Chapter 2
Lipase Action on Milk Fat

Hilton C. Deeth

1 Introduction

Lipases have important functions in almost all living organisms and play a major role in the metabolism of lipids. True lipases (EC 3.1.1.1) act on triglycerides (TG), diglycerides (DG) and monoglycerides (MG) (tri-, di- and monoacylglycerols), with triglycerides being the major substrate. Other lipases act on other types of lipids, for example, phospholipases act on phospholipids. This chapter largely concerns true lipases.

While lipases are best known for catalysing hydrolysis (lipolysis) of triglycerides, they can also catalyse acyl transfer reactions. Lipolysis reactions occur in aqueous conditions but acyl-transfer reactions occur in organic solvents containing just enough water to maintain the activity of the enzyme but minimising the hydrolysis reaction. Acyl transfer reactions include alcoholysis, acidolysis and interesterification (Fig. 2.1). Alcoholysis occurs where an alcohol is incorporated as the nucleophilic receptor and a new ester is formed; if methanol is the alcohol, methyl esters are formed whereas if glycerol is incorporated, di- and monoglycerides are formed. Acidolysis occurs where an introduced carboxylic acid replaces one of the fatty acids of the TG, and interesterification occurs where acyl transfer occurs between an ester and a TG, often between two TGs or TG mixtures. Bourlieu, Bouhallab, and Lopez (2009) reviewed 28 recent publications on the use of enzymes on milk fat and found 44% on interesterification, 36% on hydrolysis, 11% on acidolysis and 7% on alcoholysis (glycerolysis). In a previous survey of 38 publications, Balcão and Malcata (1998b) found 46% concerned interesterification and 43% hydrolysis.

H. C. Deeth (✉)
School of Agriculture and Food Sciences, University of Queensland,
St Lucia, QLD, Australia
e-mail: h.deeth@uq.edu.au

T. Truong et al. (eds.), *Dairy Fat Products and Functionality*,
https://doi.org/10.1007/978-3-030-41661-4_2

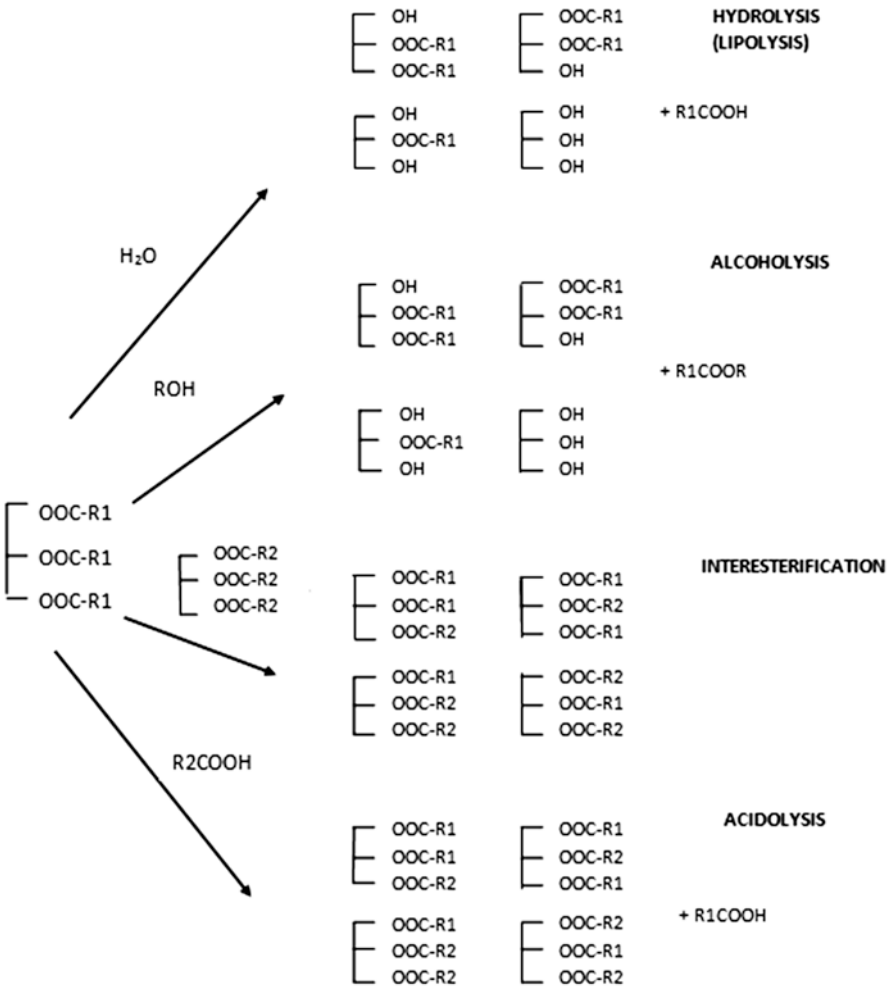

Fig. 2.1 Schematic representation of hydrolysis (lipolysis) and acyl transfer reactions catalysed by lipases

Like most enzymes, lipases are characterised by their substrate specificity. A major type of specificity is in relation to the position of the fatty acid on the glyceride. Since most natural triglycerides are asymmetric, the three positions on the glycerol backbone are different; they are designated *sn*-1, *sn*-2 and *sn*-3, where *sn* stands for stereospecific numbering. Most commonly, lipases have a strong preference for the primary esters, those in *sn*-1 and *sn*-3 positions, although a few lipases, e.g., from *Candida parapsilosis* (Akoh, Sellaopoan, Fomuso, & Yankah, 2003; Riaublanc, Ratomahenina, Galzy, & Nicolas, 1993), have a preference for esters in the *sn*-2 position. A summary of the specificities of some enzymes is given in Table 2.1.

Table 2.1 Specificities of some lipases and esterases

Specificity	Enzyme name/source
Non-specific	*Candida rugosa*, *Pseudomonas fluorescens*, *Pseudomonas cepacia*, *Candida antarctica*, *Chromobactrerium viscosum*, *Penicillium cyclopium*, *Candida cyclindracea*
sn-1,3	*Rhizopus niveus*, *Aspergillus niger*, *Rhizopus oryzae*, *Penicillium roqueforti*, *Rhizopus delemar*, *Mucor javanicus*, *Thermomyces lanuginosa*, *Rhizomucor miehei*, *Mucor miehei*, *Candida deformans*, pancreatic lipase
sn-1,3; *sn*-1 > *sn*-3	Lipoprotein lipase
sn-1,3; *sn*-3 > *sn*-1	Pregastric esterases
sn-2	*Candida parapsilosis*
MG/DG	*Penicillium camemberti*
Cis *n*-9 unsaturated FAs	*Geotricum candidum*
Linoleic acid	*Aspergillus lipolyticum*
Medium/long chain FAs	*Rhizopus niveus*, *Aspergillus niger*, *Rhizopus oryzae*
Small/medium chain FAs	*Penicillium roqueforti*
Short chain FAs	Pregastric esterases

From: Morley and Kuksis (1977), Balcão and Malcata (2002), Bourlieu et al. (2009), Jooyandeh et al. (2009)

As a consequence of the various functions of lipases, this chapter is divided into sections relating to the hydrolysis reaction (lipolysis) and its consequences for the dairy and other food industries, and the acyl transfer reactions which relate more to the biotechnology industry than to the dairy industry. Reviews related to the first have been published by Sørhaug and Stepaniak (1997), Olivecrona, Vilaró, and Olivecrona (2003) and Deeth and Fitz-Gerald (2006) while reviews related to the biotechnological topics have been published by Seitz (1974), Balcão and Malcata (1998b, 2002), Bourlieu et al. (2009), Jooyandeh, Kaur, and Minhas (2009), Kontkanen et al. (2011) and Javed et al. (2018).

2 Lipolysis

Lipolysis, the term used for lipase-catalysed hydrolysis of lipids, occurs at the ester bond between a fatty acid and an alcohol group. The products of lipolysis of TGs are therefore an unesterified or free fatty acid (FFA) and a DG. The DG can be further hydrolysed to a MG which, in some cases, can be further hydrolysed to glycerol, each time releasing a FFA, as shown below.

DGs and MGs are sometimes collectively categorised as partial glycerides, and, as discussed below, have significant surface-active properties.

The hydrolysis action catalysed by lipases differs from that of most other enzymes in that it occurs at a surface rather than in a solution of the lipid substrates. Commonly the lipid surface is a part of an oil-in-water emulsion. Enzymes which act on the ester bond of soluble esters are referred to as esterases. In general, lipids which are substrates for esterases tend to be glycerides of short-chain fatty acids, although esterases also act on a wide range of soluble esters. However, there is some overlap between lipases and esterases with some lipases acting on glycerides of short-chain FFAs such as tributyrin. Several types of esterase have been identified according to their preferred substrate, e.g., carboxylesterase, arylesterase.

The lipases of most relevance for their hydrolytic action on the fat in milk and milk products are the natural milk lipase, a lipoprotein lipase (LPL), and bacterial lipases produced by a wide range of bacteria. The bacterial lipases of significance in the dairy industry are those produced by bacteria which contaminate milk and dairy products and cause flavour and other defects. Another important category of lipases is those added in the manufacture of some products such as feta, romano and parmesan cheeses. The enzymes traditionally used for this purpose originate from the stomachs of young ruminant animals, calves, lambs or kids; they are sometimes referred to as pre-gastric esterases because of their preference for releasing short-chain FFAs.

2.1 The Lipases Responsible for Lipolysis in Milk and Milk Products

2.1.1 Milk Lipoprotein Lipase: The Natural Milk Lipase

Cows' milk and the milk of other species, including humans, naturally contain LPL (Olivecrona et al., 2003). The enzyme is so-called because it is activated by lipoproteins or their fragments (apo-lipoproteins). This can be demonstrated with milk LPL by adding blood serum to milk and observing the consequent lipolysis.

Milk LPL is the same enzyme as the one in the mammary gland where its function is the synthesis of milk fat triglycerides (Barber, Clegg, Travers, & Vernon, 1997). Its presence in milk appears to be due to spill-over from the mammary gland as its biological function in milk is unclear. It may play a role in fat digestion in the young animal, but this does not appear to be a significant function because it is inactivated at low pH, such as encountered in the stomach.

In cows' milk, LPL accounts for virtually all of the lipase activity. The LPL content is high and, under ideal conditions, is capable of causing the hydrolysis of a

large proportion of the fat in milk. Cows' milk also contains some minor esterase activity which has been designated aryl esterase (Marquardt & Forster, 1965) and carboxylesterase (Fitz-Gerald, Deeth, & Kitchen, 1981). The esterase activity is elevated in colostrum and mastitic milk (milk with high somatic cell count). However, the total esterase activity in these abnormal milks is small compared with LPL activity and has little if any relevance to lipolysis of milk fat (Deeth, 1978; Marquardt & Forster, 1962). By contrast, human milk contains a bile-salt-stimulated lipase, in addition to LPL, which is believed to have a major role in digestion of milk fat by the newborn (Hernell & Bläckberg, 1991, 1994). The following discussion relates specifically to LPL in cows' milk.

Milk LPL is a dimeric glycoprotein with a molecular weight of ~100,000 Da (Kinnunen, Huttunen, & Ehnholm, 1976). Most of it is normally associated with the casein micelle in milk through both electrostatic and hydrophobic bonding. It is physically separated from its substrate, milk fat, which is present in the form of fat globules, encased within the milk fat globule membrane (MFGM).

LPLs bind strongly to heparin and this feature is utilised in the isolation of LPL from milk using heparin-sepharose affinity chromatography (Iverius, Olivecrona, Egelrud, & Lindahl, 1972). LPL has maximum activity at a pH of ~9.0 and at a temperature of ~37 °C. It is quite heat-labile, being almost completely inactivated by pasteurisation at 72 °C for 15 s (Andrews, Anderson, & Goodenough, 1987).

LPL releases fatty acids attached to the *sn*-1 and *sn*-3 positions of the triglyceride molecule with a preference for the *sn*-1 position. It does not have a fatty acid specificity (Morley & Kuksis, 1977); however, it does tend to release a higher proportion of short-chain fatty acids that are present in the parent milk fat. This is attributable to the higher proportion of these fatty acids in the *sn*-3 position of cows' milk triglycerides. LPL action normally only progresses as far as 2-monoglycerides; it can only release fatty acids from 2-monoglycerides if they first rearrange to 1(3)-monoglycerides (Nilsson-Ehle, Egelrud, Belfrage, Olivecrona, & Borgstrom, 1973).

2.1.2 Bacterial Lipases from Milk-Contaminating Bacteria

Bacterial exocellular lipases are the other major type of lipases which act on milk fat in milk and dairy products. While there is potentially a huge range of such enzymes, the main ones of relevance to the quality of milk and dairy products are those produced during growth of psychrotrophic bacterial contaminants, principally in raw milk. These enzymes differ from the natural milk lipase (LPL) in two major ways which make them highly significant in the dairy industry: firstly, they are much more heat-stable and secondly, the MFGM is much less of a barrier to accessing triglycerides inside native, intact fat globules.

The major sources of bacterial lipases in milk and milk products are pseudomonads, particularly *Pseudomonas fluorescens* and *P. fragi* (Shelley, Deeth, & MacRae, 1986). Others include *Serratia*, *Acinetobacter*, *Achromobacter*, *Aeromonas, Alcaligenes*, *Bacillus, Flavobacterium, Micrococcus* and *Moraxella* (Muir, Phillips, & Dalgleish, 1979; Stewart, Murray, & Neill, 1975). The occurrence and significance

of lipases produced by psychrotrophic bacteria in milk and other foods have been reviewed by McKellar (1989) and Sørhaug and Stepaniak (1997).

Psychrotrophic bacteria produce lipase during the late log and early stationary phases of growth; the activity of the lipase produced sometimes reaches a peak and then decreases (Rowe, Johnston, Kilpatrick, Dunstall, & Murphy, 1990). Little lipase is produced before cell numbers reach 10^6–10^7/mL. However, different species and strains of bacteria produce lipase at different cell densities and the rate of bacterial growth is not a reliable guide to lipase production. McKellar (1989) reviewed the effects of several environmental and nutritional factors on the production of enzymes by psychrotrophic bacteria.

The molecular weights of lipases from psychrotrophic bacteria are mostly in the range 14,000–50,000 Da. For example, *P. fluorescens* lipases with molecular weights of ~16,000 Da (isolated under dissociating conditions) (Dring & Fox, 1983; Stepaniak, Birkeland, Sørhaug, & Vagias, 1987), 33,000 Da (Sugiura, Oikawa, Hirano, & Inukai, 1977), and ~50,000 Da (Chung, Lee, Jeohn, Yoo, & Rhee, 1991; Dieckelmann, Johnson, & Beacham, 1998) have been reported.

The pH optimum of the lipases is usually between 7 and 9 and the optimum temperature is 40–50 °C, although the optimum temperature may vary according to the assay conditions used (Fitz-Gerald & Deeth, 1983). Of interest in relation to chilled dairy products is that many of these lipases are active at low temperatures, e.g., ≤10 °C (Landaas & Solberg, 1978; Te Whaiti & Fryer, 1978).

One of the most important properties of these lipases is their heat stability. This varies with the species and strain of the bacteria but many are stable to pasteurization (Fitz-Gerald, Deeth, & Coghill, 1982; Kalogridou-Vassiliadou, 1984; Law, Sharpe, & Chapman, 1976) and even after UHT treatment (Christen, Wang, & Ren, 1986; Kishonti, 1975; Mottar, 1981). It is particularly significant for UHT milk in which only a small residual activity can cause lipolysis during room-temperature storage for several months. Some of the lipases are susceptible to 'low-temperature inactivation' (LTI), e.g., heating at 55 °C for 1 h, which is also effective for inactivating the corresponding proteases (Barach, Adams, & Speck, 1976). However, in fat-containing media, considerable lipolysis can occur during prolonged heating at 55 °C and hence treatment at this temperature may have limited value for eliminating these lipases from milk products (Fitz-Gerald et al., 1982; Griffith, Phillips, & Muir, 1981). Bucky, Hayes, and Robinson (1987) combined UHT treatment with a subsequent LTI treatment of 60 °C for 5 min in a patented process which is reported to considerably enhance the effectiveness of UHT treatment in reducing lipase activity.

Bacterial lipases are usually capable of acting on triglycerides in intact milk fat globules (Fitz-Gerald & Deeth, 1983), a property not exhibited by the indigenous milk lipase because of the protection afforded by the MFGM (Danthine, Blecker, Paquot, Innocente, & Deroanne, 2000). This may be because the lipases can penetrate the MFGM or their access to the substrate is facilitated by activating factors or by their enzymes such as glycosidases, proteases and phospholipases (Alkanhal, Frank, & Christen, 1985; Griffiths, 1983; Marin, Mawhinney, & Marshall, 1984). Based on the behaviour of three bacterial enzymes preparations, Bourlieu, Rousseau, Briard-Bion, Madec, and Bouhallab (2012) concluded that access by the lipases to globular fat was facilitated by the action of annex phospholipase activity.

2.2 Lipolysis in Milk

In milk, the fat is contained within fat globules which vary in diameter from <0.1 to 15 μm. Each globule is covered by a biological membrane, the MFGM. The fat contained inside the membrane is neutral lipid, of which about 98% is triglyceride and about 2% is other lipids including DGs, MGs, FFAs and cholesterol. The lipid component of the MFGM consists of phospholipids, sphingolipids, glycolipids and neutral lipids; the complex lipids—phospholipids, sphingolipids and glycolipids—which make up about 35% of the MFGM material (but only about 0.25% of the milk) are important for the stability of the MFGM (Deeth, 1997).

Lipolysis encountered in raw milk is almost always caused by the native milk LPL. Normally it is prevented from accessing its substrate, triglycerides, by the MFGM. However, there are situations where the triglycerides come into contact with the milk LPL and lipolysis results. The lipolysis can be divided into two categories: spontaneous and induced (Deeth, 2006; Deeth & Fitz-Gerald, 2006).

Spontaneous lipolysis occurs at the farm in raw milk from certain cows. In general, cows on poor quality feed and in late lactation are the most likely to produce milk which is susceptible to this type of lipolysis. The lipolysis is initiated when the milk is cooled, at which time some of the LPL moves from the casein micelle to the MFGM (Dickow, Larsen, Hammershoj, & Wiking, 2011; Hohe, Dimick, & Kilara, 1985). When attached to the MFGM, it is able to cause lipolysis of the triglycerides in the fat globule. The exact mechanism for this has not been fully elucidated but it is clear that at least three factors determine the extent of spontaneous lipolysis: the total LPL in the milk; the nature of the MFGM; and the balance between inhibiting and activating factors (Cartier & Chilliard, 1990; Deeth & Fitz-Gerald, 1975a; Sundheim, 1988). The last factor is probably the most important; addition of normal milk to milk susceptible to spontaneous lipolysis causes a reduction in the expected level of lipolysis, indicating the inhibitor-activator balance has been altered towards inhibition. This is beneficial as it means that herd bulk milks will have a lower overall lipolysis level than would be expected from the susceptibilities of individual milks. From a practical viewpoint, it has been shown that the bulk milk from certain producers is more likely to present with high levels of lipolysis, i.e., with high FFA, than other producers. The reason for this is not entirely clear.

Induced lipolysis can occur on farm or in factory. It occurs when the MFGM is disrupted in some way, thereby allowing the LPL access to the exposed triglyceride. It can be caused by agitation and pumping of raw milk, especially if foaming is involved as the fat globules at the interfacial surfaces of the foam become damaged when the bubbles are formed. A most effective way of inducing lipolysis is by homogenisation of raw whole milk which provides a triglyceride substrate with a high surface area. Similarly, mixing raw milk with (pasteurised) homogenised milk is an effective means of inducing lipolysis and hence should never occur in the dairy industry.

Above a certain level, FFAs produced by lipolysis in milk are detrimental to its quality. The major defects caused are the development of a rancid, unclean off-flavour and a reduction in the foaming capacity of milk. The latter is very important in milk for making cappuccino coffee. There is little effect on the properties of milk

when the FFA level is <1 mmol/L; an off-flavour will be noticed by many people and the foaming capacity will be noticeably reduced when it reaches 1.5 mmol/L, and when it reaches 2 mmol/L almost all people will detect the rancid flavour and the foaming capacity will be negligible.

Reduced foaming capacity of milk is an ongoing issue for the dairy industry and makers of cappuccino coffee (Huppertz, 2010). Lipolysis of the milk is known to be a major cause of reduced foaming capacity through reducing its surface tension (Buchanan, 1965; Deeth & Smith, 1983; Kamath, Wulandewi, & Deeth, 2008) but other factors, including milk compositional factors, may also be involved (Corrandini & Innocente, 1994; Gambini, Castagnetti, & Losi, 1995). The effect of lipolysis is mainly through the surface-active partial glycerides produced during lipolysis in milk caused by milk LPL although some FFAs also have surface-active properties. Commercial milk-processing operations, pasteurization and homogenization, markedly enhance the steam-frothing capacity of milk (Deeth & Smith, 1983) and hence pasteurised milk foams much better than raw milk; however, heat treatment and homogenisation are unable to significantly enhance the foaming ability of milk with a high level of lipolysis, e.g., with FFA ≥2.0 mmol/L.

Since native milk LPL is almost completely inactivated by the minimum pasteurisation conditions, 72 °C for 15 s, any lipolysis which occurs after heat processing is due to bacterial lipases. Such lipolysis is rare in pasteurised milk but can occur in UHT milk which typically has a long storage time and is stored at room temperature. The lipases responsible for lipolysis in this type of milk are the heat-stable lipases produced by psychrotrophic bacteria in raw milk as discussed above.

2.3 Lipolysis in Milk Products

As for lipolysis in milk, lipolysis in milk products can be detrimental; however, it can also be beneficial. For example, lipolysis is important in the ripening of most cheese varieties. It results from the action of added lipases, lipases produced by microorganisms, and, in raw milk cheese, milk LPL. In some pasteurised cheese varieties, such as cheddar, lipolysis is usually not extensive but is an important contributor to flavour, especially of ripened cheese (Law, 1984). However, excessive lipolysis can render the cheese unacceptable. This occurs when the FFA level reaches 2.8–3.0 mmol/100 g fat (Deeth & Fitz-Gerald, 1975b). High FFA levels in cheddar cheese are usually caused by heat-stable enzymes produced by psychrotrophic bacteria in the cheese-milk before pasteurisation, as discussed above for UHT milk.

In some cheeses, such as blue vein and hard Italian varieties, high FFA levels are characteristic of the variety (Fox & Law, 1991; Gripon, 1987). For example, a FFA content of >66,000 mg/kg is acceptable in blue vein cheese (Horwood, Lloyd, & Stark, 1981) (compared to <4000 mg/kg for good cheddar (Bills & Day, 1964) and <~400 mg/kg for milk. The most flavoursome of the FFAs is butyric acid and high levels of this acid are characteristic of Italian hard cheeses, certain pickled cheeses (Fox & Guinee, 1987) and feta cheese. Butyric acid levels can be >3000 mg/kg in

Romano (Woo & Lindsay, 1984) and up to 520 mg/kg for feta cheese (Horwood et al., 1981). The high FFA levels in these cheese varieties are due to the action of added pregastric esterases which have a preference for hydrolysis of short-chain fatty acids such as butyric and caprylic acids.

In Swiss cheese varieties, FFAs can arise through the action of lipases of propionibacteria (Oterholm, Ordal, & Witter, 1970) while in internal mould-ripened cheeses such as gorgonzola, roquefort and stilton, the FFAs result from the action of *Penicillium roqueforti* lipase. These FFAs contribute to the flavour of these cheese varieties but are also precursors for production of methyl ketones to which the peppery taste of such cheeses is attributable (Kinsella & Hwang, 1976). Lipase produced by *P. camemberti* in surface mould cheeses such and brie and camembert performs a similar function.

The level of lipolysis in raw milk cheese is generally greater than that in pasteurised milk cheese. For example, Hickey, Kilcawley, Beresford, and Wilkinson (2007) reported that after 168 days of ripening, raw milk cheddar cheese had a total FFA level of 1223 mg/kg while corresponding pasteurised cheese had a level of 856 mg/kg. The difference is largely attributable to the action of milk LPL. Buffa, Guamis, Pavia, and Trujillo (2001) also obtained higher FFA levels in cheese made from raw milk than in cheese made from pasteurised milk. Interestingly they found a similar level of lipolysis in cheese made from high-pressure-processed milk and cheese made from raw milk, indicating that high pressure does not inactivate the native milk LPL.

2.4 Lipolysis for Production of Dairy Flavours

Besides the detrimental effects of off-flavour and reduced foaming capacity of milk, lipolysed milk fat can have several benefits. At levels below those which impart a definite rancid flavour, FFAs provide richness and creaminess, and can, therefore, contribute positively to the flavour of foods. Even at higher concentrations which may make milk undrinkable, FFAs contribute a characteristic flavour to some food products.

Controlled lipolysis of milk fat is used to produce creamy and buttery flavours for bakery and cereal products, confectionery (milk chocolate, fudge), coffee whiteners and other imitation dairy products (Arnold, Shahani, & Dwivedi, 1975; Fox, 1980; Kilara, 1985). Lipolysed products with the most flavour tend to be those produced with lipases having *sn*-3 or *sn*-1,3 regioselectivity since the most flavoursome, short-chain fatty acids are largely located in the *sn*-3 position of milk fat TGs. These products are particularly useful for enhancing the flavour of reduced-fat products such as cheese (Noronha, Cronin, O'Riordan, & O'Sullivan, 2008). A good example of a commercial lipolysed milk fat product is Butter Buds® which is a highly flavoured powder made from butter, cream and cheese which can be used to enhance the dairy flavour of food products (http://www.butterbuds.com/). Another example of the use of lipolysed fat in a food product is chocolate; it accounts for the characteristic flavour of some brands.

Balcão and Malcata (2002) reviewed the potential of lipolysis of milk fat to produce dairy-type flavours. They pointed out that the fatty acid profile produced is dependent on the source of lipase and hence judicious choice of the lipase used for the hydrolysis can enable tailoring of the flavour in the product. They also reported studies showing that lipase produced by *Aspergillus lipolyticum, Penicillium roqueforti,* and *Candida candidum* produced soapy and sometimes musty flavours when used in bread, kid and lamb pregastric esterases produce rancid flavours and *P. roqueforti* lipase produces a cheese-like product when used to lipolyse milk fat emulsified in lactic-fermented condensed skim milk. *P. roqueforti* lipases have been shown to be largely responsible for the flavour of blue-veined cheese (Seitz, 1974).

Lipolysis in cheese can also occur through the addition of lipases or esterases to accelerate flavour development in varieties such as cheddar (Jooyandeh et al., 2009; Law & Wigmore, 1985). Furthermore, preparations with flavours typical of particular varieties of cheese can be produced with the aid of lipases of appropriate specificities (Kilara, 1985). Such flavours, sometimes called enzyme-modified cheese flavours, are used in processed cheese, analogue cheese, biscuits, sauces, soups, dips and spreads (Jolly & Kosikowski, 1975a, 1975b; Jooyandeh et al., 2009).

3 Alcoholysis

Alcoholysis is closely related to hydrolysis in that an alcohol replaces water in the lipase reaction. As for hydrolysis, the reaction results in an alcohol group replacing a fatty acid on the glycerol backbone; however, instead of a fatty acid being also produced, an ester of the fatty acid is produced (Fig. 2.1).

One application of alcoholysis involves the use of glycerol as the alcohol. The products of the lipase-catalysed alcoholysis, in this case, are DGs and MGs. Because of their surface-active properties, DG/MG mixtures are widely used in the food industry for stabilising emulsions; they account for about 75% of all emulsifiers used (Balcão & Malcata, 2002). Yang, Harper, Parkin, and Chen (1994) screened nine lipases for their ability to catalyse glycerolysis of milk fat. They found a range of abilities among the lipases, with two lipases from *Pseudomonas* species presenting the highest yield of MG (55–60%) and DG (24–39%) and three lipases, from *Geotricum candidum, Mucor javanicus* and *Candida cylindracea* (*C. rugosa*), yielding no partial glycerides.

Lubary, Hofland, and ter Horst (2011) adopted a different approach to producing a DG-rich milk fat. DG-rich oils have some physiological advantages over TG-rich oils. They used ethanolysis catalysed by a *Pseudomonas fluorescens* lipase. The reaction product mix contained 1,2-DGs together with fatty acid ethyl esters. The latter were removed with supercritical carbon dioxide and the 1,2-DGs isomerised using the same *Pseudomonas* lipase to give mostly (63%) 1,3-DGs.

Schmid, Bornscheuer, Soumanou, McNeill, and Schmid (1998) used alcoholysis with ethanol as the first step in the synthesis of structured triglycerides with palmitic acid in the *sn*-2 position. Such a structure is very significant as it occurs predominantly in human milk fat. This is physiologically preferable to having such a long-

chain saturated fatty acid in either the *sn*-1 or *sn*-3 positions because fatty acids removed from the primary positions by 1,3-regiospecific lipases such as pancreatic lipase form calcium salts in the body which are difficult to absorb. By contrast, fatty acids in the *sn*-2 position are readily absorbed as the 2-MG. The alcoholysis reaction was carried out on tripalmitin (PPP) in a solvent, such as acetone or methyl tert-butyl ether, with anhydrous ethanol at 3.5 mM with a 1,3-regiospecific lipase. The highest yield (88%) of 2-monopalmitin was obtained with a *Rhizopus delemar* lipase immobilised on celite in the solvent methyl tert-butyl ether. The targeted TG (OPO) was then produced by acidolysis with oleic acid using the same lipase.

An interesting application of the alcoholysis reaction was recently proposed by Andrewes (2018) for the analysis of lipase activity in UHT milk. The principle was demonstrated by the addition of methanol and a commercial enzyme preparation, produced by *Rhizopus oryzae*, to the milk and, after incubation, measuring the fatty acid methyl esters formed. The amount of methyl esters formed is taken as a measure of the lipase activity in the milk.

4 Acidolysis

Acidolysis involves the reaction of a TG with a carboxylic acid, usually a fatty acid, in the presence of a lipase. The added acid substitutes some of the fatty acids on the TG, thus producing TGs enriched in the acid added and releasing some of the original fatty acids as FFAs. It can also be used to esterify MGs or DGs, as used by Schmid et al. (1998) to esterify monopalmitin in the example given above.

A good example of acidolysis is the work of Balcão and Malcata (1997, 1998a, 1998b) and Balcão, Kemppinen, Malcata, and Kalo (1998) who reported acidolysis of milk fat with oleic acid using lipases produced by *Mucor circinelloides* and *M. javanicus* immobilized by on hydrophobic hollow fibers. After acidolysis, the milk fat contained 27–30% more oleic acid, 8% less lauric acid, and 2–6% less myristic acid. For the reaction catalysed by the *Mucor circinelloides* lipase, the modified milk fat product had 19% more low-melting fraction and 83% less high-melting fraction than the original milk fat. The authors concluded that even though there was some hydrolysis, the enzymatic acidolysis was able to produce milk fat with improved nutritional quality (Balcão et al., 1998).

One of the most important applications of the acidolysis reactions involving milk fat is modifying its structure to resemble that of human milk fat; this is to "humanise" the fat for use in infant formulae. The modification is aimed at changing not only the fatty acids but also their position on the TG. To simulate human milk fat, the *sn*-2 position of the humanised fat needs to contain mostly saturated fatty acids such as palmitic acid, and the *sn*-1 and *sn*-3 positions need to carry all of the unsaturated fatty acids. For example, Christensen and Holmer (1993) interesterified bovine milk fat with unsaturated fatty acids using the *sn*-1,3-regiospecific lipase of *Rhizomucor miehei* and produced a modified milk fat enriched in unsaturated fatty acids in the *sn*-1 and -3 positions, as occurs in human milk. Similarly, Sørensen et al. (2010) interesterified milk fat with a 7:3 mixture of rapeseed and soybean oil

fatty acids using Lipozyme® RM IM lipase (from *Rhizomucor miehei*). These authors fractionated the interesterified fat and retained a solid fraction as a human milk fat substitute (HMFS). It contained 56% palmitic acid in the *sn*-2 position compared with 72% in human milk fat. Interestingly, these authors also produced a similar HMFS by interesterifying a solid milk fat fraction with rapeseed and soybean oil fatty acids. Production of HMFSs has been reviewed by Soumanou, Perignon, and Villeneuve (2013).

Acidolysis can be used for incorporating fatty acids with particular therapeutic properties. Conjugated linoleic acid (CLA) is one such fatty acid as it is considered to be an anticarcinogenic agent (Parodi, 1999). Garcia, Keough, Arcos, and Hill (2000) used six commercial immobilised lipases, of which they found *Candida antarctica* lipase to exhibit the best activity, in a batch reactor to effect acidolysis of milk fat with CLA in a solvent-free system containing water contents of 0.15 to 2%. The optimum temperature for the reaction was 50 °C. Similar results were reported by Sehanputri and Hill (2003) using the same enzyme in a packed bed reactor.

The spreadability of butter has been a serious issue for the dairy industry. Several approaches have been used to improve this property, including mixing milk fat or cream with an unsaturated oil such as canola oil before churning into butter. Another approach is to interesterify milk fat with unsaturated fatty acids in order to substitute some long-chain saturated fatty acids with these acids. An example of this was reported by Kim et al. (2002) who used *Rhizopus arrhizus* lipase to incorporate α-linolenic acid from perilla oil into milk fat. They achieved an incorporation of up to ~24% (w/w) in the milk fat. The modified milk fat had better spreadability than the original milk fat.

5 Interesterification

As shown in Fig. 2.1, enzymic interesterification of milk fat involves reacting it with a lipase, usually in the presence of a different TG or TG mixture. If carried out in the absence of water or with a very low water content, the hydrolysis reaction is minimised and the fatty acyl interchange is maximised. The major product is a modified milk fat. This product will still contain the fatty acids from the original milk fat TGs as well as incorporated fatty acids from any added TG. Separation of the newly formed TGs from TGs containing the original fatty acids is very difficult as they have similar properties. This contrasts with the situation with alcoholysis and acidolysis where the non-TG products differ in properties from the modified TG and can be separated from it. However, where the mixed TG is a desirable end-product, this is not an issue.

As for alcoholysis and acidolysis, the intended changes can be tailored by the regiospecificity of the lipase used. Therefore, if a *sn*-1,3 specific lipase is used, the fatty acids on the *sn*-1 and -3 positions will be exchanged. In some circumstances, however, small changes in the fatty acids on the *sn*-2 may occur. Rønne, Yang, Mu, Jacobsen, and Xu (2005) reported that this occurred when a *Thermomyces lanugi-*

nosus lipase was used in interesterifying milk fat and rapeseed oil but it did not happen when *Rhizimucor miehei* lipase was used. If a non-specific lipase is used, random exchange of fatty acids will result. Such random exchange occurs during chemical interesterification. However, if random exchange is required, enzymic interesterification is preferable to chemical as it can be carried out under relatively mild conditions.

A major reason why interesterification is carried out is to change the physical properties of the fat. If milk fat alone is reacted with a lipase, the product has different melting characteristics. For example, Hayati et al. (2000) reported that after interesterification with a *sn*-1,3-specific lipase, the solid fat content at various temperatures was altered; it was lower at 5 and 10 °C but higher at 15–35 °C. This is consistent with the finding of Chmura, Staniewski, Panfil-Kuncewicz, Szpendowski, and Zawadzka (2008) that interesterification of milk fat with a *sn*-1,3-specific lipase of *Rhizomucor miehei* resulted in a decrease in C34 to C42 TGs with a simultaneous increase of C44 to C52 TGs, and an increase in penetration value at 5 °C. By contrast, chemical interesterification of milk fat with sodium methoxide decreases the solid fat content at all temperatures from 5 to 40 °C (Rousseau, Forestiere, Hill, & Marangoni, 1996).

Blending of fats is used commercially for modifying their melting behaviour and other properties. This method can be used for overcoming some of the problems associated with milk fat's lack of solid content at high temperature, which limits its use in certain food products such as bakery and confectionery. In their study, Hayati et al. (2000) interesterified binary mixtures of milk fat and two palm oil fractions, soft and hard stearin fractions. They reported that the interesterified blend generally had a higher maximum peak temperature (T_p) than the untreated mixture, and that the DSC curves were smoother than those of milk fat (milk fat typically exhibits three peaks) indicating a more homogeneous mixture of TGs. Interestingly, the interesterified blends of milk fat-soft palm stearin had similar melting behaviour to that of milk fat high melting fractions obtained by dry fractionation, leading the authors to suggest that they have commercial potential in several food applications such as in pastry and confectionery. While blends with between 25 and 75% soft palm stearin were considered to have this potential, the authors recommended that blends containing no more than 25% of the hard palm stearin have similar potential as they retained the desired milk fat flavour in the final product.

Interesterification of milk fat with unsaturated fats/oils has been reported by several authors (with canola, Rousseau, Hill, & Marangoni, 1996, Morais Nunes, de Paula, de Castro, & dos Santos, 2011; rapeseed, Kalo, Kemppinen, & Antila, 1987, Rønne et al., 2005, Giet et al., 2009, linseed (flaxseed), Aguedo et al., 2008; Shin, Akoh, & Lee, 2010; Giet et al., 2009). These studies were mainly aimed at modifying the milk fat to have a purportedly nutritionally better fatty acid profile (Kontkanen et al., 2011). In the study by Shin et al. (2010), this was assessed by the so-called "atherogenic index". Nutritionally, another benefit of employing enzymic rather than chemical methods for the interesterification is to produce a low-*trans* fat suitable for spreadable spreads (Shin et al., 2010). Enzymic interesterification does not cause *cis*/*trans* isomerisation of double bonds (Aguedo et al., 2008). This is because of the

milder conditions used for enzymic interesterification, as exemplified in the study by De et al. (2007) in which chemical interesterification was carried out at 180 °C for 4 h while enzymic interesterification (using a *Mucor miehei* lipase) was carried out at 60 °C for 4 h. Milder reaction conditions have been reported by other authors for chemical interesterification, e.g., ~80 °C for 2 h (Rousseau, Forestiere, et al., 1996) and shorter reaction times have been reported for enzymic, reactions e.g., 30 and 60 min by Rønne et al. (2005) reactions. Even milder conditions may be possible for enzymic interesterifications carried out under ultrasonication (Lerin et al., 2014).

HMFSs can also be produced by interesterification of milk fat. For example, Kalo et al. (1987) prepared a HMFS by interesterifying a milk fat solid fraction with rapeseed oil. However, starting with bovine milk fat is not a common pathway for producing HMFS. More commonly, unsaturated oils such as marine and vegetable oils are interesterified with TGs containing a high percentage of palmitic acid in the *sn*-2 position, even tripalmitin. For example, Karabulut, Turan, Vural, and Kayahan (2007) interesterified palm oil, palm kernel oil, olive oil, sunflower oil, and marine oil blend, in the ratio of 4.0:3.5:1.0:1.5:0.2, with Lipozyme® TL IM, a lipase from *Thermomyces lanuginosa*, and produced a product with mostly unsaturated fatty acids in the *sn*-1 and *sn*-3 positions and saturated fatty acids in the *sn*-2 position of the TGs, similar to that in human milk fat TGs.

6 Conclusion

The effects of lipases on milk fat are of ongoing interest to the dairy and wider food industry. In the dairy industry, they can cause flavour problems but also contribute substantially to the desirable flavour of some products. Several commercial lipases are now available and their different specificities enable them to be used for a range of modifications of milk fat. These include changes to the fatty acid composition and the structure of the TGs; these can be tailored to meet nutritional and physical functionality criteria. Lipase reactions occur under relatively mild conditions and this makes them attractive for use in a range of applications. The potential for their future commercial use with milk fat is substantial provided the costs and availability of the enzymes and the required processing equipment are favourable.

References

Aguedo, M., Hanon, E., Danthine, S., Paquot, M., Lognay, G., Thomas, A., et al. (2008). Enrichment of anhydrous milk fat in polyunsaturated fatty acid residues from linseed and rapeseed oils through enzymatic interesterification. *Journal of Agricultural and Food Chemistry, 56*, 1757–1765.

Akoh, C. C., Sellaopoan, S., Fomuso, L. B., & Yankah, V. V. (2003). Enzymatic synthesis of structured lipids. In T. M. Kuo & H. W. Gardner (Eds.), *Lipid biotechnology* (pp. 433–460). New York, NY: Marcel Dekker, Inc.

Alkanhal, H. A., Frank, J. F., & Christen, G. L. (1985). Microbial protease and phospholipase C stimulate lipolysis of washed cream. *Journal of Dairy Science, 68*, 3162–3170.

Andrewes, P. (2018). Indirect detection of lipase in UHT milk by measuring methyl ester formation. *International Dairy Journal, 79*, 1–4.

Andrews, A. T., Anderson, M., & Goodenough, P. W. (1987). A study of the heat stabilities of a number of indigenous milk enzymes. *The Journal of Dairy Research, 54*, 237–246.

Arnold, R. G., Shahani, K. M., & Dwivedi, B. K. (1975). Application of lipolytic enzymes to flavor development in dairy products. *Journal of Dairy Science, 58*, 1127–1143.

Balcăo, V. M., Kemppinen, A., Malcata, F. X., & Kalo, P. J. (1998). Lipase-catalyzed acidolysis of butterfat with oleic acid: Characterization of process and product. *Enzyme and Microbial Technology, 23*, 118–128.

Balcăo, V. M., & Malcata, F. X. (1997). Lipase-catalyzed modification of butterfat via acidolysis with oleic acid. *Journal of Molecular Catalysis B: Enzymatic, 3*, 161–169.

Balcăo, V. M., & Malcata, F. X. (1998a). Interesterification and acidolysis of butterfat with oleic acid by *Mucor javanicus* lipase: Changes in the pool of fatty acid residues. *Enzyme and Microbial Technology, 22*, 511–519.

Balcăo, V. M., & Malcata, F. X. (1998b). Lipase catalyzed modification of milkfat. *Biotechnology Advances, 16*, 309–341.

Balcăo, V. M., & Malcata, F. X. (2002). Enzyme-mediated modification of milkfat. In T. M. Kuo & H. W. Gardner (Eds.), *Lipid biotechnology* (pp. 479–492). New York, NY: Marcel Dekker.

Barach, J. T., Adams, D. M., & Speck, M. L. (1976). Low temperature inactivation in milk of heat-resistant proteases from psychrotrophic bacteria. *Journal of Dairy Science, 59*, 391–395.

Barber, M. C., Clegg, R. A., Travers, M. T., & Vernon, R. G. (1997). Lipid metabolism in the lactating mammary gland. *Biochimica et Biophysica Acta, Lipids and Lipid Metabolism, 1347*, 101–126.

Bills, D. D., & Day, E. A. (1964). Determination of the major free fatty acids in Cheddar cheese. *Journal of Dairy Science, 47*, 733–738.

Bourlieu, C., Bouhallab, S., & Lopez, C. (2009). Biocatalyzed modifications of milk lipids: Applications and potentialities. *Trends in Food Science and Technology, 20*, 458–469.

Bourlieu, C., Rousseau, F., Briard-Bion, V., Madec, M. N., & Bouhallab, S. (2012). Hydrolysis of native milk fat globules by microbial lipases: Mechanisms and modulation of interfacial quality. *Food Research International, 49*, 533–544.

Buchanan, R. A. (1965). Lipolysis and the frothing of milk. *Australian Journal of Dairy Technology, 20*, 62–66.

Bucky, A. R., Hayes, P. R., & Robinson, D. S. (1987). A modified ultra-high temperature treatment for reducing microbial lipolysis in stored milk. *The Journal of Dairy Research, 54*, 275–282.

Buffa, M., Guamis, B., Pavia, M., & Trujillo, A. J. (2001). Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk. *International Dairy Journal, 11*, 175–179.

Cartier, P., & Chilliard, Y. (1990). Spontaneous lipolysis in bovine milk: Combined effects of nine characteristics in native milk. *Journal of Dairy Science, 73*, 1178–1186.

Chmura, M., Staniewski, B., Panfil-Kuncewicz, H., Szpendowski, J., & Zawadzka, J. (2008). The effects of enzymatic interesterification on selected physicochemical characteristics of milk fat. *Milchwissenschaft, 63*, 37–40.

Christen, G. L., Wang, W.-C., & Ren, T.-J. (1986). Comparison of the heat resistance of bacterial lipases and proteases and the effect on ultra-high temperature milk quality. *Journal of Dairy Science, 69*, 2769–2778.

Christensen, T. C., & Holmer, G. (1993). Lipase-catalyzed acyl-exchange reactions of butter oil - synthesis of a human-milk fat substitute for infant formulas. *Milchwissenschaft, 48*, 543–548.

Chung, G. H., Lee, Y. P., Jeohn, G. H., Yoo, O. J., & Rhee, J. S. (1991). Cloning and nucleotide sequence of thermostable lipase gene from *Pseudomonas fluorescens* SIK W1. *Agricultural and Biological Chemistry, 55*, 2359–2365.

Corrandini, C., & Innocente, N. (1994). Influence of proteose peptone fraction on milk foaming capacity. *Scienza Technica Lattiero-Caseria, 45*, 107–113.

Danthine, S., Blecker, C., Paquot, M., Innocente, N., & Deroanne, C. (2000). Progress in milk fat globule membrane research: A review. *Le Lait, 80*, 209–222.

De, B. K., Hakimji, M., Patel, A., Sharma, D., Desai, H., & Kumar, T. (2007). Plastic fats and margarines through fractionation, blending and interesterification of milk fat. *European Journal of Lipid Science and Technology, 109*, 32–37.

Deeth, H. C. (1978). Fluorimetric detection of carboxyl-esterase activity in milk. In *Proceedings of 20th International Dairy Congress E* (pp. 364–365). Brussels, Belgium: International Dairy Federation.

Deeth, H. C. (1997). The role of phospholipids in the stability of the milk fat globule membrane. *Australian Journal of Dairy Technology, 52*, 44–46.

Deeth, H. C. (2006). Lipoprotein lipase and lipolysis in milk. *International Dairy Journal, 16*, 555–562.

Deeth, H. C., & Fitz-Gerald, C. H. (1975a). Factors governing the susceptibility of milk to spontaneous lipolysis. In *IDF document 86* (pp. 24–34). Brussels, Belgium: International Dairy Federation.

Deeth, H. C., & Fitz-Gerald, C. H. (1975b). The relevance of milk lipase activation to rancidity in Cheddar cheese. *Australian Journal of Dairy Technology, 30*, 74–76.

Deeth, H. C., & Fitz-Gerald, C. H. (2006). Lipolytic enzymes and hydrolytic rancidity. In P. F. Fox & P. McSweeney (Eds.), *Advanced dairy chemistry, volume: Lipids* (pp. 481–556). New York, NY: Springer.

Deeth, H. C., & Smith, R. A. D. (1983). Lipolysis and other factors affecting the steam frothing capacity of milk. *Australian Journal of Dairy Technology, 38*, 14–19.

Dickow, J. A., Larsen, L. B., Hammershoj, M., & Wiking, L. (2011). Cooling causes changes in the distribution of lipoprotein lipase and milk fat globule membrane proteins between the skim milk and cream phase. *Journal of Dairy Science, 94*, 646–656.

Dieckelmann, M., Johnson, L. A., & Beacham, I. R. (1998). The diversity of lipases from psychrotrophic strains of *Pseudomonas*: A novel lipase from a highly lipolytic strain of *Pseudomonas fluorescens*. *Journal of Applied Microbiology, 85*, 527–536.

Dring, R., & Fox, P. F. (1983). Purification and characterization of a heat-stable lipase from *Pseudomonas fluorescens* AFT 29. *Irish Journal of Food Science and Technology, 7*, 157–171.

Fitz-Gerald, C. H., & Deeth, H. C. (1983). Factors influencing lipolysis by skim milk cultures of some psychrotrophic microorganisms. *Australian Journal of Dairy Technology, 38*, 97–103.

Fitz-Gerald, C. H., Deeth, H. C., & Coghill, D. M. (1982). Low-temperature inactivation of lipases from psychrotrophic bacteria. *Australian Journal of Dairy Technology, 37*, 51–54.

Fitz-Gerald, C. H., Deeth, H. C., & Kitchen, B. J. (1981). The relationship between the levels of free fatty acids, lipoprotein lipase, carboxylesterase, *N*-acetyl-β-D-glucosaminidase, somatic cell count and other mastitis indices in bovine milk. *The Journal of Dairy Research, 48*, 253–265.

Fox, P. F. (1980). Enzymes other than rennets in dairy technology. *Journal of the Society of Dairy Technology, 33*, 118–128.

Fox, P. F., & Guinee, T. P. (1987). Italian cheeses. In P. F. Fox (Ed.), *Cheese: Chemistry, physics and microbiology* (Vol. 2, pp. 221–255). London, UK: Elsevier Applied Science.

Fox, P. F., & Law, B. A. (1991). Enzymology of cheese ripening. *Food Biotechnology, 5*, 239–262.

Gambini, G., Castagnetti, G. B., & Losi, G. (1995). Influence of somatic cell count and heat treatments on milk foam formation and stability. *Industrie Alimentari, 34*, 247–252.

Garcia, H. S., Keough, K. J., Arcos, J. A., & Hill, C. G. (2000). Interesterification (acidolysis) of butterfat with conjugated linoleic acid in a batch reactor. *Journal of Dairy Science, 83*(3), 371–377.

Giet, J.-M., Aguedo, M., Danthine, S., Paquot, M., Thomas, A., Vandenbol, M., et al. (2009). Enzymatic interesterification of anhydrous milk fat with rapeseed and/or linseed oil: Oxidative stability. *Journal of Agricultural and Food Chemistry, 57*, 6787–6794.

Griffith, M. W., Phillips, J. D., & Muir, D. D. (1981). Thermostability of proteases and lipases number of species of psychrotrophic bacteria of dairy origin. *The Journal of Applied Bacteriology, 50*, 289–303.

Griffiths, M. W. (1983). Synergistic effects of various lipases and phospholipase C on milk fat. *Journal of Food Technology, 18*, 495–505.

Gripon, J. C. (1987). Mould-ripened cheeses. In P. F. Fox (Ed.), *Cheese: Chemistry, physics and microbiology* (Vol. 2, pp. 121–149). London, UK: Elsevier Applied Science.

Hayati, I. N., Aminah, A., Mamot, S., Aini, I. N., Lida, H. M. N., & Sabariah, S. (2000). Melting characteristic and solid fat content of milk fat and palm stearin blends before and after enzymatic interesterification. *Journal of Food Lipids, 7*, 175–193.

Hernell, O., & Bläckberg, L. (1991). Digestion and absorption of human milk lipids. In R. Dulbecco (Ed.), *Encyclopedia of human biology* (Vol. 3, pp. 47–56). New York, NY: Academic Press.

Hernell, O., & Bläckberg, L. (1994). Human milk bile salt-stimulated lipase: Functional and molecular aspects. *The Journal of Pediatrics, 125*(Suppl), S56–S61.

Hickey, D. K., Kilcawley, K. N., Beresford, T. P., & Wilkinson, M. G. (2007). Lipolysis in cheddar cheese made from raw, thermized, and pasteurized milks. *Journal of Dairy Science, 90*, 47–56.

Hohe, K. A., Dimick, P. S., & Kilara, A. (1985). Milk lipoprotein-lipase distribution in the major fractions of bovine-milk. *Journal of Dairy Science, 68*, 1067–1073.

Horwood, J. F., Lloyd, G. T., & Stark, W. (1981). Some flavour components of Feta cheese. *Australian Journal of Dairy Technology, 36*, 34–37.

Huppertz, T. (2010). Foaming properties of milk: A review of the influence of composition and processing. *International Journal of Dairy Technology, 63*, 477–488.

Iverius, P. H., Olivecrona, T., Egelrud, T., & Lindahl, U. (1972). Effects of heparin on lipoprotein-lipase from bovine milk. *The Journal of Biological Chemistry, 247*, 6610.

Javed, S., Azeem, F., Hussain, S., Rasul, I., Siddique, M. H., Riaz, M., et al. (2018). Bacterial lipases: A review on purification and characterization. *Progress in Biophysics and Molecular Biology, 132*, 23–34.

Jolly, R. C., & Kosikowski, F. V. (1975a). Flavor development in pasteurized milk blue cheese by animal and microbial lipase preparations. *Journal of Dairy Science, 58*, 846–852.

Jolly, R. C., & Kosikowski, F. V. (1975b). A new blue cheese food material from ultrafiltrated skim milk and microbial enzyme - Mold spore reacted fat. *Journal of Dairy Science, 58*, 272–1275.

Jooyandeh, H., Kaur, A., & Minhas, K. S. (2009). Lipases in dairy industry: A review. *Journal of Food Science and Technology, 46*, 181–189.

Kalo, P., Kemppinen, A., & Antila, M. (1987). *Candida cylindracae* lipase-catalyzed interesterification of butterfat and butterfat solid fraction rapeseed oil mixtures. *Journal of the American Oil Chemists' Society, 64*, 1263–1263.

Kalogridou-Vassiliadou, D. (1984). Lipolytic activity and heat resistance of extracellular lipases of some Gram-negative bacteria. *Milchwissenschaft, 39*, 601–603.

Kamath, S., Wulandewi, A., & Deeth, H. C. (2008). Relationship between surface tension, free fatty acid concentration and foaming properties of milk. *Food Research International, 41*, 623–929.

Karabulut, I., Turan, S., Vural, H., & Kayahan, M. (2007). Human milk fat substitute produced by enzymatic interesterification of vegetable oil blend. *Food Technology and Biotechnology, 45*, 434–438.

Kilara, A. (1985). Enzyme-modified lipid food ingredients. *Process Biochemistry, 20*, 35–45.

Kim, J. S., Maeng, I. K., Lee, B. O., Kim, C. K., Kwon, Y. H., & Kim, Y. J. (2002). Lipase-catalyzed acidolysis of butterfat with α-linolenic acid from perilla oil. *Food Science and Biotechnology, 11*, 66–70.

Kinnunen, P. K. J., Huttunen, J. K., & Ehnholm, C. (1976). Properties of purified bovine milk lipoprotein lipase. *Biochimica et Biophysica Acta, 450*, 342–351.

Kinsella, J. E., & Hwang, D. (1976). Biosynthesis of flavours by *Penicillium roqueforti*. *Biotechnology and Bioengineering, 18*, 927–938.

Kishonti, E. (1975). Influence of heat resistant lipases and proteases in psychrotrophic bacteria on product quality. In *IDF document 86* (pp. 121–124). Brussels, Belgium: International Dairy Federation.

Kontkanen, H., Rokka, S., Kemppinen, A., Miettinen, H., Hellstrom, J., Kruus, K., et al. (2011). Enzymatic and physical modification of milk fat: A review. *International Dairy Journal, 21*, 3–13.

Landaas, A., & Solberg, P. (1978). Production and characterization of lipase from a fluorescent pseudomonad. In *Proceedings of 20th International Dairy Congress (Paris) E* (pp. 304–305). Brussels, Belgium: International Dairy Federation.

Law, B. A. (1984). Flavour development in cheeses. In F. L. Davies & B. A. Law (Eds.), *Advances in the microbiology and biochemistry of cheese and fermented milk* (pp. 187–208). London, UK: Elsevier Applied Science.

Law, B. A., Sharpe, M. E., & Chapman, H. R. (1976). The effect of lipolytic Gram-negative psychrotrophs in stored milk on the development of rancidity in Cheddar cheese. *The Journal of Dairy Research, 43*, 459–468.

Law, B. A., & Wigmore, A. S. (1985). Effect of commercial lipolytic enzymes on flavor development in cheddar cheese. *Journal of the Society of Dairy Technology, 38*, 86–88.

Lerin, L. A., Loss, R. A., Remonatto, D., Zenevicz, M. C., Balen, M., Oenning Netto, V., et al. (2014). A review on lipase-catalyzed reactions in ultrasound-assisted systems. *Bioprocess and Biosystems Engineering, 37*, 2381–2394.

Lubary, M., Hofland, G. W., & ter Horst, J. H. (2011). A process for the production of a diacylglycerol-based milk fat analogue. *European Journal of Lipid Science and Technology, 113*, 459–468.

Marin, A., Mawhinney, T. P., & Marshall, R. T. (1984). Glycosidic activities of *Pseudomonas fluorescens* on fat-extracted skim milk, buttermilk and milk fat globule membranes. *Journal of Dairy Science, 67*, 52–59.

Marquardt, R. R., & Forster, T. L. (1962). Arylesterase activity of bovine milk as related to incidence of mastitis. *Journal of Dairy Science, 45*, 653.

Marquardt, R. R., & Forster, T. L. (1965). Milk A-esterase levels as influenced by stage of lactation. *Journal of Dairy Science, 48*, 1526–1528.

McKellar, R. C. (Ed.). (1989). *Enzymes of psychrotrophs in raw food*. Boca Raton, FL: CRC Press.

Morais Nunes, G. F., de Paula, A. V., de Castro, H. F., & dos Santos, J. C. (2011). Optimization of the enzymatic interesterification of milk fat and canola oil blends using immobilized *Rhizopus oryzae* lipase by response surface methodology. *Food Technology and Biotechnology, 49*, 385–390.

Morley, N., & Kuksis, A. (1977). Lack of fatty-acid specificity in lipolysis of oligo-saturated and polyunsaturated triacylglycerols by milk lipoprotein-lipase. *Biochimica et Biophysica Acta, 487*, 332–342.

Mottar, J. (1981). Heat resistant enzymes in UHT milk and their influence on sensoric changes during uncooled storage. *Milchwissenschaft, 36*, 87–91.

Muir, D. D., Phillips, J. D., & Dalgleish, D. G. (1979). The lipolytic and proteolytic activity of bacteria isolated from blended raw milk. *Journal of the Society of Dairy Technology, 32*, 19–23.

Nilsson-Ehle, P., Egelrud, T., Belfrage, P., Olivecrona, T., & Borgstrom, B. (1973). Positional specificity of purified milk lipoprotein-lipase. *The Journal of Biological Chemistry, 248*, 6734–6737.

Noronha, N., Cronin, D., O'Riordan, D., & O'Sullivan, M. (2008). Flavouring reduced fat high fibre cheese products with enzyme modified cheeses (EMCs). *Food Chemistry, 110*, 973–978.

Olivecrona, T., Vilaró, S., & Olivecrona, G. (2003). Lipases in milk. In P. F. Fox & P. L. H. McSweeney (Eds.), *Advanced dairy chemistry: Proteins* (Vol. 1, 3rd ed., pp. 473–494). New York, NY: Kluwer Academic.

Oterholm, A., Ordal, Z. J., & Witter, L. D. (1970). Glycerol ester hydrolase activity of *Propionibacterium shermanii*. *Journal of Dairy Science, 53*, 592–593.

Parodi, P. W. (1999). Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *Journal of Dairy Science, 82*, 1339–1349.

Riaublanc, A., Ratomahenina, R., Galzy, P., & Nicolas, M. (1993). Peculiar properties of lipase from *Candida-parapsilosis* (Ashford) Langeron and Talice. *Journal of the American Oil Chemists' Society, 70*, 497–500.

Rønne, T. H., Yang, T. K., Mu, H. L., Jacobsen, C., & Xu, X. B. (2005). Enzymatic interesterification of butterfat with rapeseed oil in a continuous packed bed reactor. *Journal of Agricultural and Food Chemistry, 53*, 5617–5624.

Rousseau, D., Forestiere, K., Hill, A. R., & Marangoni, A. G. (1996). Restructuring butterfat through blending and chemical interesterification. 1. Melting behavior and triacylglycerol modifications. *Journal of the American Oil Chemists' Society, 73*, 963–972.

Rousseau, D., Hill, A. R., & Marangoni, A. G. (1996). Restructuring butterfat through blending and chemical interesterification. 3. Rheology. *Journal of the American Oil Chemists' Society, 73*, 983–989.

Rowe, M. T., Johnston, D. E., Kilpatrick, D. J., Dunstall, G., & Murphy, R. J. (1990). Growth and extracellular enzyme production by psychrotrophic bacteria in raw milk stored at a low temperature. *Milchwissenschaft, 45*, 495–499.

Schmid, U., Bornscheuer, U. T., Soumanou, M. M., McNeill, G. P., & Schmid, R. D. (1998). Optimization of the reaction conditions in the lipase-catalyzed synthesis of structured triglycerides. *Journal of the American Oil Chemists' Society, 75*, 1527–1531.

Sehanputri, P. S., & Hill, C. G. (2003). Lipase-mediated acidolysis of butteroil with free conjugated linoleic acid in a packed bed reactor. *Biotechnology and Bioengineering, 83*, 608–617.

Seitz, E. W. (1974). Industrial application of microbial lipases - Review. *Journal of the American Oil Chemists' Society, 51*, 12–16.

Shelley, A. W., Deeth, H. C., & MacRae, I. C. (1986). Growth of lipolytic pschrotrophic pseudomonads in raw and ultra-heat-treated milk. *The Journal of Applied Bacteriology, 61*, 395–400.

Shin, J.-A., Akoh, C. C., & Lee, K.-T. (2010). Enzymatic interesterification of anhydrous butterfat with flaxseed oil and palm stearin to produce low-trans spreadable fat. *Food Chemistry, 120*, 1–9.

Sørensen, A. D. M., Xu, X., Zhang, L., Kristensen, J. B., Jacobsen C. (2010) Human Milk Fat Substitute from Butterfat: Production by Enzymatic Interesterification and Evaluation of Oxidative Stability. *Journal of the American Oil Chemists' Society, 87*, (2):185–194.

Sørhaug, T., & Stepaniak, L. (1997). Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. *Trends in Food Science and Technology, 8*, 35–41.

Soumanou, M. M., Perignon, M., & Villeneuve, P. (2013). Lipase-catalyzed interesterification reactions for human milk fat substitutes production: A review. *European Journal of Lipid Science and Technology, 115*, 270–285.

Stepaniak, L., Birkeland, S.-E., Sørhaug, T., & Vagias, G. (1987). Isolation and partial characterization of heat stable proteinase, lipase and phospholipase C from *Pseudomonas fluorescens* P1. *Milchwissenschaft, 42*, 75–79.

Stewart, D. B., Murray, J. G., & Neill, S. D. (1975). Lipolytic activity of organisms isolated from refrigerated bulk milk. In *IDF document 86* (pp. 38–50). Brussels, Belgium: International Dairy Federation.

Sugiura, M., Oikawa, T., Hirano, K., & Inukai, T. (1977). Purification, crystallization and properties of triacylglycerol lipase from *Pseudomonas fluorescens*. *Biochimica et Biophysica Acta, 488*, 353–358.

Sundheim, G. (1988). Spontaneous lipolysis in bovine milk: Combined effects of cream, skim milk, and lipoprotein lipase activity. *Journal of Dairy Science, 71*, 620–626.

Te Whaiti, I. E., & Fryer, T. F. (1978). Production and heat stability in milk of proteinases and lipases of psychrotrophic pseudomonads. In *Proceedings of 20th International Dairy Congress (Paris) E* (pp. 303–304). Brussels, Belgium: International Dairy Federation.

Woo, A. H., & Lindsay, R. C. (1984). Concentration of major free fatty acids and flavor development in Italian cheese varieties. *Journal of Dairy Science, 67*, 960–968.

Yang, B., Harper, W. J., Parkin, K. L., & Chen, J. (1994). Screening of commercial lipases for production of mono- and diacylglycerols from butteroil by enzymic glycerolysis. *International Dairy Journal, 4*, 1–13.