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Effect of Processing on Milk Components

This bulletin includes technical information based on latest developments on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue is **"Effect of Processing on Milk Components"** It may be understood that the information given here is by no means complete.

In this issue:

INTRODUCTION

Milk is a complex colloidal dispersion of fat globules, casein micelles, and whey proteins in an aqueous solution of lactose, minerals, and a number of minor compounds. Its physical and physico-chemical properties (see table 1) depend on intrinsic compositional and structural factors, extrinsic factors such as temperature, and post milking treatment.

There is no clear distinction between physical properties and physico-chemical properties. However, physical properties may be thought of as measures of the bulk behaviour of milk and of how milk interacts with energy, while physico-chemical properties are measures of how bulk behaviour and energy interactions depend on milk's constituent colloidal particles, molecules, atoms, and ions.

Information and knowledge of these properties of milk and milk products are of importance particularly in the technological and engineering design and control of milk processes and processing equipment. Basis for the design of modern methods of milk analysis, structural aspects of milk, and elucidation of the complex chemical reactions that occur in milk can be decided based on its properties.

CHEMISTRY OF PROCESSING

Fluid milk and further processed dairy products are subjected to a series of processing treatments beginning with milking, pumping, cooling, mixing, and storage on the farm; transportation to the processing plant; and clarification, separation, standardization, pasteurization, vacuum off-flavor removal, homogenization, and packaging at the processing plant. Other processing treatments, e.g., acid and rennet coagulation, fermentation, vacuum

evaporation, drying, churning, freezing, and sterilization, are used for manufacturing a variety of further processed dairy products.

Table 1. Physicochemical Characteristics of Cow and Buffalo Milks

Characteristics	Buffalo milk	Cow Milk
pH	6.7	6.6
Buffer value (pH 5.1)	0.0417	0.0359
Density at 20◦C	1.0310	1.0287
Viscosity (cP)	2.0400	1.8600
Specific refractive index	0.2061	0.2059
Surface tension	5.4000	5.5900
Acidity (%)	0.1500	0.1400
Fat globule size (µm)	5.0100	3.8500
Phosphatase (units)	28.000	83.000
UV fluorescenc	Greenish yellow	Pale bluish

Reference: Chemical Composition, Physical and Functional Properties of Milk and Milk Ingredients. K.Kailasapathy, Chapter 4, Dairy Processing & Quality Assurance, Editor Ramesh C. Chandan.

Milking Systems

Since the milk quality payment globally focuses on criteria such as compositional (Fat & SNF) standards and bacteria and somatic cell count, milk producers do not pay much attention to the problems of chemical changes like acidity, accumulation of FFAs in the milk. Cooling, agitation, and pumping of cold milk cause a number of chemical and physicochemical changes in the milk fat system. For example, up to 65-70% of the milk fat crystallizes within 30 min, and crystallization is complete within 2-3 hr at 0-5°C. Prolonged storage of raw milk and use of high-speed pumps, agitators, and blenders has resulted in an increase

in the prevalence of off flavors due to chemical, biochemical, and microbiochemical deterioration.

Air Intake: In milking systems, the milk is mixed with air, especially when air is used as a transport medium for the milk. An excessive absorption of air in the milk. i.e., foaming also stimulates lipolysis *(Jamotte P. 1974).* Foaming in milk can be caused by air absorption into the teat cups, leaking pipeline joints, different pipeline diameters, or large pipeline diameters through which only a small volume of milk is transported and by idling of centrifugal pumps (continuous operation of the milk pump even in case of insufficient milk volume) *(Whittlestone WG. (1968) & Worstorff H. et al., 1972).*

The stability of the Milk Fat Globule (MFG) is lowered by mixing with air or any other gas during pumping or agitation of the milk. The contact between an MFG and an air bubble results in rupture of the MFG, since the membrane material and part of the core fat will spread over the air/milk plasma interface and will be released into the milk plasma when air bubbles collapse or coalesce (Walstra & Jenness, 1984; van Boekel & Walstra, 1989.) The total air admission in the cluster should be between 4 and 12 1/min; however, in automatic milking systems (AMSs), it is often higher.

Increasing air intake has great impact on the formation of FFAs, and even if milk and air are mixed only for a short pumping distance, it causes damage. Exposure of milk to oxygen during blending, mixing, and pumping promotes oxidation of milk fat and development of off flavors from the oxidation products. The free fat concentration in milk was increased 2.6- fold when air was present in the milk processing equipment; the free fatty acid concentration

under the same conditions was increased 4.3-fold (*Aule &. Worstorff H. 1975).*

Pumping

Higher flow velocities during pumping in pipes result in a greater friction in the liquid itself and between the liquid and the pipe wall. These relative differences in flow velocity perpendicular to the flow direction are called shear rates. The shear rate depends on the diameter of the pipe and the flow velocity. When milk is transported through long pipelines at velocities below 1.5m/s, the free fat content is affected only to a very small extent. An increase in free fat by only 0.42% was reported. High flow velocities through constricted pipes (e.g., at samplers), however, led to an increase by almost 10% *(Kirst E. 1980).* Lipolysis can also take place due to foaming developed during transportation of the milk through elevated pipelines in the milk processing plant *(Grosserhode J. 1974*).

The presence of air, the temperature of the milk, and the fat content affect the stability of the MFG during mechanical treatments of milk. The milk temperature is also a very important factor when milk is exposed to mechanical treatments. Several studies have reported that the maximum accumulation of FFAs upon agitation is at a temperature of ~15°C and again after ~30°C, with low formation of FFAs between 20 and 30°C. At low temperatures, the milk fat is more resistant to mechanical stress, since large parts of the triglycerides in the MFGs are crystallized, and this stabilizes the globules against coalescence. One minor factor is that the temperature affects the activity of LPL (lipoprotein lipase). Diets with high levels of saturated fat supplements result in high milk fat production and in MFGs with large average diameters.

These milk types with large MFGs are more susceptible toward coalescence and lipolysis during pumping compared with milk from cows fed a low-fat diet or unsaturated fat supplements. The reason for the lesser stability of large milk globules during pumping is presumably that the surface potential is lower for large globules than it is for smaller globules. Therefore, less energy is needed to coalesce large globules, and at the same time the collision energy is greater for large MFGs.

Cooling and Storage of milk

Cooling of milk also causes important changes in the chemical and physicochemical properties of casein micelles *(Morr 1975; Farrell and Thompson 1974; Brunner 1974; Harper 1976; Reimerdes 1982)* . These changes include release of proteolytic enzymes from micelles, which attack milk proteins and render them susceptible to slow coagulation and incomplete curd formation during cheese manufacture and may also result in flavor and texture defects in cheese and cultured milk products (.

Partial hydrolysis of milk proteins by residual proteolytic enzymes has also been implicated in the age thickening of ultra-high temperature (UHT) sterile milk products *(Harwalker 1982).* Casein micelles in cooled milk undergo partial disaggregation to release β-casein and other casein components that may function as lipolytic enzymes to promote hydrolytic rancidity of the milk fat. This release of casein subunits causes several important changes in the physicochemical properties of the casein micelles. For example, casein micelles undergo increased solvation at 0°-5°C compared to 35-40°C, and they also release inorganic phosphorus upon cooling. Similarly, the calcium content of cooled milk micelles is substantially lowered from the values observed at 35-40°C (Morr 1973). The ratio

of micelle to total casein content in milk is lowered from about 85-95% at 35-40°C to 75-80% at 0-5°C. As a result of these changes, casein micelles reversibly disaggregate from 2-3 μm aggregates at 35-40°C to sizes ranging from 100 to 250 μm at 0-5°C, become more translucent, and are less electron dense upon cooling *(Morr 1973*). Because of these chemical and physicochemical changes in the casein micelles upon cooling of milk, the milk becomes more viscous and displays an increased tendency to foam. Cold milk foams readily. Milk proteins concentrate in the lamellae of the foam where b-lacto globulin acts as a surface active agent. Foams are formed by the preferential adsorption of surface active materials at an air liquid interface with orientation of the material to form an air bubble.

Also, casein micelles in cold milk commonly exhibit incomplete coagulation upon acidification and treatment with rennet. The cold aging of milk increased the rennet coagulation time at 30°C. The increased coagulation time was inversely related to the ratio of colloidal calcium-phosphate, and could be reversed by heating to 40°C for 10 minutes or by addition of calcium chloride to the milk prior to cold aging.

The initial FFA concentration in fresh drawn milk is around 0.15–0.5 mmol per 100 g fat. During cool storage of the milk in the tank, the concentration of FFAs increases. The increase in the concentration of FFAs is largest during the first 24 h after milking. The cooling itself renders plasma proteins to adsorb to the MFG membrane. Hereby, the LPL is brought into contact with the lipids, since it is bound to the caseins. The composition and the structure of the MFG membrane are altered when raw milk is subjected to cooling. Mixing warm fresh milk with cold

milk from the previous milking results in a fast temperature increase before cooling starts again. Temperature fluctuations in milk can cause major increases in the concentration of FFAs.

Crystal structure and size vary as a function of both cooling rate and cooling temperature and regulate the hardness of the milk fat. More fat passes into the solid state by direct cooling than in stepwise cooling. The sensitivity of the fat globule membrane to shear and subsequent release of free fat is greater in milk that has a higher proportion of solid to liquid fat. Thus, milk rapidly cooled, to 0–5°C, is more sensitive to shear damage than that is cooled more slowly and in a stepwise manner.

Storage of milk at 2 to 5°C, both raw and pasteurized, caused an increase in the viscosity of the product which may be related to changes in the protein system, since viscosity is influenced largely by the colloidal components of milk. Probably, conversion of colloidal calcium partly to soluble form may uncoil the casein micelle. The change in viscosity with storage at low temperature (2 to 5°C) was greatest during the first 24 hours and reaches maximum after about 72 hours.

Stirring/Agitation: Excessive agitation of cold milk and incorporation of air during pumping and agitation cause partial removal of the protective milk fat globule membrane, resulting in partially denuded globules that are more susceptible to lipase-catalyzed rancidity, churning, and development of oxidative off flavors. A wrong dimension of the stirring unit in the bulk tank results in inexpedient mechanical treatments of milk, which again leads to churning of the MFGs and thereby susceptibility of fat to lipolysis. Incorporation of air into milk using wrongly dimensioned stirring unit also contributes to the

rupture of MFGs. The problem is presumably largest when the milk volume in the tank is low and the stirring unit therefore touches only the milk surface. At temperatures below 40°C, fat crystals start to form in the fat core of the globule. Upon deformation of the fat globule, such fat crystals can cause local structural changes to the membrane, for example by piercing it. This can lead to fat globule aggregation and partial coalescence (Walstra et al., 1999).

EFFECT OF HEAT ON MILK

Effects of heating on quality and technological properties of milk includes degradation of lactose to organic acids and formation of lactulose, denaturation of whey proteins, destruction of vitamins and enzymes, hydrolysis of proteins and lipids and disturbance of calcium/phosphorus equilibrium. Other effects include cooked flavor and nutritional value loss due to new substances formed by the Maillard reaction, which continues during storage of heated milks *(Elliott et al., 2005)*. Up to ~90°C, heat-induced changes are relatively slow and largely reversible (with the exception of whey protein denaturation), while at temperatures >100 °C, reactions occur rapidly and are irreversible. Caseins lack typical stable secondary and tertiary structures, they are very heat-stable molecules. Milk at its natural pH may withstand heating at 100°C for hours without apparent changes in casein solubility. In contrast, whey proteins are typical globular proteins and, when exposed to high temperatures, undergo unfolding, whey proteins are relatively heat labile, extensive denaturation occurring at 80°C. β-Lactoglobulin is more heat-labile than α-lactabumin as a consequence of its one free sulphohydryl

group, which permits the initiation of autocatalytic disulphide exchange reactions. Upon heating, whey proteins can either self-aggregate or form stable aggregates with casein, mainly involving disulphide bonds. Under the usual conditions of heat processing of milk, whey protein denaturation begins at about 65°C. Summary of heat induced changes are listed in table 3.

Table 3. Summary of effect of heat on the components of milk		
Substance	**Modification**	**Major consequences**
Lactose	Decomposition with formation of organic acids and furfural	Influences growth of lactic acid bacteria Reduces pH Forms ether-soluble material Carmelization
	Decomposition with formation of lactulose, heptulose	Potential nutritional significance
Lactose and proteins	Reaction between aldehyde and amino groups producing brown condense products (Maillard reaction	Reduction in nutritive value of proteins, primarily lysine Browning
Milk Fat	Formation of lactones Formation of methyl ketones	Coconut flavour in concentrated products, contribute the flavour imparted bakery products containing butter
Whey proteins (mainly β-)	Appearance of active SH groups and H_2S Protein denaturation Inactivation of immune globulins	Cooked flavor Reduction in oxidation-reduction potential Production of lipid antioxidation properties Protein aggregation Loss of creaming ability

Whey proteins and casein	Formation of ammonia Concentration and insolubilization of liquid-air interface Formation of complex between K-casein and β-1actoglobulin	Influences flavor Formation of "scum" layer on boiling Assists in stabilization to further heat processes; Improved body in cultured products
Casein	Dephosphorytation, peptide bond cleavage, loss of glyco-macropeptide from K-casein, accompanied by modification in the casein micelle structure. Lysino alanine formation under alkaline conditions	Increased sensitivity of calcium precipitation Coagulation of caseins at high temperature Coagulation of milk Potential antinutritional factor
Minerals	Displacement of the equilibrium of Ca/P soluble to Ca/P insoluble salts Modification of the nature of the surface of the micelles	A factor in forwarming process Precipitation of calcium salts and a reduction in pH Retardation of rennet coagulation Affects stability of the casein micelles

Reference: Alais, C. 1965 & Harper, W. J., and C. W. Hall. 1976

Milk Proteins during Processing: Milk proteins display unique chemical properties that influence milk processing characteristics. During heating and storage, milk proteins are subjected to a number of modifications involving the molecule itself or resulting from interactions with other milk constituents. Both the nature and extent of these modifications are temperature and time-dependent, although other factors, like amino acid sequence and protein conformation, pH, and water activity (a_w), may play a relevant role. The most important non-enzymatic mechanisms involved are related to (1) denaturation, with conformational changes of the protein molecule and irreversible modification of the three dimensional structure, and (2) covalent modifications, in which the side-chains of amino acids participate. Most of the time, these two types of mechanism occur in combination, as covalent changes may imply a change in protein conformation and vice versa, and both affect stability and technological properties of the protein fraction in heated milk products.

The main heat induced changes in milk proteins are

- aggregation of micelles,
- dissociation of κ-casein from the micelles,
- acidification
- dephosphorylation of caseins,
- proteolysis of caseins,
- hydrolysis of the caseins,
- Maillard reaction,
- covalent polymerization of protein, and
- Changes in micellar hydration and zeta potential.

The most important reactions of the milk proteins are those which involve destabilization of the protein micelle. In some cases, these are technologically desirable

reactions, such as the formation of a gel either when the pH of milk is reduced (e.g., manufacture of fermented milks and acid-set cheese- denatured whey protein molecules help in determining the target firmness of the gel and improving its water-holding capacity), or when k-casein undergoes selective proteolysis (e.g., manufacture of Cheddar cheese- whey protein aggregation with casein in milk intended for cheese making progressively results in an increased rennet coagulation time and a weaker gel). Under the correct conditions, acidification may also be used to fractionate the milk proteins (e.g., manufacture of acid casein). In other cases, however, reactions involving destabilization of micelles are technologically undesirable. Examples include the various reactions involving aggregation of casein which occur during age thickening of UHT treated milks and concentrated milks. On heating at the assay temperature (≥120°C), the casein micelles do not disintegrate but appear distorted; at pH values <6.7, the whey proteins denature and complex with the casein micelles, presumably through sulfhydryl–disulfide interchange reactions between κ-casein and the whey proteins, particularly β-lactoglobulin. At pH values >6.7, κ-casein, which plays an essential role in protecting the other caseins against calcium-induced coagulation, dissociates into the serum phase. It appears that on heating, dissociation occurs owing to electrostatic repulsion; at elevated temperatures, colloidal calcium phosphate links and hydrophobic bonds are weakened and are no longer strong enough to prevent the dissociation of κ-casein due to electrostatic repulsion. β-Lactoglobulin is very pH sensitive and thermal behavior of the whey proteins is ruled primarily by the properties of β-lactoglobulin, which are affected by the pH, lactose, sodium chloride, calcium, and other ions. Denaturation is

slower at pH 4 than at pH 6 or 9. β-Lactoglobulin is the most prominent sulfhydryl-containing milk protein. Heat treatment of milk causes a deterioration in flavor related to free sulfhydryl groups of β-lactoglobulin which appears before the protein is completely denatured. Prolonged exposure to heat causes more extensive unfolding of individual protein chains, leading to cleavage of disulfide linkages and exchange reactions with other proteins. Heating of β-lactoglobulin to denaturation allows its sulfhydryl groups to become very active. In-depth structural stability analyses revealed that even though processing of milk imposed little impairment on the secondary structural stability, the tertiary structural stability of whey protein was altered significantly. The following order was derived based on the studies: raw whole > HTST, homogenized, homogenized and pasteurized > skimmed and pasteurized, and skimmed UHT > homogenized UHT. Major whey proteins exhibit thermostability to structural unfolding in the order α-lactalbumin<albumin<immunoglobulin<β-lactoglobulin. However, the thermal unfolding of α-lactalbumin is reversible so that denaturation, as measured by irreversible changes, indicates an order of increasing the thermostability of IgG <serum albumin<β-lactoglobulin< α-lactalbumin.

Heat-induced acidification likely plays an important role in the coagulation of milk proteins, as shown by the fact that milk may be heated for at least 3 h at 140°C without coagulation if the pH is readjusted periodically to its original value. Acidification is likely to directly affect the stability of milk by reducing the micellar zeta potential and hydration, and consequently should promote protein–protein interactions. Other heat induce changes (See table 2) that may result in an increase in protein–protein

interactions include thermal dephosphorylation, deamidation of glutamine and asparagine residues, proteolysis, and the formation of covalent crosslinks.

Table 2. Main effects of various heat-induced non-enzymatic modifications on some milk protein characteristics

Modification	Conformation	Chemical composition
Amino acid racemization	Yes	no
Amino acid isomerization	Yes	Yes
Deamidation	Yes/no	Yes
Dephosphorylation	Yes	Yes
β-Elimination	Yes/no	Yes
Glycation	Yes /no	Yes
Cross-linking	Yes	Yes/no
Oxidation	Yes	Yes

L Pellegrino et al., Effects of Processing on Protein Quality of Milk and Milk Products. Chapter in Encyclopedia of Dairy Science.

At pasteurization temperatures, agglutinins are denatured and this influences the structure of the fat globules and the form of their association in the cream layer of un-homogenized milk. A well-known consequence is the reduction of the cream layer in heat-treated milk, a factor which has been of much importance in the development of pasteurization processes. A secondary effect may be to encourage the formation of cream plug, in which the cream in unhomogenized, heat-treated milk forms an unpleasant solid layer during storage. All sterilized whole and semi-skimmed milks are homogenized to avoid this problem.

Table 4. Functional Characteristics of Milk Proteins		
Functionality	**Casein or Caseinates**	**Whey Proteins**
Hydration, water binding	Very high, minimum at pH 4.6	Water-binding capacity increases with denaturation of the protein
Solubility	Insoluble at pH 4.6	Soluble at all pH levels. If denatured, insoluble at pH 5
Viscosity	High at or above pH 6	Low for native protein. Higher if denatured
Gelation	No thermal gelation except in the presence of Ca+2. Micelles gel with rennin	Heat gelation at 70°C (158°F) or higher; influenced by pH and salts
Emulsifying ability	Excellent at neutral and basic pH	Good except at pH 4–5, if heat denatured
Foam formation	Good overrun. κ-Casein best, followed by β- and αs1-caseins. Poor foam stability	Good overruns. β-Lactoglobulin better than α-lactalbumin
Flavor binding	Good	Retention varies with degree of denaturation

Milk Fat Physical Properties of Milk

Globule Size: Within the wide size range of Milk Fat Globule (MFG), the smallest globules are approximately 100- fold smaller in diameter compared to the largest ones. For the same bulk volume of fat, milk, with smaller MFG will have a higher total number of MFG. Within these milks, the smaller MFG will tend to have greater surface curvature, and a larger surface area/volume ratio, compared to larger MFG. These differences can give rise to marked differences in the physical properties of MFG size-differentiated milk and milk fat as summarized in figure.

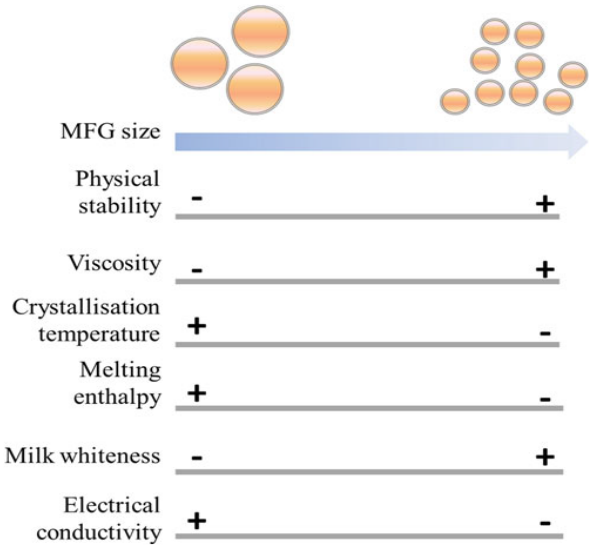


Illustration of impact of milk fat globule size on selected fundamental properties. ("+": increased; "–": decreased)

Reference: Effect of Milk Fat Globule Size on Physical Properties of Milk, Chapter 6, Effect of Milk Fat Globule Size on the Physical Functionality of Dairy Products, Edited by Tuyen Truong et al., 2017, Springer edition.

Physical Stability: fat globules are dispersed in the continuous phase of milk plasma containing casein micelles, serum proteins, sugars and minerals, can be considered as both a colloidal suspension and an oil-in-water emulsion. Within the emulsion, the MFGM maintains the integrity of the lipid droplets and helps to protect them from destabilization *(Walstra et al. 1999)*. However, as a natural oil-in- water emulsion, milk is thermodynamically unstable and readily subject to various forms of physical instability over time, leading to changes of structural organisation or spatial distribution of MFG. These instability mechanisms include gravitational

separation, droplet aggregation, flocculation, coalescence, and partial coalescence, which are governed by three colloidal interactions, i.e. van der Waals attractions, electrostatic repulsion and steric stabilization (Huppertz and Kelly 2006). Creaming phenomena can be prevented by reducing the emulsion droplet size. Indeed, for nanoemulsions (i.e. below 200 nm) in general, Brownian motion can be sufficient to overcome the influence of gravitational force (Tadros et al. 2004; Mason et al. 2006; McClements and Rao 2011), thereby providing enhanced long-term physical stability. However, particles in nanoemulsions can be subjected to sedimentation if they are coated by a thick adsorbed protein layer to such an extent that their overall density is higher than that of water. It is well established that homogenized milks (down to about 0.4 μm) are relatively stable against creaming and it is reasonable to project that the physical stability of MFG may also be enhanced in nanoemulsions. In general, the smaller the fat globule size, the more stable it is.

Viscosity: Viscosity of milk and dairy emulsions is also dependent on MFG size. In homogenised milk, the higher the homogenising pressure, the higher the viscosity. When milk was homogenised from 70 to 245 bar (1015 to 3550psi), there was a corresponding increase in viscosity from 7.1 to 15.0 % (Kessler 1981). It was also reported that smaller MFG size causes a slight increase in (apparent) viscosity (Long et al. 2012; Truong et al. 2014a; Kietczewska et al. 2003). A decrease in MFG size of 3.3 % fat milk from 2.7 to 1.0 μm resulted in corresponding higher viscosities (1.8–1.96 mPa s) (Kietczewska et al. 2003).

Optical Properties & Electrical Conductivity

Turbidity of milk is governed by light scattering from milk components, notably fat globules and casein micelles (Goulden 1958 ; Walstra et al. 2005). Light scattering is stronger with MFG than casein micelles in milk due to a high polydispersity of fat globule size (Walstra et al. 2005). Both fat globule size and fat concentration contribute to light scattering (Goulden 1958 ; Walstra et al. 2005). The color and opacity of milk is attributable to both light scattering and absorbance of visible light. It is known that homogenised milk appears whiter than raw milk (Walstra et al. 2005)..

Milk generally exhibits good electrical conductivity, largely due to the presence of dissociated soluble salts. The presence of milk fat reduces electrical conductivity, due to the poor conductivity of the fat itself, as well as the immobilisation of conducting ions by MFG. Increasing fat content generally leads to a decrease in electrical conductance.

Milk Fat Globular Membrane (MFGM):

The MFGM is subject to changes in composition and structure from the moment the fat globule leaves the mammary secretory cell. Upon milk harvesting and further milk handling, further changes to the MFGM take place. Depending on the type and degree of treatment, this may involve different physico-chemical interactions between various membrane components, the loss of membrane components and/or adsorption of components from the milk plasma *(MM. El-Loly 2011*).

The native membrane that surrounds milk fat globules consists of a complex mixture of proteins, glycoproteins, enzymes, phospholipids, triglycerides, and other minor components *(McPherson, A. V., and B. J. Kitchen. 1983).* MFGM is highly structured and contains unique polar

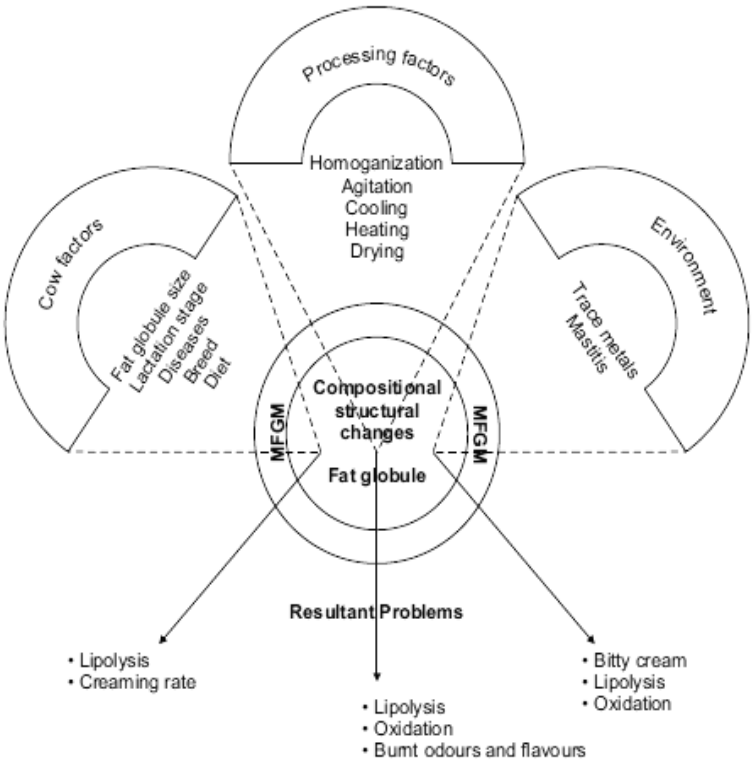
lipids and membrane-specific proteins. Sphingolipids (highly bioactive molecules, mainly present in polar lipids from animal origin) account for up to one third of the MFGM polar lipid fraction. MFGM proteins represent only 1–4% of total milk protein content; this milk fat globule membrane (MFGM) acts as a natural emulsifying agent that prevents flocculation and coalescence of milk fat globules and also protects the fat against enzymatic action (Walstra, P and R Jenness. 1984).

The stabilizing membrane acts as reactive sorts on the interface between the barriers of milk serum. As such, it can be globule and rate-controlling for a host of physical and of enzymes chemical interactions, e.g., binding mace elements; controlled release of the polar materials products of lipolysis; transfer of milk serum; maintenance of emulsion into stability by prevention of globule fusion; availability of fatty acids and cholesterol for micellar absorption in the small intestine; and destabilization by creaming, clumping, churning, freeze-thaw, and heating, resulting in loosely bound substances into milk transfer serum. Worthy of notice is that the interactions are dynamic . Table 5 & Figure indicates the effect of Heating, cooling and ageing of milk on physical changes in the MFGM (*MM. El-Loly 2011).*

Factor	**Effect**	**Comments/ References**
Air bubbles	Substantial loss of membrane material	Walstra & Jenness [1984]
Cooling	Loss of copper Loss of phospholipids	Mulder & Walstra [1974]; Baumrucker & Keenan [1973]; Patton et al. [1980]
Heating	Adsorption of copper, Adsorption of whey	Mulder & Walstra [1974]; Iametti et al. [1997]; Ye et al. [2002];

	proteins, Aggregation of BTN and XO, Loss of PAS 6/7,Loss of phospholipids	Lee & Sherbon [2002]; Koops & Tarassuk [1959]; Greenbank & Pallansch [1961]; Houlihan et al. [1992]
Ageing	The effects depend on temperature	Little is known about how the MFGM is affected by ageing as a function of temperature
Agitation	Depends on the degree of air incorporation. High shear forces are required to change the MFGM in the absence of air	Mulder & Walstra [1974]; Stannard [1975]; Te Whaiti & Fryer [1975]; Miller & Puhan [1986]
Bacterial growth	Production of lipases, Phospholipases, Proteinases and glycosidic hydrolyses may change the MFGM	McPherson & Kitchen [1983]
Stage of lactation – season	Affect the amount of membrane material FG size distribution	Few controlled studies investigating the effects of stage of lactation, season and other factors have been reported in the literature

Table 5. Summary of various factors and their effects on the bovine MFGM after the milk leaves the udder [Evers, 2004].



Summary of various factors affecting the milk fat globule membrane [McPherson & Kitchen, 1983].

Agitation, cooling, natural creaming, and lipolysis during the processing of milk alter the MFGM, but heat treatment and homogenization produce the greatest changes in the MFGM. Heat treatment of milk causes changes in the MFGM by promoting interactions between plasma proteins and native MFGM components *(Dalgleish, & Banks 1991,*

Houlihan et al., 1992, Kim & Flores 1995, Sharma, and Dalgleish 1993). Homogenization of milk causes a reduction of fat globule size and a concurrent increase in the milk fat surface area, which alters the original MFGM because the concentration of native MFGM is insufficient to cover the fat surfaces that are formed during homogenization. *Nordlund and Jleikonen* discussed a theory on the formation of free fat during cooling of the milk. The theory postulates that due to radial solidification of the milk fat, low- melting, mostly non - solidified triglycerides are present in the core of the milk fat globules during cooling. The non-solidified fat occupies a larger volume than the same amount of solidified fat. Thus, the liquid glyceride part in the center of the milk fat globules is subjected to a pressure caused by the inner stress of the molecules. Compressibility of liquid fat is low, and shifts in the crystal structures thus occur in the milk fat globules as well as in the solidified fat layers. This may lead to the destruction of the fat globule membranes. i.e., to the formation of free fat. Therefore milk fat having a higher content of short - chain or unsaturated fatty acids is more sensitive to lipolytic change.

Lactose during Processing: Lactose is the major carbohydrate in milk and its concentration varies with milk yield between 4.2 and 5%. Lactose is a disaccharide and comprises α-D-glucose and β-D molecules. Three solid forms of lactose exist, α-lactose monohydrate, anhydrous α-, and β-lactose.

Lactose is a reducing sugar and undergoes Maillard reactions with amino acids in milk resulting in brownish or burnt colour of milk. Lactose is one of the least soluble of the common sugars, having solubility in water of only 17.8% at 25◦C. This low solubility has consequences

during the production of concentrated milk and frozen dairy products and it is sometimes necessary to induce crystallization to produce a large number of small crystals in order to avoid the "sandiness" defect. Apart from the disruption and formation of disulphide bonds, leading to whey protein denaturation and casein– whey protein bonding, protein glycation via the Maillard reaction (MR), also known as non-enzymatic browning, is mainly involved in heat-induced covalent interactions in heated milk and milk products.

Lactose makes a major contribution to the colligative properties of milk: osmotic pressure, freezing point depression, and boiling point elevation. Lactose, for example, accounts for about 50% of the osmotic pressure of milk. Changes in the lactose content of milk are associated with reciprocal changes in the content of other water-soluble constituents, especially sodium and chloride.

Minerals: The minerals in milk consist principally of bicarbonates, chlorides, citrates, and bicarbonates of calcium, magnesium, potassium, and sodium. Most of the minerals are distributed between a soluble phase and a colloidal phase, as much as 60% calcium and 50% phosphorous may be in colloidal phase. The distribution of calcium, citrate, magnesium, and phosphate between soluble and colloidal phases and their interaction with milk proteins have important consequences for the stability of milk and milk products (See table 6).

Table 6. Partition of Major Minerals in Colloidal and Soluble Phases (% of Total Minerals)		
Mineral	**Colloidal**	**Dissolved**
Calcium	67	33
Magnesium	36	64
Sodium	4	96
Potassium	6	94
Phosphate	55	45
Citrate	6	94
Chloride	0	100
Sulphate	0	100

The minerals are present in a complex equilibrium consisting of colloidal state and soluble state. The ratio of colloidal to soluble state can influence the following characteristics:

- Heat stability and alcohol coagulation of raw milk.
- Aggregate of fat globules during homogenization of milk fat.
- Calcium content of milk influence firmness of curd during cheese making and viscosity of fermented milks.

The citrate concentration, in turn, can affect the soluble calcium content and milk stability. This has consequences for milk processing and may require the addition of anions to complex to ionic calcium, to reduce calcium available for binding to casein and stabilize milk against aggregation.

Vitamins:

It contains the fat-soluble vitamins A, D, E, and K, and the water soluble B-group vitamins B1, B2, niacin, biotin, panthothenic acid, B6, folate and B12, and ascorbic acid

(vitamin C). Processing methods have the potential to alter the stability of vitamins in milk. From a technological point of view, a number of physical and chemical factors may negatively affect nutrients' stability, either naturally present or added to food. Vitamins are sensitive to heat, light, and humidity, as well as oxidizing and reducing agents.

Van Eeklen and Heijne reported that that losses of thiamine vary according to the method of sterilization. With prolonged in-bottle sterilization, 25 to 50% of the thiamine may be lost. When sterilization time was shortened by continuous UHT processing, the loss was reduced (3 to 10%). Ford et al. reported 10% loss of thiamine on processing in direct or indirect heat transfer plants with no further loss on storage for up to 180 days at ambient temperature. Gregory and Burton found negligible loss of thiamine on treatment in eight direct and indirect UHT plants, but a small loss occurred on storage for 45 days. Burton reviewed effects of UHT processes and prolonged storage on stability of vitamins in milk; he found that losses of thiamine varied. Some workers found 15% loss on processing with milk from an early uperization direct heating plant; others found 20% loss with a similar plant. Some plants reported 20% thiamine loss, but there was no difference between direct and indirect heat transfer systems. A loss of 10% was found after 28 days storage with both direct and indirect heating methods. Gorner and Uherova reported the loss of 18% of thiamine from milk during direct UHT sterilization at 140°C for 2 to 3 s.

Riboflavin is stable to heat but rapidly destroyed by light according to some investigators. Van Eeklen and Heijne stated that sterilization caused no loss of riboflavin; others found losses of 5% at most. Ford et al. found 10% loss of

riboflavin with direct and indirect sterilization methods. Gorner and Uherova reported the destruction of 2.4% of riboflavin in milk during direct UHT treatment at 140°C for 2 to 3 s.

Van Eeklen and Heijne reported vitamin C losses of 40 to 60% from in-bottle sterilization and of 30 to 40% from UHT processing. When milk was stored after sterilization, its ascorbic acid disappeared. Ford et al. (5) found complete destruction of dehydroascorbic acid from direct and indirect UHT processes; this represented about a quarter of the original vitamin C content and 20% loss of the reduced ascorbic acid.

Reuter and Hope found that after a holding time of 400 s, 6 to 7% more vitamin C was destroyed at 100 than at 60°C. There was no difference of vitamin C content of milk heated at 130 and 140°C with equal holding time. Mortar and Naudts (10) found mean losses of vitamin C following pasteurization were small (12.8%). Following UHT treatment, mean losses were higher - 17.7% for direct UHT and 31.6% for indirect UHT treatment. In-bottle sterilization caused losses of 50 to 66.5%.GS Haddad and Morrison L observed that processing milk at the four temperature-time heat treatments resulted in no significant loss of thiamine or riboflavin. Losses of these vitamins were not significant during 2 wk frozen storage. Losses of vitamin C from heat processed milks were significant and increased with increased severity of treatment. Vitamin C content of raw and heat treated milks decreased significantly during 2 wk frozen storage.

Table 7 Stability of Vitamins during storage of Milk	
Vitamins A & carotene content	• Vitamin A is stable in pasteurized milk at refrigeration temperatures provided the milk is protected from light, but substantial losses can occur in milk packaged in translucent bottles. (See Table 5 for retention in different packaging materials). • Losses of vitamin A can occur in UHT milk during its long shelf-life at ambient temperatures • Added vitamin A is less stable to light than the indigenous vitamin. • The increased surface area of dried milk products accelerates the loss of vitamin A. 30% loss during 5 Months storage of dried milk at 4-15°C. • Vitamin A stable for up to 6months in sterilised milk at 4-20°C in dark, 50% loss after 6 weeks storage at 38°C.
Vitamin B Complex	• Thiamine(B_1): the storage of pasteurized milk- 10%, UHT Milk stored for 1-2 years: 20-40% • Pyridoxine (B_6): 35-50% in UHT milk during its shelf life. • Cynocobalamine (B_{12}): Losses is significant in UHT milk stored few weeks in ambient temperature and no loss at 7 for 6 months. • Riboflavin (B_2): Liquid milk exposed to light can lose between 20 and 80% of its riboflavin content in two hours, with the rate and extent of loss being dependent upon the light intensity, the temperature and the surface area of the container exposed.
Vitamin D	• Extended exposure to light and oxygen are needed to cause significant losses of vitamin D. • Vitamin D_2 is stable in milk during heat treatments (pasteurization, boiling and sterilization). • Vitamin D_2 was stable during storage at refrigerated temperature (4-7°C for 7days) in glass and plastic bottles, whereas in polyethylene pouches the loss was significantly higher.
Vitamin C	• Losses of ascorbic acid from pasteurized milk, with and without added ferrous lactate, were 35–40 %. • Decrease in vitamin C content after 3 days storage at room temperature represent 35% of the initial value, after 1 month in a 3-layered packaging material 99%, in a 6-layered packaging 51%, and after 4 months in the 6-layered packaging material 75% of the vitamin C degraded in Sterilized and UHT treated fortified milks.

CHANGES IN SENSORY ATTRIBUTES

Effect on Color: There are several independent effects of heat on the color of milk. At relatively low temperatures, below about 50°C, there is a reversible whitening with increase in temperature. This is very slight, and it is doubtful whether it can be perceived by the eye. It can however be measured as a change in the reflectance of the milk: the reflectance of light throughout the visible spectrum, i.e. between 400nm and 700nm wavelength, increases with increase in temperature over the range 5-50°C, and conversely falls again as the temperature falls (Burton,1956). It seems probable that these changes arise from the migration of calcium into the micelle with increase in temperature, so causing an increase in micelle size and an increase in light scattering (Burton, 1955a).

At higher temperatures, above about 60°C, there is an irreversible whitening of milk which increases with time at any temperature (Burton, 1955b)., and also appears to reach higher levels with higher temperatures. The denaturation of the serum proteins of the milk is responsible for this change. It was originally suggested that the denaturation caused an aggregation of the serum proteins into particles having a high ratio of length to breadth (Burton, 1956). It is now known that denatured serum protein associates with the casein in milk, so an alternative possibility is that the size of the casein micelles is being increased by the adsorption of the denatured serum protein.

The whitening effect is of significance in UHT milk and milk products. UHT milk and cream are invariably whiter than the unprocessed product. This effect is quite independent of the fact that UHT products containing fat

are almost invariably homogenized, which gives rise to changes in appearance because of the more uniform distribution of the fat.

It is well known that milk becomes browner with increased severity of heating. The browning is a consequence of the Maillard reaction between the lysine of the milk proteins and the lactose. The Maillard reaction in milk is an extremely complex one, in which colorless intermediate compounds are first produced and are then converted into melanoidins, dark colored compounds which give rise to the brown color. In spite of this complexity, the overall progress of the reaction can be followed by color measurement. The rate of browning increases with pH. Over the normal range for milk, the change in color development with pH is comparatively slight, but an increase in pH to above 7 can cause a marked change. Since browning development takes place in the non-fat part of the milk, the color is more marked in separated milk for the same intensity of heat treatment and less marked in creams, particularly those of high fat content.

Effect on flavor:

Heated flavor: flavor is developed when milk is heated for times and to temperatures which denature the serum proteins, particularly β-lactoglobulin, i.e. when the milk is heated to temperatures above about 70°C. It is associated with the liberation of free -SH groups arising from the denatured β-Iactoglobulin (Hutton & Patton, 1952). The free –SH groups may be oxidized to hydrogen sulphide which is volatile and gives a characteristic smell to freshly heated milk. The level of free -SH groups falls rapidly in the presence of oxygen or in the presence of other oxidizing systems. As the level falls, the intensity of the heated flavor also falls. As its intensity falls, the flavor may change in

character. This is particularly true with separated milk: the changed flavor in this case has been sometimes called a 'degenerate heated flavor' and likened to the smell of boiled cabbage. These changes take place during the first few days of the life of packaged UHT milk.

Sterilized flavor: At higher temperatures, of the order of 90°C and above, the level of free -SH groups begins to fall (Burton, 1959a; Lyster, 1964). A new flavor develops which is characteristic of sterilized milk or milk products. It is unproven, but almost certain, that this flavor is formed as a consequence of the Maillard reaction, as it appears at the same time as the brown color and other measurable products of the reaction. It is stable, and does not decrease in intensity during storage: in fact it increases in intensity during prolonged storage even at room temperature, as does the brown color. Many compounds have been identified in sterilized concentrated milks and in UHT-processed milks, originating from both proteins and fat. Diacetyl, Lactones, Alcohol ketones, Maltol, Vanillin, Benzaldehyde and Acetophenone are might contribute to the sterilized flavor. UHT-processed milk when fresh has a more-or-less strong 'heated' flavor with a sulphurous odour. The odour disappears rapidly and the heated flavor disappears in a few days to leave a characteristic 'UHT' flavor which is not a recognizable 'sterilized' flavor. On storage, this flavor becomes stronger, to become that which is often described as 'stale'. It may be that this is a third flavor in addition to the 'heated' and 'sterilized' types.

It is possible that the characteristic UHT flavor, and the 'stale' flavor which succeeds it during storage, are in fact precursors of the true sterilized flavor. There is, however, some difference between the UHT flavor and the sterilized flavor, because a UHT process with an extended holding

time to give a milk color similar to that of in-container sterilized milk gives a flavor which, although similar to, is clearly distinguishable from sterilized milk of the same color. The brown color and sterilized flavor appear in milk at the same time in a heat treatment process as a fall occurs in the content of the heat-activated -SH groups, and it is possible that the -SH groups play a part in the initial stages of the development of the color and flavor.

Proteolysis/Protein Hydrolysis: Excessive proteolytic activity is undesirable as it hydrolyzes caseins to water-soluble peptides that are lost to varying degrees in whey and not recovered fully during the manufacture of products such as casein or cheese. Moreover, hydrolysis may alter the functionality (e.g., molecular mass, charge) and interactivity of the remaining (recovered) protein and thus the techno-functionality of the resultant products, such as the ability of the resultant cheese to shred or grate, or the ability of casein to hydrate, form gels, or impart structure/texture to products in which it is used as an ingredient (e.g., gluten substitute in bakery products, imitation cheese, and processed cheese products).

Proteolysis occurs with following three enzyme source: native milk proteinase (plasmin), proteinases of somatic cells, and proteinases of psychrotrophic bacteria.

a. **Native Milk Protinases (Plasmin):** native proteinase system of milk comprises plasmin as the active enzyme, its zymogen (plasminogen), and enzyme activators/inhibitors. Whereas plasminogen, plasminogen activator, and plasmin are all very heat stable, the plasmin inhibitor is heat labile. Plasmin and plasminogen in milk fully survive pasteurization at pH 6.8. Plasmin is associated with the casein micelles and readily hydrolyzes αs1-, αs2-, and β-caseins, resulting

in an increase in γ-caseins. κ-Casein can also undergo some degree of hydrolysis by plasmin. The levels of plasmin and plasminogen of milk have been found to increase significantly with advancing lactation. The process of involution involves para-cellular leakage from the blood, assisted by disruption of tight junctions between mammary epithelial cells. It is suggested that there may be a positive correlation between loosening of the mammary tight junctions and plasmin and plasminogen derived activity in milk.

b. Proteinases of somatic cells: The lysosomes of somatic cells in milk are a significant source of proteinases (e.g., cathepsins D and B). The level of cathepsin D in milk is correlated significantly with SCC, which is an indicator of the intensity of the cellular immune defense in cows. Milk SCC generally increases with stage of lactation, a trend that may be associated with a reduction in the milk yield and/or increased infection where late-lactation milk coincides with a deterioration in environmental conditions of the animal (e.g., wet lying conditions). Infection with pathogens such as *S. aureus*, *S. chromogenes, E. coli,* and *S. dysgalactiae* is conducive to the development of mastitis and udder inflammation in the cow. Cellular damage at the site of this infection initiates chemical signals that attract white blood cells to the area of infection. Some of these cells are transferred to milk and, therefore, the SCC of milk increases during mastitis. An increase in SCC of milk is associated with a higher rennet clotting time and decreases in curd firmness, cheese yield, and the percentage fat recovered from milk to cheese. The magnitude of these effects varies with SCC and cheese type. An SCC standard of 400000 cells /ml for bulk milk is adopted in European

milk quality schemes with many milk purchasers now applying bonus payments for milk with 200000 cells ml/1, and this has reduced the effects of mastitis and high SCC on product quality. Effects of mastitis on raw milk and dairy products are listed in table 8.

Product	**Effects**
Cheese	Reduced yields and yield efficiencies Elevated moisture content Increased rennet clotting time Soft cheese and textural defects Higher loss of solids in whey Inferior organoleptic properties
UHT milk	Accelerated age gelation
Pasteurized fluid milk	Reduced shelf life Organoleptic defects
Cultured products	Increased coagulation time Inferior organoleptic properties
Butter	Extended churning times Reduced shelf life Inferior organoleptic properties
Milk powder	Altered heat stability Reduced shelf life
Cream	Altered whipping properties

Table 8. Effects of mastitis on raw milk and dairy products.
Reference: Auldist MJ and Hubble IB (1998) Australian Journal of Dairy Technology 53: 28–36.

c. Proteinases of psychrotrophic bacteria:

Refrigerated storage of milk avoids the mesophillic bacterial spoilage of milk, it favors the growth of psychrotrophic microorganisms, which produce heat-resistant extracellular enzymes such as proteinases and lipases. These proteinases hydrolyze the caseins in milk, to a degree dependent on temperature (2–7°C) and duration of cold storage. The contribution of these enzymes is more when milk quantity is less during lean season where refrigeration storage is extended. The caseins are particularly susceptible to hydrolysis at low temperatures because of the solubilization of colloidal calcium phosphate, lower degree of hydrophobic- induced casein

interactions, loosening of the micelle structure, and the solubilization and dissociation of all caseins, especially β-casein, into the serum phase. Hydrolysis of casein by proteinases from psychrotrophic bacteria is undesirable because of the deterioration in cheese making quality of the milk, especially when counts of the psychrotrophic bacteria are high eg 10,00,000 cfu/ml. Adverse effects include increased rennet coagulation time, reduced curd firmness, higher losses of protein in cheese whey, lower cheese yield, higher cheese moisture, and development of off-flavors such as bitterness and rancidity. The extent of these defects is exacerbated by an increase in the temperature of cold storage in the range 2–10°C, as a consequence of higher levels of bacterial growth and activities of lipases and proteinases, especially during the first 48h. Most proteinases of Pseudomonas can survive heat treatment at 149°C for 10 s and, for example, one proteinase from Pseudomonas is about 4000 and 400 times more heat resistant than spores of *Geobacillus stearothermophilus* and *Clostridium sporogenes*, respectively. This heat resistance and the ability to hydrolyze casein at temperatures as low as 2°C are among the main characteristics of these proteinases. *Pseudomonas aeruginosa* is able to produce an exocellular proteinase that can remain active at 2°C for up to 1 month and can hydrolyze casein at this temperature; most of the proteinases show optimum activity at pH 6.5–8.0.

Lipolysis/ Fat Hydrolysis: milk fat lipolysis in milk leads to elevated levels of FFAs, which are carried over into the products and result in off-flavors (rancid, soapy, and bitter) and flavor inconsistency. This is undesirable in all cheeses, even in those where the cheese making procedure is designed to promote controlled hydrolysis of the milk fat (triacylglycerols) by the addition of exogenous

lipases/esterases and/or lipolytic cultures. Most lipolysis in milk is caused by the native lipoprotein lipase (LPL). LPL acts on the milk fat triacylglycerols, with the resultant production of FFAs, which are carried over to the cheese.

Lipolysis in milk can be broadly described as induced or spontaneous depending on the means of activation of the LPL. Induced lipolysis is defined as lipolysis promoted by both mechanical damage to (homogenization, pumping, and temperature fluctuations) and temperature changes of the milk. Mechanical damage to milk involves disruption of the membrane surrounding the fat globule, thus allowing direct contact between fat and LPL. Such damage may be accelerated by subjecting the milk to mechanical processes and/or temperature cycling (cooling/reheating). Physical actions that promote mechanically induced lipolysis include excessive shear/whipping and cavitation, which may be caused by a number of factors such as over-agitation and pumping, air incorporation, and poor design of milk-handling systems; the resultant damage may be accentuated by freezing and thawing of milk. Milk may be particularly vulnerable to these actions when volumes are low, depending on time of year. Milk as it leaves the cow is at ~37°C. The activity of LPL on milk fat is at a maximum at 30°C and markedly decreases at temperatures >37°C and <12°C. However, change in temperature can also promote the development of lipolysis, for example, cooling to 5°C followed by rewarming to 25–37°C and recooling.

Apart from this, spontaneous lipolysis is defined as that which develops in the milk of some cows during cold storage without being subjected to any physical or mechanical treatment. The major predisposing factors associated with spontaneous lipolysis in the cow are late

stage of lactation, poor quality feed, and mastitis, milking frequencies.

The main biochemical factors include the amount of lipase activity, the integrity of the MFGM, and the balance of lipolysis activating and inhibiting factors. A number of studies have reported that milk from cows in late lactation has a higher FFA level than that from cows in early lactation. This may be due to changes in the lipolytic activity of the milk and/or greater damage to the MFGM, especially when milk volumes are relatively small, making the milk more susceptible to agitation, pumping, and freezing (freezing onto the cooling surface of direct expansion milk storage tanks).

Psychrotrophic bacteria are capable of spoiling milk by biochemically altering the compounds present in milk. Psychrotrophs can cause the decomposition of urea, reduction of nitrate to nitrite and hydrolyses of proteins and lipids at temperatures as low as subzero. During the early stage of growth of psychrotrophic microorganisms, biochemical changes occur at a low level, resulting in a lack of freshness or a stale taste. At the later stages, biochemical transformations gain velocity and aroma and flavor defects become apparent. Development of these off-flavors and odors is usually a result of proteolysis or lipolysis, and both are of major concern to the dairy industry. The heat-stable lipases secreted by Pseudomonas, Acinetobacter, and Moraxella are able to hydrolyze tributyrin and milk fat at both 6°C and 20°C. Fluorescent Pseudomonas species and Flavobacterium and Alcaligenes species are recognized as the most active lipolytic bacteria.

Psychrotrophic bacteria produce heat-stable lipases that survive pasteurization ((HTST-72°C for 15 s treatments.)

and ultra-high temperature (UHT) treatments (UHT (138°C for 2 s). *P. fluorescens, P. mucidolens*, and some strains of *P. fragi* produce lipases that are stable at temperatures as high as 100°C, some strains of *P. fragi, S. aureus, Geotrichum candidum, Candida lipolytica, Penicillium roqueforti*, and other *Penicillium spp.* have been reported to produce lipases that are active at -7°C, -19 and even -29°C.

Even though the bacterial lipase is not inactivated by pasteurization (unlike indigenous LPL), the psychrotrophic bacteria that produced them are destroyed. However, lipolysis associated with psychrotrophic lipases in cold-stored milk is generally only a problem where bacterial counts are very high (>1 X 10^6 to 1X10^7cfu /ml) and not an issue with modern milk production practices, except perhaps where holding times prior to processing are long (eg., >5days).

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"Effect of Processing on Milk Components"

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