

Modified Milk Fat and Its Applications in Food Products

Kamal Gandhi*, Prabin Sarkar, Ashok Aghav, Tanmay Hazra, Darshan Lal
Dairy Chemistry Division, National Dairy Research Institute, Karnal, Haryana, India

Abstract

Natural fats have complex composition as they are composed of several fatty acids present primarily as triacylglycerols, which give fat highly diverse functional and nutritional properties. This complexity of fat provides opportunities for modifying themselves for different applications. The negative nutritional and physical image of the fat, especially certain saturated long chain fatty acids, trans-fats, cholesterol as well as poor spreadability and oxidative susceptibility has driven the development of technologies to produce modified fat with different physicochemical or nutritional properties. Modified fats have wide area of application. They can substitute the conventional costlier fats and can also be used in functional foods, as nutraceuticals. Moreover, one can use different fractions derived from single source of fat for different applications. Therefore, modification of fat can be considered as a tool which enhances the functionality and nutritional value of natural fats and broaden their area of application.

Keywords: Triacylglycerols, trans-fats, spreadability, nutritional properties, nutraceuticals

***Author for Correspondence** E-mail: kamalgandhi4444@gmail.com

INTRODUCTION

The milk of major bovine breeds contains 3.5–5% fat which is composed of more than 400 different fatty acids, mainly present as triglycerol [1]. It is widely used in a variety of food products due to many favorable physical, chemical and nutritional properties of milk fat. Milk fat has a large number of fatty acids (> 400) and a very heterogeneous triacylglycerol composition. It has a wide melting range (between about –40 and 40 °C). Triacylglycerols are the major storage forms of energy in animals.

Milk fat is an important nutrient for energy; it provides 9 kcal/gm of milk fat, essential fatty acids (linoleic, linolenic, and arachidonic acids), and fat-soluble vitamins. They also play a very important role in growth and development, disease prevention, and maintenance of good health. It is not only an essential component of human diet but also provides a high level of palatability, flavor, satiety, and mouth feel to food [2].

Why Modification of Milk Fat?

- ✓ To improve physical and nutritional properties of milk fat and to fractionate the milk fat for its application
- ✓ To change the profile of fatty acid, its physical form can be altered and lowering the caloric contribution of fat and to reduce the cholesterol from milk fat
- ✓ To improve its function and nutritional properties, physical property and its application in different industries like chocolate industry, bakery, and dairy industry

Technology Available to Modify Milk Fat

1) Physical modification of milk fat

- A) Tempering
- B) Texturization/crystallization
- C) Fractionation
 - a) Dry fractionation
 - b) Solvent fractionation

2) Chemical modification

Two widely used chemical methods are:

- a) Hydrogenation
- b) Inter-esterification
- 3) Enzymatic modification:

It includes “enzymatic inter-esterification of milk fat.”

4) Cholesterol Reduction:

- a) Supercritical fluid extraction and treatment with adsorbents
- b) Distillation processes
- c) Treatment with enzymes

1) *Physical Modification of Milk Fat*

Physical modification of milk fat by fractionating milk fat or by blending milk fat or milk fat fractions with other oils and fats results in products with an altered triacylglycerol composition, but one in which the fatty acids in milk fat maintain their original position in the triacylglycerol molecules [3]. Other methods of physical modification can involve changes in the texture of the milk fat solely by application of physical treatments to the fat without changing the triacylglycerol composition.

This involves:

- A. Crystallization
- B. Fractionation
 - a) Dry fractionation
 - b) Solvent fractionation

A) Crystallization: In the Alnarp cream crystallization process and variations thereof, cream is cooled rapidly to a temperature between 8 and 4 °C to initiate crystallization prior to butter-making to produce softer butter. After a short holding time, the cream is warmed to about 20 °C. These treatments alter the physical and nutritional properties of fat. High melting triacylglycerols crystallize; they are then cooled to the churning temperature for further crystallization of the mild-melting of triacylglycerols. It is then held, typically, overnight before churning [4].

B) Fractionation: Milk fat has a large number of fatty acids (> 400) and a very heterogeneous triacylglycerol composition. It has a wide melting range (between about -40 and 40 °C). Milk fat lends itself to separation into a series of fractions with different chemical compositions and physical properties and this broadens its application range [5].

Fractionation techniques examined include

- a) Dry fractionation.
- b) Solvent fractionation.

Fractionation techniques offer opportunities for more enhanced and permanent modifications to physical properties.

a) Dry Fractionation: Dry fractionation involves melting the milk fat, controlled cooling and crystallization of molten milk fat while cooling to or at a desired temperature and separation of crystals from the liquid phase. The process is attractive because of its simplicity, relatively low cost and ability to select between fractions based on the melting or functional properties of the fats, which is usually the reason for fractionation. It does not involve the use of solvents, detergents or other additives and furthermore, the desirable flavor is not lost although they are partitioned differently between various fractions. Dry fractionation is the most commonly used method in industry for fractionation of milk fat.

The characteristics of fractions obtained are affected by many factors, including the equipment design, the associated process, the initial temperature of the molten fat, the crystallization conditions (e.g., degree of initial super cooling), the rate of subsequent cooling and agitation after crystallization commences, the final temperature of fractionation and the method used to separate the fractions. Commercially, separation is mostly performed by vacuum filtration on belt or drums filters or by pressure in membrane filters [6].

Membrane filters have a much better separation efficiency, and oil entrainment is reduced to 40–45%. New centrifugal systems such as a filter or worm centrifuge and nozzle centrifuges [7] are becoming more popular in dry fractionation. The separation efficiency of the filter centrifuge is comparable to the membrane filter. Further advantage of centrifuges include enclosed, hygienic and continuous operation.

b) Solvent Fractionation: Milk fat can be separated into fractions with different melting points by crystallization in an organic solvent. Many studies have examined crystallization of milk fat in solvents such as acetone or ethanol and other solvents including hexane, pentane, ethyl acetate and isopropanol. The properties

of fractions crystallized from solvents have been summarized by Ref. [5]. The phase behavior and crystallization kinetics of the milk fat are dependent on the choice of solvent used for fractionation. Larsen and Samuelson [8] examined the use of acetone for fractionation of milk fat and suggested that the use of polar solvents has advantages over the use of non-polar solvents.

Fractionation can be used to obtain groups of TAGs with distinct chemical and physical properties and altered rheological characteristics. For example, the spreadability of butter can be improved by fractionating milk fat and then recombining the fractions in various proportions [9].

The very high melting TAGs in milk fat seem to provide structural integrity in recombined butters, while the lower melting fractions (LMFs) serve to reduce hardness [9]. Increasing the proportion of LMF in butter increases the levels of short-chain fatty acids and oleic acid [10]. Butters enriched in the high melting TAGs have a higher solid fat content and viscosity at higher temperatures. This translates into improved structural stability and a reduced tendency for oiling of and moisture migration [11]. The cost of fractionation can be prohibitive [12] although, from a functional standpoint, fractionation is a good method for improving the physical properties of butter [11, 13]. Milk fat fractions may also have applications in pastry-making,

as in chocolate bloom inhibitors [14] as butter flavor-rich concentrates [15], or to improve the rheology of reduced-fat cheese curds [16].

1) Chemical Modification

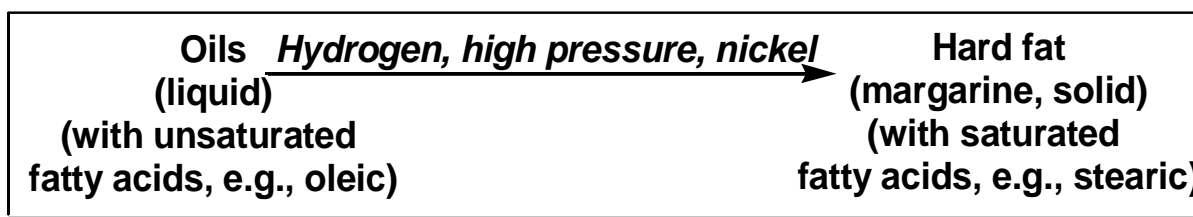
Most widely used chemical methods are:

- a) Hydrogenation
- b) Inter-esterification

a) Hydrogenation

It is a type of addition reactions accepting hydrogen at the double bonds of unsaturated fatty acids. The hydrogenation is done under high pressure of hydrogen and is catalyzed by finely divided nickel or copper and heat.

Hydrogenation involves the addition of hydrogen atoms to the double bonds in the fatty acid chain. The process is carried out at a high temperature in the presence of a catalyst. Hydrogenation reduces the degree of unsaturation of fat and increases its hardness. Hydrogenation of milk fat improves its oxidative stability [17], an effect that is to be expected because of the decrease in unsaturation. Hydrogenation of milk fat to different extents enables the production of fats with higher melting points and blends of these can be made to suit specific end-uses [18]. It is unlikely that hydrogenation will be used in the industry because making milk fat more saturated is not desirable from a nutritional viewpoint. Hydrogenation increases the level of trans-fatty acids, which are of concern from a nutritional point of view [7].



b) Inter-esterification

Inter-esterification involves the exchange of fatty acids within and between TAGs. It can be used to produce fats and oils with desired functionalities, including health benefits. Milk fat with improved cold-spreadability has been produced successfully using chemical inter-esterification [19]. Enzymatic inter-esterification can also be used to produce milk

fat with altered chemical and melting properties [20]. Unfortunately, when milk fat is inter-esterified, its butter flavor is typically reduced [21]. Inter-esterification may be useful in incorporating healthful fatty acids, such as conjugated linoleic acid, into milk and other fats [22]. The re-distribution of the fatty acids results in modification of the physical properties and nutritional properties of fat

[23]. Chemical catalyst such as sodium alkoxide, sodium potassium alloy, metallic sodium and sodium hydroxide are used for the purpose.

2) Enzymic Modification of Milk Fat

Lipases may be used to lipolyse milk fat to produce dairy flavor enhancers or for inter-esterification of milk fat systems to produce milk fat with improved nutritional or physical properties. Lipases may be used with or without an organic solvent in the system [24]. Lipases can be classified into groups that reflect their specificity. The action of the lipase, its stability and rate of reaction are influenced by many factors, including temperature, pH, type of solvent, water activity and whether it is in an immobilized or free form [24]. Liquid butter oil by itself can act as a solvent as well as a substrate and inter-esterification is enhanced in the presence of an organic solvent such as hexane [25].

Enzymic Inter-esterification of Milk Fat

Inter-esterification of milk fat has been carried out by various free and immobilized lipases in both solvent and solvent-free systems. Safari *et al.* [26] examined the inter-esterification of milk fat by the lipase from *Rhizomucor miehei* in various organic solvents (hexane, hexane:chloroform (70:30, v/v), and hexane:ethyl acetate (70:30, v/v)). The addition of chloroform or ethyl acetate to hexane increased lipase activity. It was suggested that the polarity of the solvent influences the partitioning of water in the system with consequent effects on enzymic activity. Bornaz *et al.* [27] examined lipase-catalyzed inter-esterification of milk fat in a solvent-free system. It was found that the use of a 1,3 specific lipase from *R. miehei* in a stirred batch reactor to effect inter-esterification raised the solid fat content at 20 °C from 21 to 46% after 48 h. Others have used enzyme treatment of milk fat to produce modified milk fat with improved nutritional properties. *R. javanicus* lipase immobilized onto hydrophobic hollow wares was found to have a reduced specificity towards short-chain fatty acids [28].

3) Cholesterol Reduction

Cholesterol is present in milk at a level of 0.25–0.46%. The interest in removing cholesterol from milk fat has been driven primarily by consumer concern about the

possible link between cholesterol and heart disease. A marketing position has been created for low-cholesterol products and this has spurred interest in examining alternate ways of cholesterol removal [29]. A number of physical, chemical and biological processes have been used to reduce the level of cholesterol in milk fat [30]. Three such processes are:

- a) Supercritical fluid extraction and treatment with adsorbents
- b) Distillation processes.
- c) Treatment with enzymes.

a) Supercritical Fluid Extraction

Supercritical CO₂ extraction may be used in batches or continuous systems to fractionate anhydrous milk fat into fractions with specific properties in order to enhance its utilization [31, 32]. Supercritical fluid extraction using CO₂ provides an alternative to the use of chemical solvents for the fractionation of milk fat. This fractionation technique exploits the solubility of components near the critical point of CO₂ and the modulation of solubility using small changes in temperature and pressure. In supercritical fluid extraction, milk fat is fractionated into different streams selectively enriched in short-chain, medium-chain or long-chain triacylglycerols. The processing conditions for fractionation of milk fat may be varied to obtain different fractions.

Supercritical CO₂ Extraction for the Removal of Cholesterol: Careful manipulation of process conditions is necessary to obtain efficient removal of cholesterol. When supercritical CO₂ extraction is used to fractionate milk fat, the liquid fraction becomes enriched in cholesterol and the solid fraction is reduced [33].

Process Conditions: The efficiency of supercritical CO₂ for removing cholesterol is temperature and pressure-dependent. Removal of about 90% cholesterol from milk fat was achieved using bench-scale supercritical CO₂ extraction using an ascending pressure profile [34]. With multistage supercritical CO₂ extraction, more than 90% cholesterol can be removed from milk fat [35].

Use of Adsorbents during Supercritical CO₂ Extraction: The use of adsorbents in conjunction with appropriate conditions for

supercritical CO₂ extraction enhances the efficiency of cholesterol extraction. Selective removal of 97% of cholesterol has been achieved with the use of silica gel as an in-line adsorbent [36]. Removal of 96% of cholesterol in milk fat fractions can be achieved by a combined supercritical CO₂ extraction and an alumina adsorption process [37].

b) Distillation Processes

Distillation processes exploit the low volatility of cholesterol compared to the major triacylglycerols of milk fat for removal of cholesterol. Vacuum and short-path molecular distillation processes can efficiently remove cholesterol but it may be achieved at the expense of removing some low-molecular-weight triacylglycerols and flavor components of the milk fat. Vacuum steam distillation is commonly used for refining fats and can also be used to refine milk fat. Cholesterol-reduced milk fat, which was produced by steam distillation, has been used successfully to formulate butter, cream and ice cream [38]. If the flavor of milk fat is to be preserved, the flavors can be trapped and re-incorporated into the milk fat that has been stripped of cholesterol [39].

c) Treatment with Enzymes

Alternatively, enzymes may be used to convert cholesterol into other products [39]. Enzyme systems that have been examined include:

- (1) Cholesterol reductase, which converts cholesterol to coprostanol, a product that is poorly adsorbed by the body, and
- (2) Cholesterol oxidase, which oxidizes cholesterol to non-steroid compounds.

Applications of Modified Milk Fat

1. Cold-Spreadable Butter

Milk fat fractions, obtained by fractionation from the melt or from solvent solution, can be combined with other fractions or with anhydrous milk fat to produce butters with improved spread ability at refrigerator temperature. Butter spreadability can be improved by an increase in the total unsaturated content of the fat or by the removal of the middle melting fraction, both of which contribute to decreased plasticity and spreadability at refrigerator temperature (5 °C). One approach is to simply combine high-melting and low-melting fractions. In this

approach, increasing the percentage of high-melting fraction results in less spreadable butter at refrigerator temperature. Conversely, as the percentage of low-melting fraction is increased, cold-spreadability improves, but the butter becomes too soft at room temperatures (20 °C). As a consequence, a simple combination of high- and low-melting fractions does not result in butters with fully functional properties over the temperature range of typical patterns of home usage. Therefore, because requirements for a fully functional cold spreadable butter require spreadability at refrigerator temperature and maintenance of structural integrity at room temperature, additional strategies are required to provide these characteristics in a consumer product that can be used to distinguish it. Good cold spreadability is exhibited in fats that are approximately 30 to 40% solid at 5 °C and 7 to 15% solid at 25 °C. The selection of milk fat fractions to improve butter spreadability is a good illustration of the use of SFC to predict the functionality and behavior of milk fat fractions in a food product.

2. Dairy Foods

Traditional dairy foods can also benefit from the use of modified milk fats. Researchers have reported that high-melting milk fat fractions increase the stability of whipped cream at fat contents as low as 10%. Milk fat fractions, generally of the low to middle-melting range, are used commercially in Europe for ice cream manufacture [40]. The low-melting milk fat fraction produces higher fat destabilization than the high melting fraction, which is a desirable feature for proper ice cream structure [41]. Several studies have been performed on use of high-melting milk fat fractions in different cheeses. Promising results were obtained with cheese spreads and processed cheese [42]. The potential for the use of milk fat fractions (high melting and low melting) in low-fat cheeses is being actively considered.

3. Chocolate Industry

The first use of milk in a solid chocolate product is commonly attributed to Daniel Peter in 1875. Before this, combinations of milk and cocoa solids were consumed as beverages during the eighteenth century. Much has changed since Daniel Peter's time with respect

to the technologies used to process both milk ingredients and chocolate. The fat phase affects the rheological properties of fluid chocolate, release from the mold, snap, gloss, prevention of bloom, melting properties and flavor release [43]. Adding increasing amounts of milk fat alters the physical and functional properties of chocolate including hardness, ability to temper and melting point.

Hydrolyzed milk fats have been produced to enhance the buttery flavor of milk chocolate [44] and of different components in chocolate; the fat phase has the greatest influence on its quality. The fat phase affects the rheological properties of fluid chocolate, release from the mold, snap, gloss, prevention of bloom, melting properties and flavor release [43]. Milk fat and cocoa butter are the two main forms of fat used in chocolate manufacture to provide these properties.

Many different compounds are responsible for the flavor of milk fat and varying their individual proportions alters its overall flavor. Some of the important classes of compounds that are present are free fatty acids, lactones, methyl ketones and esters. One of the most important functions of milk fat in chocolate is its role as a flavor precursor. The fats in dairy ingredients can provide flavors by a number of mechanisms that occur during the manufacture of chocolate. These include hydrolysis or lipolysis, dehydration and decarboxylation. Although lipolysis is considered to be undesirable in most dairy products, it can be used to advantage in milk chocolate. Fresh milk contains lipases, which hydrolyze the triglyceride molecules and release fatty acids including butyric, capric and capric acids. These volatile flavorful fatty acids can impart a "buttery," "creamy" flavor in milk chocolate. Hydrolyzed milk fats have been produced to enhance the buttery flavor of milk chocolate [44].

Milk fat alters the temperatures at which the various crystal forms of cocoa butter occur. Milk fat also tends to slow the rate of cocoa butter crystallization in mixtures of cocoa butter and milk fat and this is expected to occur in chocolate-containing mixtures of these two fats [43]. Adding increasing amounts of milk fat alters the physical and functional properties of chocolate including

hardness, ability to temper and melting point. The incompatibility of milk fat and cocoa butter at levels greater than this is not of practical concern to chocolate manufacturers because the level of addition of milk fat is usually limited to 30% of the total fat. At levels of milk fat addition above 30% of the total fat, chocolate becomes soft because more liquid fat is present at these very-high proportions of milk fat. Most commercial milk chocolate products are made with a ratio of milk fat to total fat of between 12 and 32%. A general guideline is that in order to achieve the desired physical properties of chocolate, a minimum solid fat content of 45% is required. Fat bloom in chocolate is often characterized by the loss of gloss and dulling of the chocolate surface due to the presence of grey/white clusters that have the appearance of mold [45]. There are a number of ways of preventing bloom, including the use of milk fat as a bloom inhibitor. Most of the work on bloom inhibition has been conducted on dark chocolate, in which bloom is more obvious on the dark background and in which milk fat levels are usually lower. The addition of 1–2% milk fat to a dark chocolate formulation is able to delay bloom formation [43]. There are various theories on how milk fat prevents bloom. Milk fat crystallizes in a solid formation with cocoa butter and the triglycerides that are unique to milk fat prevent or slow down the transformation of the form V crystal structure of cocoa butter to form VI [43]. When chocolate crystallizes more slowly, as happens when milk fat is present, microscopic cracks are less likely to occur within it [46]. A further theory claims that milk fat inhibits bloom by maintaining a solution in which the unstable forms of cocoa butter are held. Higher melting fractions (HMFs) of milk fat are more effective in preventing bloom than non-fractionated milk fat. Lohman and Hartel [47] had shown that HMFs, in chocolate containing 30% of the fat as milk fat, inhibit bloom.

4. Bakery Industry

The applications of milk fat are extended by fractionation. The solid fraction had extended and improved plasticity like in commercial vanaspati and could find application in baking, confectionery, etc. The liquid fraction had a lower solid-fat content at ambient temperature and could be used as a cooking oil or as salad

oil. Milk fat may be made harder by blending with high melting triacylglycerols or fats. Blending of milk fat with tripalmitin has been suggested to make it more suitable for applications. Blending milk fat with 10–20% of tripalmitin is an alternative to the use of hard milk fat fractions to increase the mechanical properties of coatings [48].

Pastry fats are an important application for modified milk fats. For this application, the fat must be plastic enough to be rolled in between layers of dough without breaking, yet be firm enough to avoid melting and absorption into the dough.

Unmodified milk fat does not perform well in this application; however, higher melting milk fat ingredients that are manufactured by crystallization from melted milk fat have been shown to be excellent pastry fats.

Pastry fats based on milk fat fractions generally impart a desirable butter flavor to the pastries compared with those made with pastry margarines. Milk fat fractions are also used to manufacture cakes and cookies. Low-melting milk fat fractions inhibit fat bloom in shortbread and Danish butter cookies and, at the same time, impart a very desirable buttery flavor [49].

Cake and cookie applications result in softness and moistness from interactions between fat and air, water, and solids. Fat-air interactions are readily illustrated by creaming of fat to incorporate air and to increase the volume of the product.

Fats used for cookies generally have melting points lower than other pastry fats, which provide a melting-in-the-mouth quality for these products. Currently, the information on interactions of milk fat fractions with other food constituents, such as air, water, and solids, is qualitative and limited. Increased understanding of these interactions would allow for more knowledgeable tailoring of milk fat and increase its use in bakery applications.

Advantages of using milk solids in bread production:

There are several advantages by adding milk fat fraction in the bread dough:

- 1) Increased dough strengthening
- 4) Better crust color
- 5) Better grain and texture
- 6) Increased loaf volume
- 7) Better keeping quality
- 8) Better nutrition

CONCLUSIONS

Modified fats have wide area of application. They can substitute the conventional costlier fats and can be used in functional foods, as nutraceuticals. Moreover, one can use different fractions derived from single source fat for different applications. It helps in incorporation of fatty acids of interest. Therefore, modification of fat can be considered as a tool which enhances the functionality and nutritional value of natural fats and broadens their area of application. The dry fractionation appears to be dominant modification process due to its simplicity and absence of chemicals and enzymes. Modification of fat helps in improving/altering physical and nutritional properties like by hydrogenating the fat, the physical property, i.e., hardness is improved. Furthermore, we can also improve nutritional value of fat by inter-esterifying essential fatty acid like arachidonic acid. By esterifying low caloric fatty acid, the calorific value of TAG can be reduced.

REFERENCES

1. RG Jensen. The composition of bovine milk lipids: January 1995 to December 2000. *J Dairy Sci* 2002; 85:295–350p.
2. J McGrady. More-healthy fats and oils. *Food Technol.* 1994 Nov; 148p.
3. KE Kaylegian. The production of specialty milk fat ingredients. *J. Dairy Sci.* 1999; 82:1433–1439p.
4. BD Dixon. Investigations into the commercial application of the modified Alnarp cream cooling method. *Aust. J. Dairy Technol.* 1969; 24:50–56p.
5. KE Kaylegian, RC Lindsay. Milk fat usage and modification. In: *Handbook of Milk fat Fractionation Technology and Application.* AOCS Press, Champaign, Illinois: 1995; 1–18p.

6. D Illingworth. Fractionation of fats. In: AG Marangoni, SS Narine (Eds.). *Physical Properties of Lipids*. New York, NY, USA: Marcel Dekker Inc: 2002; 411–448p.
7. RE Timms. Fractional crystallization – The fat modification process for the 21st century. *European Journal of Lipid Science and Technology* 2005; 107:48–57p.
8. NE Larsen, EG Samuelson. Some technological aspects on fractionation of anhydrous butter-fat. *Milchwissenschaft*. 1979; 34:663–665p.
9. KE Kaylegian, RC Lindsay. Performance of selected milk fat fractions in cold spreadable butter. *J. Dairy Sci.* 1992; 75:3307–3317p.
10. S Kulkarni, MK Rama Murthy. Relation between properties of fat and rheological characteristics of butter. *Indian J. Dairy Sci.* 1987; 40:232–237p.
11. A Shukla, AR Bhaskar, SSH Rizvi, et al. Physicochemical and rheological properties of butter made from supercritically fractionated milk fat. *Journal of Dairy Science*, 1994; 77:45–54p.
12. S Gupta, JM DeMan. Modification of rheological properties of butter. *Milchwissenschaft*. 1985; 40: 321–325p.
13. PK Pal, DK Bhattacharyya, S Ghosh. Modifications of butter stearin by blending and inter-esterification for better utilization of edible fat products. *Journal of American Oil Chemist Society* 2001; 78:31–36p.
14. KE Kaylegian. The production of specialty milk fat ingredients. *Journal of Dairy Science* 1999; 82:1433–1439p.
15. AR Bhaskar, SSH Rizvi, C Bertoli. A comparison of physical and chemical properties of milk fat fractions obtained by two processing techniques. *Journal of American Oil Chemist Society*, 1998; 75:1249–1264p.
16. M Rosenberg. Applications for fractionated milk fat in modulated rheological properties of milk and whey composite gels. *Australian Journal of Dairy Technology*, 2000; 55:56–60p.
17. RN Mukherjea, JG Leeder, SS Change. Improvement of keeping quality by selective trace-hydrogenation and winterisation. *J. Dairy Sci.* 1966; 49:1381–1385p.
18. A Huyghebaert, H de Moor, J Decatelle. Milk fat chemical modification. In: KK Rajah, KJ Burgess (Eds.). *Milk Fat: Production, Technology and Utilization*. Society of Dairy Technology, Huntingdon: 1986; 44–62p.
19. J.B Mickle, RL Gunten von, RD Morrison. Rearrangement of milk fat as a means of adjusting milk fat like products. *J. Dairy Sci.* 1963; 46:1357–1361p.
20. P Kalo, P Parviainen, K Vaara, et al. Changes in the triglyceride composition of butter fat induced by lipase and sodium methoxide catalyzed inter-esterification reactions. *Milchwissenschaft*. 1986; 41:82–85p.
21. DH Kleyn. Textural aspects of butter. *Food Technol.* 1992; 46:118–121p.
22. HS Garcia, JM Storkson, MW Pariza, et al. Enrichment of butter oil with conjugated linoleic acid via enzymatic inter-esterification (acidolysis) reactions. *Biotechnol. Lett.* 1998; 20:393–395p.
23. E Frede. Physical and chemical modification of milk fat, 2.3 Inter-esterification. In: *Monograph on Utilizations of Milkfat, Bulletin*, No. 260 International Dairy Federation, Brussels: 1991; 12p.
24. D Rousseau, AG Marangoni. The effects of inter-esterification on the physical properties of fats. In: AG Marangoni, SS Narine (Eds.). *Physical Properties of Lipids*. Marcel Dekker, New York. 2002; 479–564p.
25. P Lee, HE Swaisgood. Modification of milk fat physical properties by immobilized *Pseudomonas fluorescens* lipase. *J. Agric. Food Chem.* 1997; 45:3343–3349p.
26. M Safari, S Kermasha, F Pabai. Inter-esterification of butter fat by lipase from *Mucor miehei* in organic solvent media. *Food Biotechnol.* 1993; 7:265–273p.
27. S Bornaz, J Fanni, M Parmentier. Limit of the solid fat content modification of butter. *J. Am. Oil. Chem. Soc.* 1994; 71:1373–1380p.
28. VM Balcao, FX Malcata. Inter-esterification and acidolysis of butterfat with oleic acid by *Mucor javanicus* lipase: changes in the pool of fatty acid residues. *Enz. Microb. Technol.* 1998; 22:511–519p.

29. E Schlimme. Removal of cholesterol from milk fat. *Eur. Dairy Mag.* 1990; 4:12–13, 16–21p.
30. A Boudreau, J Arul. Cholesterol reduction and fat fractionation technologies for milk fat: an overview. *J. Dairy Sci.* 1993; 76:1772–1781p.
31. J Arul, A Boudreau, J Maklouf, et al. Fractionation of milk fat by supercritical carbon dioxide. *J. Food Sci.* 1987; 52:1231–1236p.
32. AR Bhaskar, SSH Rizvi, JW Sherbon. Anhydrous milk fat fractionation with continuous countercurrent supercritical carbon dioxide. *J. Food Sci.* 1993; 58:748–752p.
33. JA Arul, A Boudreau, J Makhlouf, et al. Fractionation of anhydrous milk fat by short-path distillation. *J. Am. Oil. Chem. Soc.* 1988a; 65:1642–1646p.
34. RL Bradley, Jr. Removal of cholesterol from milk fat using supercritical carbon dioxide. *J. Dairy Sci.* 1989; 72:2834–2840p.
35. Anon. Supercritical extraction holds promise for cholesterol-free butter. *Food Eng.* 1989; 61:83–84, 86p.
36. W Huber, A Molero, C Pereyra, et al. Dynamic supercritical CO₂ extraction for removal of cholesterol from anhydrous milk fat. *Int. J. Food Sci. Technol.* 1996; 31:143–151p.
37. RS Mohamed, GBM Neves, TG Kieckbusch. Reduction in cholesterol and fractionation of butter oil using supercritical CO₂ with adsorption on alumina. *Int. J. Food Sci. Technol.* 1998; 33:445–454p.
38. BG Schroder, RJ Baer. Consumer evaluation of reduced-cholesterol butter. *Food Technol.* 1991; 45(10):104–107p.
39. A Boudreau, J Arul. Cholesterol reduction and fat fractionation technologies for milk fat: an overview. *J. Dairy Sci.* 1993; 76:1772–1781p.
40. HD Goff, RW Hartel. *Ice Cream*, 7th Edn. New York: Springer. 2013. (Published in February, 2013: the newest edition of the long-standing Arbuckle Ice Cream text and a complete revision of our 6th ed.)
41. HD Goff, WK Jordan. The action of emulsifiers in promoting fat destabilization during the manufacture of ice cream. *J. Dairy Sci.* 1988.
42. MA Thomas. The use of hard milk fat fraction in processed cheese. *Aust. J. Dairy Technol.* 1973; 28:77–83p.
43. RE Timms. The phase behavior and polymorphism of milk fat, milk fat fractions and fully hardened milk fat. *Aust. J. Dairy Technol.* 1980; 35:47–53p.
44. LB Campbell, SJ Pavlasek. Dairy products as ingredients in chocolate and confections. *Food Technol.* 1987; 41(10):78–85p.
45. JM Aguilera, G Mayor, M Michel. Fat migration in chocolate: diffusion or capillary flow in a particulate solid. A hypothesis paper. *Journal of Food Science* 2004; 69(7):R167–R174p.
46. J Kleinert. Etude sur la formation du blanchiment gras et les moyens propres a le retarder. *International Chocolate Review.* 1961; 16:345–368p.
47. MH Lohman, RW Hartel. Effect of milk fat fractions on fat bloom in dark chocolates. *J. Amer. Oil Chem. Soc.* 1994; 71(3):267–276p.
48. P Fairley, JB German, JM Krochta. Phase behavior and mechanical properties of tripalmitin/butterfat mixtures. *J. Food Sci.* 1994; 59(2):321–325, 327p.
49. O Tolboe. Physical characteristics of butter fat and their influence on the quality of Danish pastry and cookies. In: R Marcuse (Ed.). *Lipidforum: Milkfat and its Modification*. SIK, Goteborg, Sweden. 1984: 43p.