trade)

An alternative and obvious way of keeping fish fresh is to keep them alive until they are delivered to the buyer or ready to be eaten. This is a common practice worldwide. Typically, the fish are placed in a container with clean water. The water temperature is then lowered and the fish are starved to reduce their **metabolic rate**. This decreases fouling of water with metabolic products (ammonia, nitrite and carbon dioxide) that become toxic and make it difficult for the fish to extract oxygen.

5.10.3 Fish products

1. **Whole fish:** the fish as it originally came from the water, with no physical processing
2. **Drawn fish:** a whole fish, which has been eviscerated, its internal organs removed
3. **Dressed fish:** fish that has been scaled and eviscerated, and is ready to cook.
4. **Filletted fish:** the fleshy sides of the fish cut lengthwise from the fish along the backbone.
5. **Fish steaks:** large dressed fish can be cut into cross section slices, usually half to one inch thick, and usually with a cross section of the backbone

5.10.4 Fish Preservation

Preservation techniques are needed to prevent fish spoilage and lengthen shelf life. Preservation techniques can be classified as follows.

a. Control of temperature

Freezing: The flesh of fish consists of muscle fibers, which in turn are composed of microscopic cells, and the constituents of the flesh are roughly 81 % water, 16% protein and fat, and 3 % organic salts. Freezing will remove as much water as possible from the fish by turning it into ice, thereby reducing the bacterial action. Briefly, the fish is dried by freezing. Deterioration of fish in cold store is usually due to oxidation or drying. Oxidation is more pronounced in fatty fish. Maintaining low storage temperature -20°F is the best safeguard against oxidative changes.

b. Control of water activity (Drying, Salting and Smoking)

Available water is necessary for the microbial and enzymatic reactions involved in spoilage. There are a number of techniques that have been or are used to tie up the available water or remove it by reducing the a_w . Traditionally, techniques such as **drying**, **salting**, and **smoking** have been used, and have been used for thousands of years. The drying, salting and smoking techniques are as discussed in the previous sections.

c. Physical control of microbial loads

Microbial loads can be physically controlled by canning and then sterilizing with heat. Heat or ionizing irradiation can be used to kill the bacteria that cause decomposition. Heat is applied by cooking, blanching or microwave heating in a manner that pasteurizes or sterilizes fish products. Sterilized products are stable at ambient temperatures up to 40°C , but to ensure they remain sterilized they need packaging in metal cans or restorable pouches before the heat treatment.

d. Chemical control of microbial loads (Bio-preservation and Fermented fish)

Microbial growth and proliferation can be inhibited by a technique called bio-preservation. **Bio-preservation** is achieved by adding antimicrobials or by increasing acidity of the fish muscle. Most

bacteria stop multiplying when the pH is less than 4.5. Acidity is increased by fermentation, or by directly adding acids (acetic, citric, lactic) to fish products.

e. Control of the oxygen reduction potential

Spoilage bacteria and lipid oxidation usually need oxygen, so reducing the oxygen around fish can increase shelf life. This is done by controlling or modifying the atmosphere around the fish, or by vacuum packaging.

5.10.5 Transport

Fish is transported widely in ships, and by land and air, and much fish is traded internationally. It is traded live, fresh, frozen, cured and canned. Live, fresh and frozen fish need special care.

Live fish: When live fish are transported, they need oxygen, and the carbon dioxide and ammonia that result from respiration must not be allowed to build up. Most fish transported live are placed in water supersaturated with oxygen.

By air: Over five percent of the global fish production is transported by air. Air transport needs special care in preparation and handling and careful scheduling. The air shipment of leaking seafood packages causes corrosion damage to aircraft, and each year, in the US, requires millions of dollars to repair the damage. Most airlines prefer fish that is packed in dry ice, and not packed in ice.

By land or sea: "The most challenging aspect of fish transportation by sea or by road is the maintenance of the cold chain, for fresh, chilled and frozen products and the optimization of the packing and goods density. Maintaining the cold chain requires the use of insulated containers or transport vehicles and adequate quantities of coolants or mechanical refrigeration. Continuous temperature monitors are used to provide evidence that the cold chain has not been broken during transportation.

5.10.6 Quality and safety

The quality of fish and fish products depends on safe and hygienic practices. Outbreaks of fish-borne illnesses are reduced if appropriate practices are followed when handling, manufacturing, refrigerating and transporting fish and fish products. Ensuring standards of quality and safety are high also to minimize the post-harvest losses. Fish processing highly involves very strict controls and measurements in order to ensure that all processing stages have been carried out hygienically. Thus, all fish processing companies are highly recommended to join a certain type of food safety system. One of the certifications that are commonly known is the Hazard Analysis Critical Control Points (HACCP).

CHAPTER 6

POULTRY PRODUCT PROCESSING

6.1 Poultry Meat Processing

Poultry processing, is preparation of meat from various types of fowl for consumption by humans. Chickens and turkeys are the most common sources of poultry; however, other commercially available poultry meats come from ducks, geese, pigeons, quails, pheasants, ostriches, and emus. Poultry (poultry meat) is derived from the skeletal muscles of various birds and is a good source of protein, fat, and vitamins, and minerals in the diet. Hence it provides an excellent medium for the growth of

both spoilage and pathogenic microorganisms; therefore it needs special care and sanitation measure. To achieve this, different processing techniques are essential.

6.2 The Slaughter of Poultry

6.2.1 Pre-slaughter handling

When the birds have reached “harvest” time, they are generally taken off of feed and water. This allows their digestive tracts to empty and reduces the potential for contamination during processing. At night the birds are caught and placed into plastic or wooden transport cages. The birds are then transported to the slaughterhouse, where the trucks are often kept between sets of fans to ventilate the cages. In the next step the birds are removed from the cages and transferred to continuously moving shackles where they are suspended by both legs. The transfer is often done in a dark room illuminated by a red light; the birds are not sensitive to the red light and this helps to keep them calm.

6.2.2 Slaughtering (Stunning and killing)

After the birds have been transferred to the moving shackles, they are usually stunned by running their heads through a water bath that conducts an electric current. Stunning produces unconsciousness, but it does not kill the birds. The birds are killed either by hand or by a mechanical rotary knife that cuts the jugular veins and the carotid arteries at the neck.

6.2.3 Scalding

Following bleeding, the birds go through scalding tanks. These tanks contain hot water that softens the skin so that the feathers can be removed. The temperature of the water is carefully controlled. If retention of the yellow skin colour is desired, a soft-scald is used (about 50 °C). If a white bird is desired, a higher scald temperature is used, resulting in the removal of the yellow pellicle. Then after evisceration, removal of wastes and offal, washing, drying, canning, chilling, freezing and packaging are the following procedures.

6.3 Egg

The high-quality egg produced under today’s large-scale integrated flock system lends itself well to handling and processing by automatic equipment. In fact, most new complexes are in-line systems designed to carry eggs from the hen house to the carton in one continuous operation. Eggs must be handled properly throughout each phase of production, processing, and transportation to maintain quality. Washing equipment washes, sanitizes, and dries eggs automatically. Grading equipment uses mass scanners to help operators detect and remove dirties, checks, irregular shells, meat and blood spots, and loss eggs. Automatic weighing equipment individually weighs each egg and sorts the eggs according to the official weight classes. Automatic packaging equipment places the eggs into cartons, closes the cartons, and stamps the cartons with a production code. Coolers reduce the temperature of the eggs and maintain humidity levels that minimize quality deterioration. In addition to people doing quality control work, a plant occasionally conducts quality segregation by hand-candling. In these situations, adequate facilities and equipment must be provided in order to grade eggs with maximum efficiency.

6.4 Egg quality measuring

While the avian egg is a vehicle for reproduction, it also serves as a source of food for humans. The size and shape of avian egg differs among the various species of birds, but all eggs have four main parts; (yolk, albumen/ egg white, shell membrane, and shell), which all are separated by membrane

from each other. The egg constituents are used for analyzing the quality of egg and to accomplish grading of the egg.

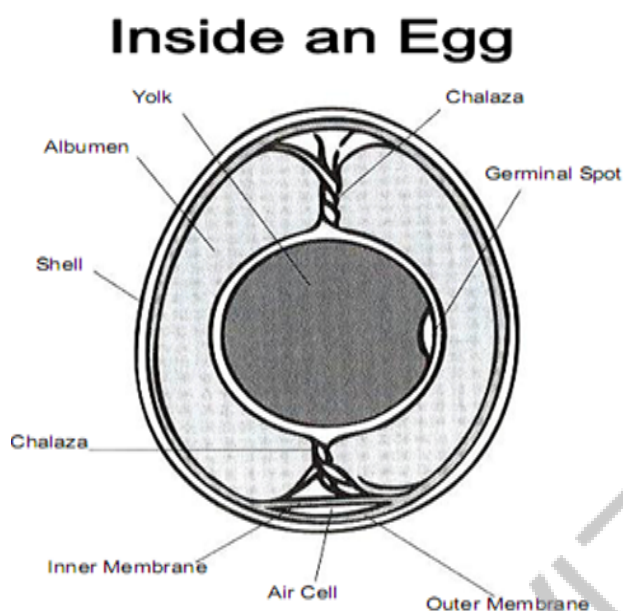


Figure 6.1: parts of egg

Egg grading: is a form of quality control used to divide variable egg constituents (products) into a number of desired classes. Commercial chicken eggs are sorted according to exterior and interior quality into three grades established by the United States Department of Agriculture (USDA) as AA, A, B and the rest is graded as **dirty**. For all grades the shell must be intact.

Grades "AA" and "A" eggs are nearly identical, the main difference being that grade A eggs are slightly older than grade "AA" eggs. Grade "AA" eggs therefore have firmer, thicker whites that hold the yolks up high and round, whereas the white of grade "A" egg is "reasonably firm."

Grade "B" eggs have stained or abnormal shells, minor blood or meat spots and other minor defects. They are used in the food industry to make liquid, frozen and powdered egg products, so you are unlikely to find them at the grocery store.

Exterior egg quality (Eggshell quality) measurement:

Exterior quality refers to a shell's appearance, cleanliness, and strength. Appearance is important because the shell is the first thing you notice about an egg. Cleanliness is important because the shell is the egg's first defense against bacterial contamination; the cleaner the shell, the easier it can do its job. The shell accounts for about 12% of the weight of a large egg. An egg shell strength is naturally influenced by the vitamins and minerals in the hen's diet/feed, especially vitamin D, Ca, P, and Mn. Shell strength is also influenced by a hen's age (older hens lay larger eggs with thinner and weaker shells). One way to test the strength of an egg is to press the ends between the palms of our hands.

Summary of egg quality measurements:

1. Egg shell (exterior egg) quality:- Cleanliness, Shape, Texture (sandy, rough, rigid or soft, and thin), color, mottling of shell (bright spot, or moist appearance, around pores; that is observed by candling), tremulous or moving air cell; observed by candling, and tainted/contaminated shell.

2. Inner egg quality:-

a. The egg white (albumen) quality:- color, weak or thin or watery white, spots (blood and meat),

microbial spoilage (detected by odor and flavor)

b. yolk quality:- color (colorless or colored), misplaced egg yolk, blood and meat spot, mottled (yolk surface covered by different spots), texture (thick, pasty, rubbery or cheese-like), odor and flavor (microbial spoilage), and stuck yolk (being immovable because of Newcastle infection).

3. Egg size: - is also taken in to consideration especially for reproduction/hatchery.

Note: - egg membranes are not ideal for quality measurement, since they are not found inseparably after the egg is broken and not occupy a significant part of the egg.

Factors affecting egg quality:

Many factors affect egg quality. A few of these problems can be prevented or reduced by good hen management. Producers should remember that genetics, feed quality, duration after the egg is laid, and environment play a role in egg quality. The most reliable factor is age. A young pullet produces smaller eggs with strong egg shell and albumen that stands high. As the hen ages, the shell become thin and the albumen begins to weaken and run.

6.5 Egg preservation techniques

Spoilage of egg is due to the entrance of air carrying germs through the shell. Normally the shell has a surface coating of mucilaginous matter, which prevents for a time the entrance of these harmful organisms. But if this coating is removed or softened by washing or otherwise, the keeping quality of the egg is much reduced. This fact explains, why many methods of egg preservation have not been entirely successful, and suggests that the methods employed should be based up on the idea of protecting and rendering more effective the natural of the shell, so that air bearing the germs that cause decomposition may be excluded. Raw eggs will last about **30** days in the refrigerator without losing any quality and stored for 2-3 months at a temperature not higher than **12°C** without doing anything on egg, but the humidity need to be close 75%. This is important in successful egg storage.

Summary of table egg preservation:-

- Keep clean and cool for a maximum of 15-30 days (hatchery egg)
- Freeze the egg (both whole shell and scrambled table egg)
- Coat the egg with different oils and compounds then keep cool (shelled table egg)
- Thermo stabilize then oiled and freeze (shelled table egg)
- Pickled the scrambled egg then keep cool
- Dehydration the scrambled egg and keep cool

In general, refrigeration and freezing is common methods used worldwide. The best method of preserving whole shell eggs is refrigeration (1°C - 4°C) in a well-sealed container, while freezing is an efficient method of long-term storage. Freezing the egg with a good-liquid tight container and holding at -18°C, enable storage of the product for about one year. But do not freeze whole eggs in the shell as the shell may burst due to expansion during freezing.

Egg Product Refrigeration/Freezing Chart

Product	Refrigerate	Freezer
Whole eggs with shell	2 months or longer	Not recommended
Whole eggs without shell	4 days	1 year
Egg whites	4 days	1 year
Egg yolks	4 days	1 year
Hard-cooked eggs	1 week	Not recommended

Care of Eggs on the Farm

Immediately after it is laid, an egg begins to lose quality, even if it is removed from the nest, cooled, packed, and marketed promptly. Keeping temperature and humidity conditions at an optimum level retards this loss in quality to a large degree. Although most eggs are produced by large in-line integrated operations, some are still produced from off-line production facilities. At off-line sites, certain steps are necessary to maintain egg quality at the highest level.

Some of these steps are:

1. Gather eggs frequently (at least 3 times a day).
2. Handle the eggs carefully to prevent breakage.
3. Cool the eggs promptly and store them under the optimum temperature and humidity.
4. Pack the eggs in clean, cool packing materials.
5. Pack clean eggs separately from dirty eggs.

GOOD LUCK!

THE END

