CC7;Unit 10

Spoilage of Milk fat (part 2)

By
Dr B.V. Balasubramanyam
Principal Scientist, Dairy Technology Section,
ICAR- National Dairy Research Institute
Adugodi, Bengaluru-560030

In cow milk fat, the triacylglycerols account for about 98% of the total milk lipids. The diacylglycerols, monoacylglycerol and free fatty acids (FFA) are mostly products of lipolysis, and the cholesterol and phospholipids are cellular membrane material which accompanies the fat globule during extrusion from the secreting cell. Chemical changes that takes place in fat content of milk and milk products is of great concern to the dairy industry due to the flavour changes that bring about in the products particularly during storage of fat rich dairy products. There are two changes that the fat undergoes chemical changes. They are hydrolytic rancidity and autoxidation. The end products of these reactions bring about sensory changes particularly the flavour changes in milk and milk products. In the first part on Milk fat we learnt on composition and characterization of milk fat. In the second part we will understand on spoilage of milk fat through autoxidation, hydrolysis and lipolysis. Hydrolytic rancidity is a result of hydrolytic degradation of milk lipids due to action of lipase enzyme on lipids, whereas, autoxidation is the oxidation of milk lipids.

The details of these reactions are covered under the lipolysis of milk fat with following sections:

Hydrolysis of lipids

- Induced lipolysis
- Spontaneous lipolysis

Autoxidation of lipids

- Conditions favoring autoxidation
- Prevention of autoxidation
- Measurement of autoxidation

Lipolysis of Milk Fat

Market milk and some products manufactured from milk may have a flavor which is described as “rancid”. The term denotes the flavour due to the presence of free fatty acids, more notably low chain volatile fatty acids. Development of rancid flavour is due to the accumulation of proper concentration and type of free fatty acids which are released as a result of the hydrolysis of milk fat by the action of lipases which are normally present in milk. In general lipolysis is caused by indigenous or natural lipase enzyme present in raw milk which accounts for the rancidity in milk, while in stored milk and milk products lipolysis produced by microorganisms is of great importance. The enzyme lipase cleaves (hydrolyses) the triglycerides to free fatty acids and glycerol. Generally glycerol is flavorless while the free fatty acids particularly low chain fatty acids possess strong rancid flavour.

Raw cow milk contains a relatively large amount of lipase activity, but seldom sufficient lipolysis takes place to bring about off flavors in milk immediately. Milk when freshly secreted from a healthy cow, has about 0.5µmol of free fatty acids per milk of milk. These acids are the result of incomplete synthesis rather than the lipolysis. The free fatty acids are responsible for the development of off flavours in milk and milk products. However at these levels of FFA in fresh milks do not contribute to any off flavours. Under proper conditions of handling and storage, we can observe only small increase in FFAs. On the other hand, if proper care is not taken during handling and storage, substantial increase in FFAs can be observed due to induced or spontaneous lipolysis. Induced lipolysis results when the milk lipase system is activated by physical or chemical means. Spontaneous lipolysis occurs in milk which has had no treatment other than cooling soon after milking. In general lipolysis is caused in milk by the indigenous (or natural) lipase enzyme preset in raw milk, while in stored products, lipolysis caused by the lipase enzyme produced by microorganisms is of great significance.

A) Induced lipolysis:

The induced lipolysis can take place in milk due to various factors, such as vigorous agitation, homogenization, temperature activation and freezing.

Vigorous agitation: lipolysis in raw milk can be initiated by vigorous agitation. Such treatment disrupts the milk fat globules membrane and renders the milk triglycerides more accessible to the natural milk lipase present in the raw milk. Incorporation of air and consequent foam formation enhances the activation of lipase activity. The amount of lipolysis induced by agitation depends upon the mode of agitation, duration of agitation, and temperature at which it is agitated. Agitation t high temperature induces higher lipolysis activity (30 – 40^0C) than at cold storage temperature (<5^0C).

Homogenization: homogenization of raw milk or cream results in very strong activation of lipolysis. Perceptibly rancid flavour in raw milk can be observed within 5 minutes after homogenization of milk. During homogenization, the protective fat globule membrane which protects the milk fat gets damages and permits ready accesses to lipase to act upon the free fat. Hence the heating of milk to around 60^0C before homogenization is mandatory which inactivates the natural lipase enzyme present in the milk and thus prevents the rancidity defect in the market milk.

Temperature activation: The lipase system can also be activated by cooling freshly drawn milk to 5^0C and rewarming to 30^0C and recooling to 5^0C. No satisfactory explanation for activation of lipase system due to temperature fluctuation is available but changes in the physical state of fat (liquid/solid ratio) have been suggested and damage/alteration of the globule surface and binding of lipoprotein co-factor may also be involved.

Freezing: Freezing and thawing leads to churning of the fat and induces the lipolysis. One of the hypothesis is that during freezing, the fat globules gets ruptured due to squeezing by the hardened ice crystals and during thawing, the free fat oozes out and comes in contact with the lipase enzyme for possible lipolytic activity.

B) Spontaneous lipolysis:

Milk which undergoes lipolysis without being subjected to any of the treatments is referred as spontaneous lipolysis. Spontaneous or cold storage lipolysis is defined as the lipolysis which occurs in some individual milk when cooled to below 15^0C soon after milking. Some of the factors which affect the spontaneous lipolysis are: stage of lactation, feed and nutrition, season, health of the animal particularly mastitis, and yield of milk.

Hydrolysis of Milk Fat: Hydrolytic rancidity can be observed in milk and milk products as a result of pre-or post manufacture contamination by microorganisms. Some microbes are potent lipase enzyme producers which when produced by the microbes, they bring about lipolytic activity in the milk and milk products.

The hydrolytic rancidity is of great importance in ripening of cheese. The lipase enzyme induced by the activity of the lactic bacteria hydrolyses the fat and releases the fatty acids which ate responsible for the development of cheese flavour in the cheese during ripening period. The breakdown of fat also helps in the development of mellow body in the ripened cheese.

The hydrolytic rancidity can be prevented or retarded by giving proper heat treatment to the milk which can inactivate the lipase enzyme. Generally the lipase enzyme gets inactivated when milk of cream heat treated to more than 60^0C for 2 to 3 minutes or 80^0C for 20 seconds. Hygiene on the farm and at the processing units is of importance in controlling the microbial growth and minimizing the microbial lipolysis problems. Once lipolysis is occurred, and free fatty acids are liberated, then there is little that can be done to reduce its effect on quality of food.

Measurement of lipolytic rancidity: the degree of hydrolytic rancidity is measured by quantifying the total free fatty acids. The methods usually involve the initial step of isolation of free fatty acids and estimation of fatty acids by titrating against the known alkali solution. The extent of lipolysis is commonly expressed as ‘acid degree value’ (ADV) of the fat as millimoles of free fatty acids per 100 g fat. ADVs greater than one is undesirable and probably perceptible by taste to most of the consumers. The rancid flavour in milk can be detectable when the Acid Degree Value exceeds 1.2 – 1.5millimoles/litre.

Autoxidation of milk fat

Lipid oxidation in fluid milk and number of its products has been a concern of the dairy industry. Because it is one of the basic chemical reaction that occurs in food that generally results in deterioration in quality of the food. To prevent or to retard this defect low-temperature refrigeration, inert gas or vacuum packing is recommended. The rate of autoxidation is influenced by the complex composition of dairy products, the physical state of the product (liquid, solid, emulsion, etc.) and the presence of natural anti or pro oxidants, as well as processing, manufacturing and storage conditions tend to influence. These factors would also influence composition and percentage of products formed due to autoxidation.

Mechanism

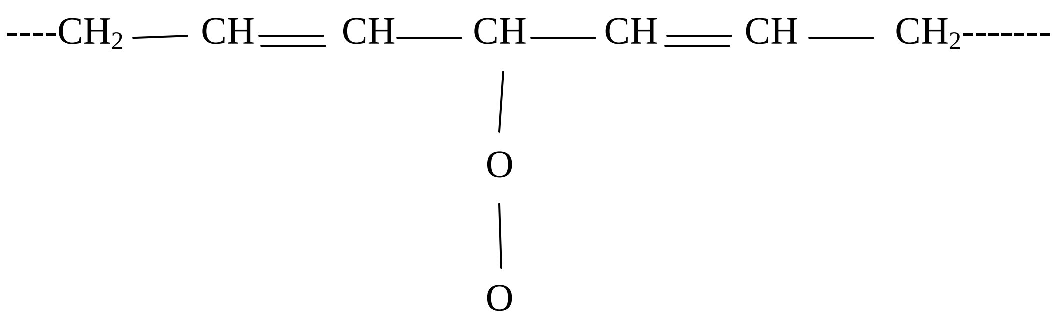
Lipid oxidation is an auto-catalysed free radical Chemical reactions involved autoxidation of milk fat are grouped in to three phases. *viz.* initiation, propagation and termination. The initial step in the autoxidation of unsaturated fatty acids is the formation of free radical. In the case of monounsaturated fatty acids the reaction is initiated by the removal of hydrogen atom from the methylene group of adjacent to the double bond. The resulting free radical stabilized combines with oxygen to form peroxide containing free radicals. These in turn react with another mole of unsaturated compound to produce two hydroperoxides in addition to free radicals capable of continuing the chain reaction. In a polyunsaturated fatty acid methylene groups located other than those located between the double bonds can also involve in these reactions but to a lesser degree. Hydroperoxides formed due to autoxidation being unstable they readily decompose forming the saturated and unsaturated aldehydes. The mechanism suggested for this is that it involves in cleavage of the hydroperoxide to the alkoxyl radical which undergoes carbon - to - carbon fission to form aldehyde. Formation of other products such as unsaturated ketones saturated and unsaturated alcohols saturated and unsaturated hydrocarbons and semialdehydes are also being observed. Saturated and unsaturated aldehydes impart characteristic off-flavours in the products. The terms often used to characterize the flavor are “painty”, nutty, melon-like, grassy, tallow, oily, card board, fishy, cucumber etc.

chain reaction which is normally divided into three phases i.e. initiation, propagation and termination. The initial step involves removal of hydrogen atom from a fatty acid forming a fatty acid free (FA) radical i.e.

$$CH_3\text{-------}CH_2 - CH = CH - CH = CH - CH_2 - COOH$$

Although saturated fatty acids may lose H^+ and undergo oxidation, the reaction principally involves unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA), the methylene, -------CH_2------ , group between double bonds being particularly sensitive. The polar lipids in milk fat are richer in PUFA than neutral lipids and are concentrated in the membrane with several pro-oxidants and are therefore sensitive to oxidation.

The initiation reaction is catalyzed by singlet oxygen (produced by ionizing radiation and other factors), polyvalent metal ions especially copper, or light. The FA free radical may lose a H from a hydrogen donor e.g. an antioxidant resulting in termination of the reaction, or may react with molecular oxygen, forming an unstable peroxy radical



In turn, the peroxy radical may obtain a H from an antioxidant resulting in termination of the reaction, or from another fatty acid forming a hydroperoxide and another FA free radical, which continues the reaction.

The intermediate products of lipid oxidation are themselves act as free radicals and more than one may be formed during each cycle, and hence the reaction is autocatalytic i.e, the rate of oxidation increases with every cycle.

The hydroperoxides are unstable and may break down to various products, including unsaturated polyphenols, which are mainly responsible for the off flavours of the oxidized lipids. The FA free radicals, peroxy radicals and hydroperoxides are flavourless.

Factors favoring the oxidation of milk fat
A range of environmental and physical factors, processing and storage conditions and certain endogenous and exogenous chemical constituents have been shown to influence the rate and extent of lipid oxidation. They are;

Oxygen: the concentration of oxygen in any system is a key parameter in oxidative reaction. Incorporation of oxygen due to agitation and/or presence of dissolved oxygen in milk may i9ncrease the chances of autoxidation of milk fat. Vacuum treatment or packing or replacement of oxygen with any inert gas prevents or retards the autoxidation to a great extent.

Metals: certain metals are pro-oxidants which favors and catalyses the autoxidation of lipids. The principal pro-oxidant metals are copper and iron. Nickel can also catalyze the autoxidation but aluminum, zinc, tin and stainless steel are practically inert to this reaction. The metals may be indigenous to milk coming as a part of co-factors of enzymes such as xanthine oxidase, lactoperoxidase, catalase etc., or enter milk as a contaminant from equipment, water, soil etc. Metals containing enzymes acts as pro-oxidants owing to the metals they contain rather than enzymatically activated the autoxidation. Riboflavin is a potent photosensitizer and catalyzes a number of oxidative reactions in milk i.e. fatty acids, proteins and ascorbic acid.

Light: light is very effective in promoting the off flavour development in milk and milk products. The water soluble riboflavin present in milk acts as a potent photosensitizer and induces photo-oxidation of milk fat. The extent of oxidation of fat depends on the wavelength of light rays, intensity of light and duration of exposure of the milk fat to the light.

Certain processing conditions such as homogenization, churning of cream to butter, exposure of fat to atmosphere at high temperature for long period also induces the off flavour development due to autoxidation.

Prevention of autoxidation of milk fat

Anti-oxidants

Antioxidants are molecules with an easily detachable H atom which they donate to fatty acid free radical or fatty acid peroxy radicals, which would otherwise takeout a H from another fatty acid forming another fatty acid free radical. The residual antioxidant i.e. the molecule without the donated H is stable and the antioxidants break the autocatalytic chain reaction. Milk and milk products have some inherent antioxidants and some are produced during processing of milk.

Tocopherols: Tocopherols generally act as anti-oxidants in lipids. Milk fat contains approximately 20µg of α tocopherol/g. They are nothing but vitamin E. it is soluble in fat medium. Vitamin activity is expressed as tocopherol equivalent (TE), where 1 TE is equivalent to the vitamin E of 1 mg α tocopherol. The biological activity of β, Ƴ – tocopherols and β- tocopherol is 50, 10 and 33% respectively of the activity of α tocopherol. Vitamin E is an effective antioxidant. It can donate hydrogen from the phenolic –OH group to free radical. Thus the vitamin protects the lipids in the body against damage caused by free radicals.

Ascorbic acid (Vitamin C) is present in low concentration in milk. At low concentrations it can act as an anti-oxidant. But at higher concentrations, it acts as pro-oxidant.

The thiol groups of β lactoglobulin and proteins of fat globule membrane are activated by heating. These thiol groups have antioxidant properties but they also produce active oxygen species which could act as pro-oxidants under certain conditions. Some products of maillard reaction are effective antioxidants.

Use of chemical antioxidants: the use of synthetic antioxidants is found to be very effective in controlling the autoxidation of milk fat. Many compounds containing two or more phenolic hydroxyl groups and dihydroquercetin have been employed as anti-oxidants. These compounds exert their influence by interrupting the chain reaction in autoxidation by reacting with the free radicals which otherwise would have resulted in continuous formation of free radicals. Synergists such as polybasic acids such as citric and phosphoric can be used in conjunction with antioxidants. These compounds have no antioxidant properties. But they increase the effectiveness of the synthetic antioxidants. The most commonly used synthetic antioxidants are Butylated Hydroxy Anisol (BHA), Butylated Hydroxy Toluene (BHT), Tertiary Butylated Hydroxy Quinone (TBHQ), and gallic acid ester. In India use of BHT is banned in dairy products by Food Safety and Standards Authority of India (FSSAI) .

The vacuum packing or replacing air by any inert gases (more commonly nitrogen or carbon dioxide) in the packet can prevent or retard the autoxidation to a great extent due to absence of oxygen. .

Measurement of lipid oxidation: in addition to the organoleptic assessment, several chemical/physical methods have been developed to assess the lipid oxidation. These include: peroxide value, thiobarbaturic acid value, UV absorption at 233 nm, and analysis of carbonyls by HPLC. The estimation of liberated iodine from potassium iodate is also used as one of the criterion for estimation of extent of autoxidation.

Conclusion: Fat is a rich source of energy. They are the triglycerides of fatty acids. Free fats are covered by fat globule membrane which protects the fat from hydrolysis. The physical constants of fats is a measure of its purity. The fat is susceptible to hydrolytic rancidity and oxidative rancidity. Hydrolytic rancidity is due to hydrolysis of fat by lipase enzyme. The liberated free fatty acids are responsible for the off flavors in the products. Oxidative rancidity is auto-oxidative in nature which causes liberation of peroxides which is responsible for the oxidative rancidity in fats. The autoxidation of unsaturated fatty acids gives rise to unstable hydroperoxides which decompose to a wide range of carbonyl compounds many of which may contribute to the off flavours in fat rich products. The principal decomposition products of hydroperoxides are unsaturated and saturated aldehydes, with lesser amounts of unsaturated ketones, saturated and unsaturated hydrocarbons, semialdehydes, and saturated and unsaturated alcohols. Milk has some anti-oxidants such as ascorbic acid, α tocopherol and thiol groups which protects the fat from the oxidation during normal course of storage. The metals such as copper and iron, light and dissolved oxygen are pro-oxidants which enhances the autoxidation of milk fat. The fat globule membrane which covers the free fat gives some degree of protection from lipolytic rancidity and from autoxidation.