Role of Milk Fat in Dairy Products

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9.1 Role of Milk Fat in Butter

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The popularity of butter is on the rise again with consumption in EU28 up to 9% since 2013 (CLAL 2019). In the 1970s, the association between saturated fats and cardiovascular disease triggered a move away from butter to vegetable oil alternatives. In recent years, customers have allowed fat, and butter, back into their diets.

Conventional dietary guidelines, which recommend a very low intake of fats, have not accounted for the diverse range of food sources, processing or specific individual complex nutrients and structures in dairy products. Following a review of butter consumption and cardiometabolic outcomes, Pimpin *et al.* (2016) found that there was no association with cardiovascular disease in a balanced diet.

Butter is defined as a "product with a milk-fat content of not less than 80% but less than 90%, a maximum water content of 16% and a maximum dry non-fat milk-material content of 2%" (EU 2013). This simple definition belies a complex microstructure of a continuous crystal network of partially coalesced fat globules, dispersed in a liquid oil phase and interrupted by intact fat globules, water and air droplets (Juriaanse and Heertje 1988). The result is an intricate interaction of texture, melting characteristics, colour, aroma and flavour.

Butter holds an almost unique place on our dining table. It possesses a delicate sweet creamy flavour, with a fat content that can act as an effective solvent to accept the flavour of other ingredients and aid their dispersion. Butter acts as a

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spreadable moisture barrier in a sandwich, but with ideal melt-in-the-mouth characteristics. As a laminate between dough layers, butter traps steam to yield the *millefeuille* of croissants and puff pastry (Mattice and Marangoni 2017). As an emulsion base for *roux*, or sautéing vegetables in a *beurre* blanc, *noisette* or *noir*, the heat sensitivity of milk solids yields a gastronomic playground as Maillard browning reactions are exploited (van Boekel 1998; Kato 2003; McGee 2004).

No essential difference exists to facilitate this wide range of applications. The multiple natures are determined by the three-dimensional solid fat crystal network, surrounded by the liquid oil phase and other milk solids (DeMan and Beers 1987).

9.1.1 Milk Fat and the Milk Fat Globule Membrane (MFGM)

Fat in milk is organized as spherical lipid droplets ranging in size from 0.1 to 10 μm, with a mean diameter of around 4 μm. The core of the droplet is comprised mainly of triglycerides (TAGs) (98%) and smaller quantities of diglycerides, monoglycerides and cholesterol esters and is stabilized by an outer biological membrane, the milk fat globule membrane (MFGM), composed of polar lipids, cholesterol and proteins. The MFGM is asymmetric in composition, having an inner layer of neutral lipids, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol and an outer layer of polar lipids, phospholipids (phosphatidylcholine and sphingomyelin), glycolipids (cerebrosides and gangliosides) and sphingolipid domains (Deeth 1997; Lopez *et al.* 2011). The phospholipids and glycolipids, together with the protein components, are important for the emulsification role of the MFGM (Thomé and Eriksson 1973).

The MFGM is sensitive to physiological, chemical, biological (enzymatic) and physical (mechanical) changes. Typical handling processes such as ageing, agitation, separation, foaming and temperature treatment can easily cause damage and loss of MFGM material (see review by Evers 2004). The MFGM cannot be regarded as intact at the beginning of buttermaking. The separation of milk to skimmed milk and cream causes some damage, resulting in loss of surface proteins and destabilizing the MFGM (Holzmüllera *et al.* 2016).

9.1.2 Buttermaking: Whipping of Cream

At the beginning of buttermaking, the cream is whipped. Air is incorporated into this oil-in-water emulsion (35–40% fat) by a shear action forming and breaking bubbles, which are initially stabilized by the adsorption of soluble proteins, especially β-casein and whey proteins, to the air/water interface (Brooker 1986). A secondary process involves the collision of fat globules inducing aggregation and partial coalescence at the interface to create a continuous enveloping matrix to stabilize the foam (Anderson *et al.* 2005; Goff 1997). The aqueous phase becomes trapped between the fat globules by capillary action. This process can only occur if there has been sufficient cooling of the cream to achieve a suitable solid to liquid fat ratio. Otherwise, the whipping process would cause a release of excess butter oil, which would spread across the interface leading to eventual bubble collapse (Prins 1986; Brooker 1993; Holtrum 2004).

9.1.3 Cooling and Crystallization

Cooling and crystallization of fat within the MFGM occurs differently than in bulk oil environments (Herrera and Hartel 2000; Fredrick *et al.* 2011; Pérez-Martínez *et al.* 2012). Within the MFGM is an environment thought to be free from impurities, which may otherwise trigger early seed crystal formation, meaning that the crystallization behaviour is affected by the triacylglycerol (TAG) composition alone (Lopez *et al.* 2001). This can generally be split into three fractions: a low melting fraction (LMF) melting in the range of −25 °C to 10 °C, a medium melting fraction (MMF) from 10 °C to 19 °C and a high melting fraction (HMF) above 20 °C (Deffense 1993). As the cream is chilled, supercooling occurs within each range, and nucleation of seed fat crystals begins. Slow cooling rates favour the formation of a small number of larger crystals with a wide size distribution (Lopez *et al.* 2001; Rønholt *et al.* 2012). Before the crystals

aggregate, they have time to become organized into a layered structure creating an internal crystalline shell that gives the globule stability. These large crystals have the potential to grow during ageing and can later pierce the MFGM when subjected to shear forces during churning (Rønholt *et al.* 2014a). Fast cooling rates, on the other hand, are shown to induce rapid nucleation of a large number of small uniform crystals, which aggregate more quickly, forming bulges in the outer surface of the MFGM (Moens *et al.* 2019). These bulges are more susceptible to rupture on collision resulting in greater partial coalescence with other globules.

Fat crystallizes in one of three forms: α, β´ or β, in increasing order of stability (Chapman 1962). Initial crystallization in the unstable α-form occurs in cream <20 °C and transitions to the desirable β´-form during ageing (Van Aken and Visser 2000; Fredrick *et al.* 2011). After cooling, cream is held at cool temperatures (typically 10–12.8 °C) to continue the crystallization of the fat for a period of 12–15 h. Lopez *et al.* (2002), using X-ray diffraction, noted that cooling cream yielded a triple layer α-phase, which transforms in the following hours into double and triple layer β´-forms. The temperature and composition of the TAGs within the globule determine the amount of fat that converts to a crystalline state or solid fat content (SFC).

9.1.4 Tempering of Milk Fat

The balance of unsaturated (UFA) and saturated fatty acids (SFA) in milk throughout the year affects the SFC for buttermaking (Phelan *et al.* 1982; Cullinane *et al.* 1984b; Couvreur *et al.* 2006). These variations are mainly attributed to changing feeding systems (grass vs mixed ration) during a season. Without modification of the buttermaking process, changes to this balance would result hard and brittle butter in the winter and soft and oily butter in the summer. To counteract these variations in preparation for churning, the pasteurized cream can be tempered through a thermal programme to manipulate size and distribution of the solid and liquid phases. "Hard" winter cream requires cooling to 8 °C to induce as few crystals as possible, before heating to 20 °C to melt the bulk oil phase leaving only the hard-fat crystals (Szakaly and Schaffer 1988; Bylund 1995). This cream is cooled back to 16 °C where any crystallizing fat will adhere to the existing crystals. This yields a higher volume of free liquid oil to soften the resultant butter. In summer, the Alnarp "6-12-6" (cold-warm-cold) method or variations can be used to crystallize a greater proportion of the total fat content. Rapid cooling leads to many small crystals and a larger crystal surface area. As a result, more liquid fat will be adsorbed to the crystal surfaces leaving less liquid fat available to form the continuous oil phase during churning and working, and a firmer butter results. Care should be taken to ensure that sufficient liquid oil remains to act as bridges between the solid fat crystals to create butter grains (Rønholt *et al.* 2014c; Lee and Martini 2018).

9.1.5 Churning and Working the Butter Grains

As churning continues, the MFGM in contact with the air interface ruptures, and the core liquid fat spreads over the surface (Evers 2004). The continued high shear forces of churning increase the air volume making the fat lamellae between bubbles progressively thinner. The fat globules concentrated in the serum phase form larger aggregates, leading to distortion and penetration of the MFGM by solid fat crystals, coalescence and subsequent leakage of internal liquid oils (Schmidt and van Hooydonk 1980; Brooker 1986). The stability of the crystals may be influenced by the ageing temperature, or starting temperature, which helps them to withstand the effect of shear and churning time (Rønholt *et al.* 2012). The spread of liquid fat collapses the foam and triggers an inversion to a water-in-oil emulsion and the formation of butter grains, with the consequent release of the bulk water volume as buttermilk containing MFGM fragments.

The butter grain mass is worked gently to disperse entrapped water (and salt, if added) in the continuous phase and serves to release additional fat crystals and liquid oil from the remaining fat globules. The deformation of the worked butter causes stress in the moisture droplets, disrupting

larger droplets and resulting in discrete water droplets of 5–10 μm (Walstra *et al.* 2006). Water droplets are prevented from recoalescing by the continuous fat lamella and are too small to support microbial growth.

9.1.6 Changes During Storage

After production, butter is stored to allow it to set but is often held for prolonged periods in either a refrigerated or frozen state for commercial reasons. During the initial period, more of the liquid fat becomes integrated into the solid fat matrix, and the SFC increases. Within the first day of storage, Rønholt *et al.* (2014b) found that all α-crystals had transformed to β´-form. However, no further increase was noted in the elastic modulus (G´) or hardness. Similar findings were observed by Méndez-Cid *et al.* (2017) for butter stored for 9 months at 4 °C and 12 °C.

Milk possesses a range of antioxidant components, including the sulphur-containing amino acid cysteine, carotenoids, vitamins, metals (selenium and zinc) and antioxidant enzyme systems, such as superoxide dismutase, catalase and glutathione peroxidase (Khan *et al.* 2019). β-Carotene comprises 90% of the carotenoids in cows' milk (Ollilainen *et al.* 1989, Hulshof *et al.* 2006). It is acquired in the diet and stored in the fat globule core (Jensen and Nielsen 1996), shielded by the intact MFGM, but contributes to the rich colour of butter and cheese when exposed. It is a strong quenching antioxidant, binding light and acting as a scavenger of free radicals, such as singlet and triplet oxygen (Foote 1976; Min and Boff 2002). β-Carotene is partially metabolized by the cow to form retinol (vitamin A_1) but is entirely converted in buffalo, goat, sheep milk resulting in white cheese and butter products from these animals.

Méndez-Cid *et al.* (2017) recorded fat oxidation in samples held at 4 °C and 12 °C, with and without salt, rising from 0.39 to 0.90 and 1.36 meq O_2/kg fat for unsalted butter, respectively, and from 0.38 to 1.72 and 3.75 meq O_2/kg fat, respectively, for salted butter. Salt, which is added to butter as a preservative and for flavour, has been identified as a pro-oxidant in food systems with the chloride ion being the active agent possibly through the inhibition of antioxidant enzymes and increasing catalytic activity of metals (Osinchak *et al.* 1992; Cui *et al.* 2018). Salted butter stored at 5 °C and -20 °C over 18 months showed a similar oxidative pattern (Krause *et al.* 2008), with a stale off-flavours developing in the refrigerated samples within 6–9 months. Off-flavours were not evident in the frozen samples until 12–18 months.

Rancid off-flavours in butter are caused by free fatty acids (FFA) released from TAGs by the action of lipases. Low levels of short-chain FAA may be perceived as pleasant, but when storage conditions permit the release of high concentrations of stronger off-flavours result causing a downgrading of the butter (McNeill *et al.* 1986). Indigenous milk lipoprotein lipase is associated with the casein micelle (Deeth 2006) and therefore absent from the lipid fraction. In addition, this enzyme is heat labile and almost totally inactivated by pasteurization. However, heat-resistant psychrotrophic bacterial lipases can remain active (Woo and Lindsay 1984). Psychrophilic bacteria, whose growth is favoured by extended storage at low temperature, can produce heat-stable lipases. Their growth is estimated to be low due to the dispersed and isolated nature of minute water droplets, despite the relatively high moisture content and water activity (a_w) (Voysey *et al.* 2009). Production variation can result in a coarse dispersion of moisture and, combined with surface contamination, may allow the post-production growth of *Pseudomonas* spp. and moulds causing hydrolytic rancidity and surface taint (Jay 2000; Suryavanshi and Ghosh 2010).

9.1.7 Rheological Properties

Texture is one of the main factors that determines the acceptance of butter as a product. It influences butter's appearance, spreadability, mouthfeel, taste and its suitability for use in a wide range of applications. The composition and structure of the milk fat are mainly responsible for the rheological properties of butter.

The spreadability and melting characteristics of milk fat and butter are complex. Milk fat contains thousands of TAGs with different fatty acid

combinations, each with their own melting point within the range of -40 to 40 °C. Within the butter, this balance of aggregated solid fat surrounded by liquid fat gives it a viscous nature. On testing, butter displays non-Newtonian behaviour and behaves as a plastic with yield values above which the butter will flow/spread (DeMan and Beers 1987). Generally, a butter with a SFC of between 20 and 40% is expected to be spreadable. It should be noted that a minimum of 9% SFC is required to prevent movement and coalescing of moisture within butter (Rousseau *et al.* 2003). For use as a "roll-in" laminate layer for Danish pastry and croissants, a SFC of 10–40% over a temperature range of 33.3–10.0 °C is desired to allow the butter to spread evenly without tearing the dough (Baldwin *et al.* 1971). Rolling of the dough to create 15 layers is performed in a three-step process with intervals of cooling at 2–4 °C to retard softening. SFC content also acts to soften bread and protect baked products against staling; saturated TAGs complex with amylopectin to prevent starch recrystallization and moisture migration (Smith and Johansson 2003).

The temperature at which butter reaches critical SFC levels is, of course, dependent on its milk fat composition, which is largely determined by the feeding system and diet of the cow, combined with age, breed, species, genotype, pregnancy and stage of lactation (Chilliard *et al.* 2007). The type of feed system is dictated by the geographic and cultural factors such as climate, land availability, feedstock and needs of the animal. In Ireland and New Zealand, the alternation between winter-indoor silage/concentrate and summer-outdoor fresh grass grazing (Cullinane *et al.* 1984a, Auldist *et al.* 1998) has a significant effect on the fatty acid compositions. Unsaturated fatty acid (UFA) levels peak in summer (April–June in the northern hemisphere) mainly due to increases in oleic (18:1), linoleic (18:0), γ-linoleic (18:3) and palmitoleic (C16:1) acids and a decrease in palmitic acid (C16:0) (Cullinane *et al.* 1984a). The resultant summer butter was found to be softer (137 kPa at 4 °C, 37 kPa at 15 °C) compared with the firmer winter (December–February) butter (412 kPa at 4 °C, 137 kPa at 15 °C) (Cullinane *et al.* 1984b). Butter is deemed to have acceptable spreadability in the range of 30–60 kPa (Rohm and Raaber 1991). Couvreur *et al.* (2006) and O'Callaghan *et al.* (2016) confirmed the benefit of feeding proportions of fresh pasture in place of maize silage with a similar correlation of a decrease in the C:16:0/C18:1 ratio with increasing grass supplementation, improved butter texture and a decrease in off-odours. The butter from pasture-fed cows also gave significantly lower atherogenic (AI)[1] and thrombogenic index (TI)[2] scores showing an added health benefit.

9.1.8 Flavour and Aroma

Butter should have a mild, sweet, clean and pleasant flavour and delicate aroma making it well suited as a food ingredient (Bradley and Smukowski 2009). The profile of butter includes a fine balance of short-chain fatty acids, such as butanoic acid (buttery, cheesy, sweaty), γ- and δ-lactones (sweet, peach, coconut), indole and skatole (mothball/faecal), phenols and hydrocarbons with a phytoene skeleton (Kato 2003, Mallia *et al.* 2008). Milk fat can act as an excellent solvent for flavours and as a carrier in cooking in a multitude of ways. Controlled fermentation with bacteria introduces a lactic acid taste and aromas of diacetyl, butanoic acid and δ-decalactone to create the distinctive lactic or cultured cream.

On heating butter and flour (white *roux*) to 120 °C, Kato (2003) detected an overlay of a slightly roasted aroma of aldehydes, carboxylic acids and lactones. At 160–180 °C (brown *roux*), flavour develops further and is strongly influenced by the Amadori rearrangement associated with Maillard browning of fats, sugars and proteins: furans, cyclic ketoenols (sweet, fruity, floral) and nitrogen-containing pyrazines (nutty, roasted) (Shigematsu *et al.* 1977, Maga and Katz 2009).

[1] *Atherogenic index: the sum of the proportion of lauric and palmitic and four times the myristic levels, divided by the total concentration of saturated fatty acids*

[2] *Thrombogenic index: an index of the tendency to form blood clots in blood vessels*

Unfortunately, butter is also susceptible to acquiring off-flavours and aromas. Consumption of highly aromatic weeds or flowers, or exposure to strong environmental odours in air, up to 30 min to 24 h prior to milking can be detected in the final butter due to direct transfer via the rumen, breakdown products or changes in the biochemistry of the cow (Urbach 1990, Viallon *et al.* 2000). During the processing of the milk, care is taken to prevent exposure to cleaning chemicals, foul water or any other compound that could taint the flavour. Some consideration should be given when selecting a milk supply for delicate products; high-fat milk products or powders made from milk from pasture are less susceptible to oxidation than from dry feed, while milk with higher levels of methyl ketones and free fatty acids may benefit butter shortening in baking or certain cheese types (Urbach 1990, 1997).

Once produced, it is essential to minimize the exposure of butter to light and oxygen (Koyuncu and Tuncturk 2017). Dairy products are sensitive to light due to the photosensitizer riboflavin (vitamin B_2), which under the influence of light produces methional (musty, potato) in a reaction with methionine to create a sunlight (burnt, oxidized) aroma (Patton 1954). Small amounts of riboflavin are retained in the aqueous phase of butter: 0.34 mg/kg of a total of 60–63.4 mg/kg in milk (USDA 2018). Foil laminates offer the greatest protection and are far superior to parchment or transparent options (Emmons *et al.* 1986). While this is the standard form in Ireland, novel consumer-driven options include parchment only or butter sculptures in clear packaging. Lozano *et al.* (2007) confirmed foil laminates as the best method of preventing styrene and aroma migration into butter during long-term storage. Buttermaking, both artisanal and industrial, has the ability to produce consistent and highly functional products to meet consumer needs and desires. Knowledge of the important role of milk fat composition and behaviour is essential in understanding the complexities of its correct use.

9.2 Creams

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9.2.1 Introduction

Cream is a fluid milk product comparatively rich in fat, in the form of a fat-in-skimmed milk emulsion, obtained by physical separation from milk (Codex Alimentarius Commission 2018). This simple definition does not reflect that the word "cream" has for a long time been considered a premium product or a value-enhancing ingredient in milk products and other foods. The special "creaminess" results from the fine dispersion of the fat globules in the hydrophilic phase and depends strongly on the fat content. In separated cream, the diameter of fat globules ranges between ca. 1 and 8 µm. During further processing to the different cream products, this typical oil-in-water (o/w) emulsion is modified or even converted into another physical state. Modification can be achieved by homogenization, which markedly reduces the average fat globule size and improves creaminess. On the other hand, mechanical treatment of chilled cream causes destabilization (i.e. coalescence of the fat globules). This treatment and the concurrent entrapment of air are essential for whipping cream into a stable foam.

The fat content of cream products varies from about 10 to 50%. Products with a low, internationally not-yet standardized, fat content are "coffee cream" (≥10% fat, Germany), "half-and-half cream" (≥10.5% fat, USA), "half cream" (≥12% fat, UK) or "light cream" (≥12% fat,

France). Traditional whipping cream has 30 to 40% fat, whereas double cream contains about 50% fat. Creams of high fat content are also essential ingredients in dairy or non-dairy products such as some fresh cheese varieties or cream liqueurs. Cream powders refer to a group of milk powder products containing 40–75% fat. These products are used as ingredients for bakery products, chocolate, ice cream or sweet desserts.

Butter is manufactured from cream (30–80% fat) by phase inversion. Reviews on cream, cream processing and cream products have been published by Towler (1994), Early (1998), Kessler (2002), Smiddy *et al.* (2009) and Hoffmann (2015a, b). Two older IDF Bulletins (IDF 1992, 1996) dealt with pasteurized and Ultra High Temperature (UHT) creams.

In summary, the significance of milk fat in the different cream products is based on fat content, fat distribution, the physical state of the fat and, last but not least, the chemical, physical and sensory properties of the non-fat ingredients. In the following, interactions between these factors are described for the most important cream products.

9.2.2 Coffee Cream

In many countries, coffee cream is still a popular long-life product. However, foamed milk in coffee specialities such as "cappuccino" or "café latte" offered in coffee bars or brewed in automatic coffee machines at work or at home increasingly competes with coffee cream. In this section, "coffee cream" does not mean a national statutory term but simply an appropriate description of the functional properties. Such creams contain usually 10 or 12% fat, less frequently 15 or 18%. Traditionally, coffee creams are sterilized in bottles or cans. During the last 30 years, continuous flow sterilization in a UHT plant, followed by aseptic packaging, has replaced the former process to a large extent. The products need good stability both during storage and in hot coffee beverages. A shelf-life of several months at ambient temperature requires particularly low creaming and sedimentation in the package, which is facilitated by a lower fat content (10 or 12%) and optimized processing conditions, mainly heat treatment and homogenization. Scientific bases for the manufacture of such products were published by Buchheim *et al.* (1986), Abrahamsson *et al.* (1988) and Geyer and Kessler (1989). Summaries were given by Hoffmann (2004, 2015a, b).

The different creams may contain stabilizing salts, which can be added as an aqueous solution after standardization and preheating (high-temperature pasteurization at 90–95 °C). They raise the pH and/or complex Ca^{2+}, resulting in reduced aggregation of casein micelles during sterilization and in hot coffee beverages. Sodium phosphates have a reduced buffering capacity and increased ion exchange ability with an increasing degree of condensation (chain length). Trisodium citrate has both buffering and sequestering properties and is used also. Whereas phosphates and citrates are essential additives in traditionally sterilized cream, high-quality flow-sterilized creams (containing 10 or 12% fat) may be produced without additives or hydrocolloids.

Homogenization of cream results in the formation of a secondary fat globule membrane, consisting predominantly of micellar casein and denatured whey protein (Walstra *et al.* 1999). To obtain desirable product properties, the formation of larger, thermally induced protein aggregates and, particularly, fat/protein complexes must be avoided (Buchheim *et al.* 1986). The number and dimensions of the particles are influenced more by temperature than by heating time during flow sterilization. In general, such adverse structures are reduced by flow sterilization at ≤130 °C rather than at UHT temperatures (≥135 °C). Frequently, a second two-stage homogenization step is performed after heating in order to disrupt heat-induced fat/protein aggregates. Sensory effects of a lower heating temperature and a necessary prolonged heating time (≥1 min) are a more pronounced cooked flavour and a more brownish colour of the resulting cream. However, these effects are of minor relevance in the coffee beverage.

Physical properties of flow-sterilized cream can be controlled by homogenizing conditions. Usually, one homogenizer is integrated upstream (i.e. before flow sterilization) and one downstream. Each of the two homogenizers often

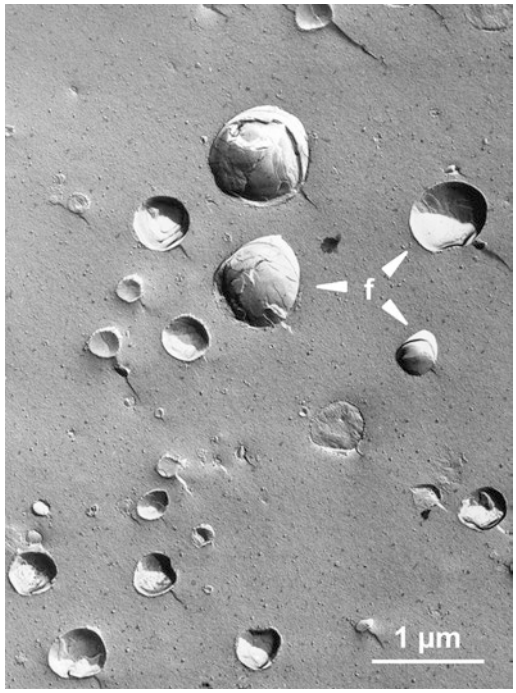

Figure 9.1. Electron micrograph of flow-sterilized coffee cream; f: homogenized fat globules.

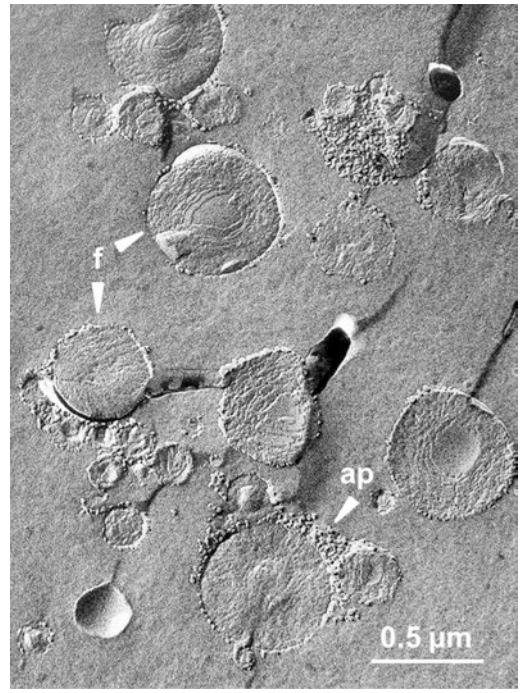

Figure 9.2. Electron micrograph of floccules in a coffee cream after feathering in a hot coffee solution (enlarged compared to Figure 9.1); f, fat globules; ap, aggregated protein.

operates at a total pressure (single- or double-stage) of about 20 MPa at 70 °C. A good coffee cream should have a narrow fat globule size distribution with a volume-mean diameter preferably between 0.4 and 0.6 µm and a very low degree of aggregation (Figure 9.1). This results in a product with low viscosity, high whitening power, slow creaming and high "coffee stability", i.e. resistance against feathering in hot coffee beverages.

The coffee stability is particularly important for the quality of the product. It is affected by the coffee brand and concentration, while high temperature (≥70 °C), low water hardness, low pH (about 5.0) and a high concentration of sulphates accelerate protein coagulation and, hence, fat/protein aggregation. Therefore, it must be ensured that this feathering remains invisible to the naked eye (Buchheim *et al.* 1986; Hoffmann *et al.* 1996) (Figure 9.2). The probability of feathering increases with the fat content of the cream. Coffee cream in small deep-drawn polystyrene (PS) cups lose 10–15% of their weight during their shelf-life of about 4 months which can result in floccules of condensed cream droplets.

9.2.3 Whipping Cream

Whereas the processing of long-life coffee cream is characterized by high-pressure homogenization and severe heat treatment, traditionally pasteurized whipping cream is produced carefully with less thermal input (ca. 85 °C for 10 s) and without homogenization (Figure 9.3). However, the demand for a longer shelf-life has led to a subsequent high-temperature pasteurization (≥110 °C) or even UHT heating with additional low-pressure homogenization. The higher thermal load results in more cooked flavour. The aim of UHT treatment is to produce sterile cream with a shelf-life of up to 3 months without refrigerated storage. Usually, indirect heating at ≥135 °C for a few seconds is applied in order to limit thermally induced physical, chemical and sensory changes. The homogenization effect

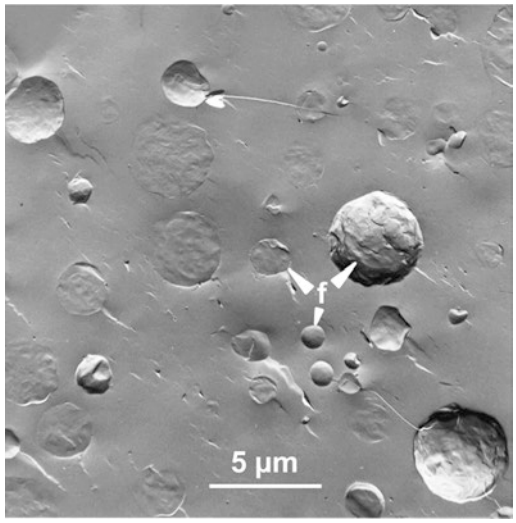

Figure 9.3. Electron micrograph of fluid whipping cream; f: fat globules.

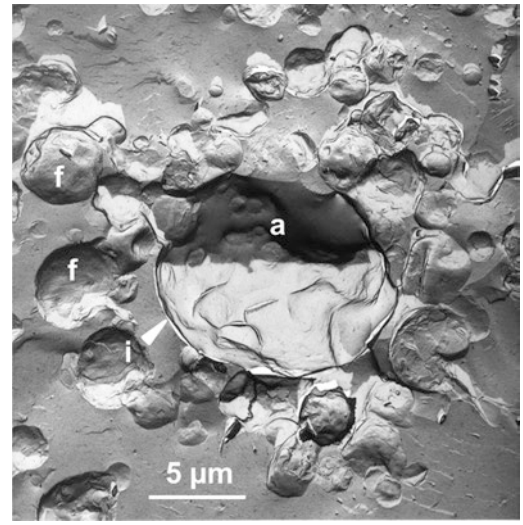

Figure 9.4. Electron micrograph of whipped cream; a, air cell; f, fat globules; I, interfacial layer.

must be moderate in order to retain acceptable whipping properties. Therefore, a downstream two-stage homogenization of cream at a total pressure of not more than 4 MPa is used frequently. Milk fat globule size of unhomogenized and slightly homogenized cream has an influence of whipping properties (Eden *et al.* 2016).

To improve the whipping properties of cream with a longer shelf-life, both stabilizers and emulsifiers may be added, if legally permitted. In particular, emulsifiers such as monoacylglycerols reduce the extended whipping time of homogenized cream (Anderson and Brooker 1988; Smiddy *et al.* 2009). The addition of stabilizers, most commonly hydrocolloids such as carrageenans (Kovácová *et al.* 2010), also in combination with milk constituents (whey proteins and high-melting fat fractions; see Precht *et al.* 1988) can slow down creaming during the shelf-life of cream and improve the stiffness and stability of whipped cream. Hydrocolloids exert their positive effect by increasing the viscosity of the milk serum phase (Smiddy *et al.* 2009).

A sufficient cooling of cream containing ≥30% fat is indispensable before whipping since the transformation of the original oil-in-water (o/w) emulsion into a stable foam requires that part of the fat is solid. The initial stage of whipping involves stabilization of the trapped air bubbles by a temporary interfacial film, largely of soluble whey proteins and non-micellar β-casein. During the second stage of whipping, the bubbles are reduced in size, and the overrun remains nearly constant. On mechanical treatment, fat globules increasingly lose at least segments of their natural membrane, thereby exposing strongly hydrophobic surface areas of pure fat. Subsequently, these partly destabilized fat globules adsorb at the air/serum interface of the air bubbles (Figure 9.4). The leakage of liquid fat from mechanically stressed and deformed fat globules supports globule agglomeration and partial coalescence. These agglomerates also interact with the air bubbles and may form bridges between them. The whole process of foam formation results in a partly coalesced fat globule network, which stabilizes the air cells, traps the serum phase and forms the characteristic stiff texture. These highly dynamic and concurrent processes also apply on the whole to UHT whipping cream subjected to low-pressure homogenization. Details of the interactions and processes during whipping are described by Anderson *et al.* (2005), Anderson and Brooker (1988), Buchheim (1991), Buchheim and Dejmek (1997), Smith *et al.* (2000), van Aken (2001) and Goff and Vega (2007). Han *et al.* (2018) presented confocal scanning laser micrographs; the

whole whipping process could be depicted and distinguished visually by different colours for fat and protein.

The whipping properties of creams are assessed by whipping time, increase in volume (expressed as overrun), foam firmness and subsequent serum leakage. Comparative studies require standardized temperature and procedure. Most test whipping devices are modifications of that described originally in 1937 by Mohr and Baur (see Hoffmann 2015b). Rheological properties of whipped cream can also be determined using a controlled-stress rheometer with parallel-plate geometry at 10 °C. The low temperature causes the fat to congeal on the plate, and a torque sweep is performed to establish the linear viscoelastic region. The subsequent frequency sweep uses the torque value from the middle of the linear region. The elastic modulus G', the viscous modulus G" and the resulting tan δ as a function of frequency are determined (Smith *et al.* 2000; Jacubczyk and Niranjan 2006).

Whipping of a typical cream increases the volume by 80–125% by inclusion of ambient air. UHT-treated creams can also be aerated by means of suitable propellants (e.g. N_2O), resulting in a volume increase in the range of about 300–600%. Such convenience products contain emulsifiers, stabilizers and usually also sugar and flavourings. These products are aseptically filled into sterilized aluminium or tin-plate cans. An obvious advantage of aerosol whipped cream is the speed and ease of foam production in controllable portions. The amount of gas dissolved in the cream must be sufficiently high to obtain satisfactory foaming properties, but is not allowed to exceed 1.5–2.0 MPa in the cans, particularly for household use, to avoid the risk of explosion. Compared with regular whipped cream, more fat globules adsorb at the air interfaces, and, simultaneously, agglomeration of fat globules is reduced substantially which results in an impaired network formation between the air bubbles. Due to the different foaming process, aerosol creams develop only soft foams with low stability (Buchheim 1991; Wijnen 1997; Smiddy *et al.* 2009).

Whipping cream ranks among premium food products and is consumed for its pure flavour. High-quality raw milk and separated cream must be handled carefully to minimize damage to the natural fat globule membrane. Excessive agitation and pumping should be avoided, and the flow velocity should not exceed the critical shear rate (Kessler 2002). Incorporated air bubbles increase the risk of damaged fat globules or can act as centres for fat globule aggregation and subsequent coalescence. During crystallization, fat globules are most sensitive to mechanical treatment. As a result of the partial or complete loss of the protective membrane, both indigenous and bacterial lipases catalyse the hydrolysis of exposed fat to fatty acids, imparting rancid taints (Kosinski 1996). When raw cream is homogenized without being subjected immediately to high-temperature pasteurization, indigenous milk lipoprotein lipase penetrates the secondary membrane of fat globules and hydrolyses triglycerides to free fatty acids within a few minutes, resulting in intense rancidity (Walstra *et al.* 1999).

Active extracellular bacterial lipases and proteinases of *Pseudomonas* spp. and most other Gram-negative psychrotrophs may be present, even in UHT cream, if refrigerated raw milk was stored for a prolonged period. They can contribute to rancid and tallowy flavours and also to bitty cream or serious physical changes such as gelation (Castberg 1992; Driessen and van den Berg; 1992; Houlihan 1992; Kosinski 1996).

Flavour defects in cream may occur not only during manufacture but also during transport or storage until the best-before date. UHT whipping cream with its long shelf-life at ambient temperature is particularly susceptible to off-flavours. Hence, adequate packaging materials must be chosen. Protection against oxygen and/or light is most important as they may induce oxidation of unsaturated fatty acids, leading to flavour deterioration. Stress history relating to light exposure during processing, packaging or retail display of cream increases the tendency of oxidation also during subsequent dark storage. Riboflavin acts as a photosensitizer and may initiate generation of radicals or other reactive species resulting in formation of lipid and protein oxidation products (Westermann *et al.* 2009). Paper cartons with a coating of polyethylene and an aluminium foil laminated to the inner carton layer are often used. Appropriate filling conditions should also be

selected to minimize the oxygen content of the package and the cream. However, a certain level of residual oxygen may be beneficial as the UHT process exposes free sulphydryl groups and releases hydrogen sulphide from β-lactoglobulin, thus creating the typical cooked flavour. During storage, oxidation of these groups occurs, and most of the cooked flavour disappears. A balanced antioxidative/oxidative action of sulphur groups and oxygen will probably help to ensure cream products of good taste and odour (Eyer *et al.* 1996).

An important factor for physical stability of cream is the temperature of the cream during transport and storage. Even a brief warming to ≥30 °C supports creaming during subsequent storage at 20 °C and may lead to a distinct thickening after cooling before whipping (Hoffmann 1999).

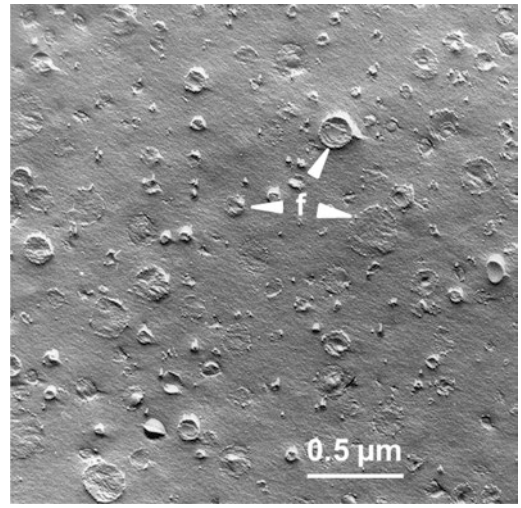

Figure 9.5. Electron micrograph of cream liqueur; f: homogenized fat globules.

9.2.4 Cream Liqueurs

Cream liqueurs combine the flavour of alcoholic drinks with the texture of cream in products. Cream liqueurs have a shelf-life of several years at ambient temperature. During that period, the liqueur must be resistant to both microbiological and physical changes. The microbiological safety is guaranteed by a sufficient concentration of alcohol (≥14%) together with a high sugar content (about 19%). Avoiding serious phase separation is the more demanding challenge. This can be achieved by optimal composition and processing. The addition of sodium caseinate (ca. 3%), trisodium citrate (ca. 0.2%) and possibly low-molecular-weight emulsifiers like monoacylglycerols (ca. 0.1%) stabilizes the o/w emulsion of the added cream (e.g. 16% of 48% fat cream) in the liqueur. This composition appears to represent many commercially available products. However, each manufacturer decides on the preferred components, and concentrations thereof, which enable a product of desired organoleptic properties (Smiddy *et al.* 2009; Hoffmann 2015a).

In the final liqueur product, more than 98% of the fat globules should have a diameter of <0.8 µm, resulting in enhanced viscosity, creaminess and whitening power (Banks and Muir 1988). The typical volume-mean diameter of about 0.2 µm is, by far, the smallest of all dairy products (Buchheim and Dejmek 1997) (Figure 9.5). This is achieved by multiple passes through a standard radial diffuser homogenizer or by a single pass through a high-pressure homogenizer (HPH) at 50–150 MPa. HPH may allow the use of lower pre-emulsion feed temperatures than conventional homogenization processes and has potential as a means of improving the shelf-life stability of cream liqueurs. However, too severe homogenization results in overprocessing of the products (Heffernan *et al.* 2009 and 2011).

Compared to unhomogenized cream, the total fat surface area increases by a factor of about 20 (up to ca. 40 m^2/g fat) in cream liqueurs. Therefore, sodium caseinate is dissolved first in hot water before adding the cream, sugar, citrate and a complementary emulsifier (if necessary). No other protein than sodium caseinate is able to provide the required long-term emulsion stability (Lynch and Mulvihill 1997). α_{S1}-Casein seems to be the fraction of sodium caseinate that is most soluble in ethanol (O'Kennedy *et al.* 2001; Mezdour *et al.* 2008). Trisodium citrate, a useful stabilizer for several dairy products, such as evaporated milk or sterilized coffee cream, complexes the Ca^{2+} and concurrently increases the pH. In cream liqueurs, trisodium citrate prevents the interaction between sodium caseinate and available calcium. Otherwise, gelation and

syneresis during storage would occur. If a cream liqueur with a substantially higher alcohol content than 14% is produced (e.g. 19%), addition of further alcohol after homogenization of cream (and other ingredients) is required in order to produce a stable emulsion. The manufacture of cream liqueurs ends with filling into brown glass bottles to prevent light-induced off-flavour. Very occasionally, during long-term storage, the formation of a non-redispersible cream or fat plug in the neck of the bottle may occur (Dickinson *et al.* 1989). The fatty solid-like cohesive structure of this plug points to unfavourable ambient temperatures, possibly accompanied by excessive mechanical agitation. The formation of neck-plug may be similar in origin to the thickening of whipping cream after warming for a short period (≥30 °C) and subsequent cooling.

9.2.5 Cultured Cream

Cultured or sour(ed) creams find various applications as valuable ingredients. They are used in cake mixes, as dip bases for snacks and vegetables and to complement sauces and dressings. Cultured creams are manufactured in many countries, and their fat content generally ranges from 10 to more than 40%. According to Codex Alimentarius Standards (Codex Commission 2018), fermented creams are soured by the action of suitable microorganisms, normally mesophilic lactic acid bacteria, to a pH of about 4.5. Cultures commonly used include *Lactococcus (Lc.) lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, citrate-positive strains of *Lc. lactis* and *Leuconostoc mesenteroides* subsp. *cremoris*, with *Lactobacillus acidophilus* also being increasingly used (Smiddy *et al.* 2009). Acidified creams are obtained by the action of food-grade acids and/or acidity regulators, often by a combination of lactic and citric acid, rarely glucono-δ-lactone. Lactic acid has bacteriostatic effects, while citric acid can be fermented by many organisms (Born 2013). Lactic acid-producing bacteria may also be added (e.g. acidified sour cream with ≥18% milk fat in the USA; Code of Federal Regulations Title 21, Part 131). The production of cultured cream is largely equivalent to that of other fermented milk products. It starts with the standardization of the fat content and may include enrichment of non-fat milk dry matter and hydrocolloids, if legally permitted. These ingredients improve texture and prevent syneresis of the final product. Adequate processing conditions and a higher fat content reduce the need for supplementation. The homogenization pressure required for cream decreases with increasing fat content. Homogenization after high-temperature pasteurization results in better consistency compared to upstream treatment. The fat globules participate directly in the following fermentation process and are integrated in the developed network (Buchheim and Dejmek 1997) (Figure 9.6). Normally, the use of mesophilic lactic acid bacteria results in long fermentation times (14–24 h). Typical cultured cream products should be uniform (without creaming), creamy and viscous with a slightly acidic, mild "cheesy" or "buttery" flavour. Advantages of direct acidification are elimination of culture-handling problems, improved production efficiency and extended shelf-life. Such creams have a similar appearance and texture as those of cultured sour creams, but the latter have a superior flavour (Smiddy *et al.* 2009). Cultured creams may also develop a nearly plastic consistency by modified composition and/or appropriate production and may then be used as low-fat spreads (o/w type).

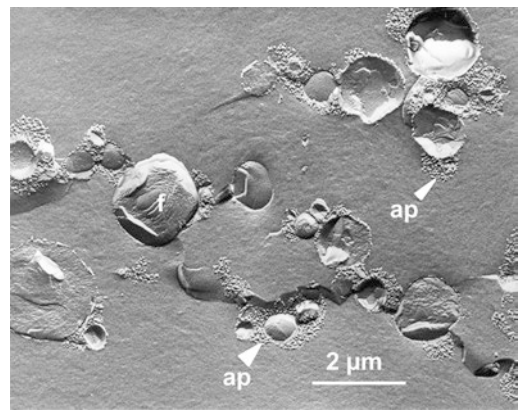

Figure 9.6. Electron micrograph of cultured cream (10% fat); f, fat globules; ap, aggregated protein.

9.2.6 Recombined Cream

These products are obtained by recombining milk products with or without the addition of potable water resulting in end product characteristics similar to those of natural cream (Codex Alimentarius Commission 2018). Over the last two decades, recombined creams have received increasing interest because of their obvious advantages in industrial production compared to fresh cream (van Lent *et al.* 2008). Unlike natural cream, the raw materials can easily be stored and transported to regions where fresh milk is not readily available and/or where suitable storage facilities are scarce. Moreover, the composition and manufacturing conditions of recombined creams can be modified for product development goals with different functional properties. Different dairy products have been used in studies over the years to emulsify anhydrous milk fat into recombined cream. Using cream residue powder instead of skim milk powder as protein source, such stabilized recombined creams mimicked fresh cream best as regards both emulsion and whipping properties (van Lent *et al.* 2008). An addition of different monoacylglycerols with fatty acids differing in chain length and/or saturation degree may influence the microstructural arrangement of the milk fat inside the fat globules and hence the whipping properties of the recombined cream (Frederick *et al.* 2013a, b). Milk fat globule membrane material from different sources has also the potential to improve whipping properties (Phan *et al.* 2014).

9.3 Role of Milk Fat in Dairy Products: Cheese

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Milk fat in cheese contributes to its flavour, texture, colour and functional behaviour. Its importance becomes apparent in any attempt at making a lower fat version of a cheese. Fat has multiple effects in cheese, including indirect effects on metabolic functioning of the microbiota during cheese ageing. This section will focus on the role of fat in *Cheddar*-like cheeses and *pasta filata* cheeses as these have been best studied.

9.3.1 Milk Fat and Cheese Flavour

Cheeses can be divided into two categories: those in which free fatty acids play a dominant role in their flavour profile and those in which excessive production of free fatty acids is considered a flavour defect. Fatty acids are released from milk fat by enzyme-catalysed lipolysis (1) through addition of lipases usually via rennet paste or pregastric esterases or (2) from production of lipases by cheese microbiota during ripening. In such cases, the contribution of fat to the flavour profile of cheese depends on fatty acid composition of the milk and activity and specificity of the lipase enzymes. The shorter-chain fatty acids such as butyric, capric, caproic and caprylic are most volatile and impact flavour the most. Their relative content in cow, sheep and goat's milk imparts slightly different flavour profiles to cheeses made from those milks. This field of lipolysis in cheese has been reviewed by Collins *et al.* (2003), and the remainder of this section will discuss the role of fat on cheeses made without exogenous lipases.

A common problem when fat content of cheese is lowered is that flavour changes and consumer liking decreases (Childs and Drake 2009). There is an obvious decrease in dairy or milk fat flavour (Drake *et al.* 2010), yet development of adverse flavours is not directly related to fat content. Carunchia Whetstine *et al.* (2006) found that removal of fat after ageing did not change cheese flavour profile or levels of volatile and aroma-active compounds. Flavour differences when lowering fat content of cheese are caused by altered biochemistry and a different array or balance of volatile compounds produced during ageing (Milo and Reineccius

1997) and differences in cheese texture (Drake *et al.* 2010).

Low-fat cheese contains the same key odorants as full-fat cheese but at different relative concentrations (Drake *et al.* 2010). Low-fat cheeses lack characteristic milk fat, sulphur and brothy flavours and instead are characterized by rosy and burnt flavours (reminiscent of burnt sugar). Rosy flavour was attributed to an increase in phenylethanal. The burnt flavour was attributed to an increase in furanones (especially homofuraneol) and 1-octen-3-one. Low-fat cheeses also had lower levels of methanethiol, a crucial precursor of compounds contributing to aged cheese flavour.

Bitterness observed in low-fat cheeses may be a cheese matrix effect rather than difference in levels of bitter compounds (Drake *et al.* 2010) as fat can alter sensory perception of bitter hydrophobic peptides. A slower rate of their release from the cheese mass during chewing would make them less noticeable in a full-fat cheese than in a low-fat cheese. Similarly, higher levels of fat in cheese present a more polar matrix which may influence detection thresholds of key odorants and influence perceived flavour.

The largest difference resulting in altered biochemistry during cheese ageing of lower-fat cheeses is that instead of being at 4.5 to 5.0% salt concentration, a low-fat cheese with the same overall 1.8% salt level may only have 3.5% salt in moisture. This alters survival and growth of bacteria as well as their metabolic activity (McMahon 2010; McMahon *et al.* 2014). If the salt concentration of a low-fat cheese is increased to a level comparable to that of a full-fat cheese, then apart from being overly salty and lacking in milk fat flavour, its other flavour attributes are similar to that of full-fat cheese.

9.3.2 Milk Fat and Cheese Colour

Removing fat from cheese imparts a translucent appearance and an increased intensity and atypical colour when annatto is added (Sipahioglu *et al.* 1999; Wadhwani *et al.* 2012). This has been attributed to fewer light-scattering centres (Pastorino *et al.* 2002) especially when fat is lowered by more than 50%. Other changes to cheese composition such as lowering calcium/protein ratio to soften the cheese and hold more moisture also contribute to this loss of opacity.

When desired flavour notes (such as buttery, nutty and cheese and milky attributes) are missing from a low-fat cheese, consumer attention is drawn to the cheese colour. In evaluations of low-fat cheeses made with the same flavour profile but different levels of annatto and titanium oxide to provide whiteness, the translucency of low-fat cheese was detrimental to consumer liking (Wadhwani *et al.* 2012). Even though trained panelists tasting cheese under red light perceived no differences in flavour, consumers rated the level of sharpness based upon colour and opacity of the cheese. Cheeses that were more opaque and whiter were considered to be mild to medium in flavour, while cheeses with the most annatto added were considered to have more flavour.

9.3.3 Milk Fat and Cheese Texture

Considerations on how fat influences cheese texture must take into account both the temperature and how texture is defined. In the cold, solid fat particles exert a reinforcing effect on the protein matrix within the cheese and increase its hardness when measured using small strain tests. However, when the cheese is being chewed and subject to large-scale deformations, the fat particles provide weak points within the protein network, allowing it to be fractured into micro-scale particles and formed into a smooth mass. Raising the temperature softens the fat particles in cheese, and they have less reinforcing effect (Rogers *et al.* 2010) and help in formation of a smooth mass when chewed such that individual particles are less easily detected (Rogers *et al.* 2009).

Lower-fat cheeses are reported to be perceived as being waxier, more fracturable and chewy, hard and springy, less sticky and cohesive, less meltable and less smooth than full-fat cheese (Johnson *et al.* 2009). If cheese is looked at as a material consisting of a hydrated protein matrix with interspersed fat particles, less fat results in less interruptions in the protein matrix and less interference of long-range interactions between

those proteins leading to a more elastic texture. When the elastic nature of cheese is expressed as hand springiness/rate of recovery (a 30% nonfracture deformations), there are clear distinctions between full-fat, 50% reduced fat, and low-fat (90% reduced fat) *Cheddar* cheeses (Rogers *et al.* 2009). More springiness is maintained during storage in low-fat cheese than full-fat cheese. Also, as they age, full-fat cheeses can be broken down into smaller particles during chewing, becoming more cohesive and adhesive than low-fat cheese. This results in a greater smoothness of mouth coating, while low-fat cheese requires more chewing before it can be swallowed.

Sensory textural properties include mixing of the cheese with saliva during oral processing being broken down during mastication as it is chewed and lubricated by saliva. To achieve similar bolus formation, a low-fat cheese should have similar breakdown patterns and saliva interactions (Rogers *et al.* 2010). This includes warming of the cheese to body temperature as it is chewed and the consequent partially melting of the fat in cheese.

When cheeses were made with the same protein/moisture ratio and same calcium/protein ratio but differing fat contents (3% to 33%), Rogers *et al.* (2010) found no differences in rheological properties when measured at 25 °C. At 10 °C when the fat is solid, cheese rigidity increases with fat content. Cheese structure can be viewed as a continuous protein gel network disrupted with interspersed fat globules. This supports a filled gel model for fat in hard and semi-hard cheeses in which storage modulus is a combination of gel network elasticity and phase volume, filler particle elasticity and phase volume and interactions (or lack of) between the filler particle and the gel network (Sala *et al.* 2009; Rogers *et al.* 2010). Stiffer filler particles (cold fat globules) produce firmer materials by reinforcing the gel network. Filler particles with similar elasticity to the gel network (such as warm fat globules) will not show any differences as the filler particle volume increases. This filled gel model for cheese was confirmed by Barden *et al.* (2015) who made cheese in which fat globules were replaced with Sephadex beads. While the Sephadex beads had greater reinforcing effect and greater energy recovery than milk fat, there were no differences based on filler type on critical strain point (the level of strain where damage or long-term relaxations take place in the network). The only influence on critical strain was filler volume with full-fat cheeses having lower critical strain. Filler particles provide sites for stress concentration and initiate fracture, thereby lowering the strain required for initial fracture (Barden *et al.* 2015).

A low-fat cheese would have similar rheological properties to a full-fat cheese if (1) a filler particle with similar properties to fat is added at a phase volume equal to the fat phase and (2) the casein gel phase has the same chemistry as in the full-fat version. Filler particles that are rigid and do not melt will result in mechanical properties of cheese that are similar to milk fat at cold temperatures but different at room temperature. The ideal filler particle would melt or soften at body temperature, just as occurs in a full-fat cheese.

9.3.4 Milk Fat and Cheese Manufacture

During manufacture of rennet-coagulated cheeses, casein micelles aggregate and form a network structure that is driven by hydrophobic (entropic-driven) interactions. The shrinkage of the curd network structure continues throughout cheesemaking causing whey to be expelled as chains of casein micelles merge with one another forming thicker protein strands in which individual casein micelles can no longer be identified (Oberg *et al.* 1993; Merrill *et al.* 1996). Within curd particles, the fat globules act as an inert filler because of their hydrophilic fat globule membrane that is non-attractive to associations taking place between the hydrophobic proteins.

A consequence of fat globules blocking the protein strand coalescence is a small volume of serum (whey) is retained as well (McMahon and Oberg 2017). When fat content is lowered, there are fewer of these serum reservoirs. Although a low-fat cheese may have higher total moisture content, when measured in proportion to the protein (or on the basis of fat-free substance), mois-

ture content is lowered compared to a full-fat cheese, and the make procedure has to be adjusted to counteract this effect (Merrill *et al.* 1994). Also, since some of the cream is separated, a low-fat cheese contains smaller fat globules and fewer clumps of fat globules (Rogers *et al.* 2010). Hence, there are fewer interrupting points in the protein matrix, and the ability to retain larger pools of serum within the protein is reduced.

A continuation of the influence of fat content on moisture content of cheese is observed when the cheese curd is cooked and stretched during the manufacture of *pasta filata* cheeses (Oberg *et al.* 1993; McMahon and Oberg 2011, 2017). As cheese curd is heated and reaches a temperature of 50 to 55 °C, the proteins in the cheese curds become plasticized and coalesce into larger strands that are oriented in the direction of stretching. This results in a redistribution of the water and fat during stretching and moulding with the larger strands (or fibres) of protein being separated by channels containing water, water-soluble cheese components, bacteria and fat globules (Figure 9.7) (McMahon *et al.* 1999).

The coalescence of the protein matrix strands continues until the fat globules are sufficiently packed to physically inhibit fusion of the protein matrix. Thus, closely packed clusters of fat globules and bacteria are formed and oriented into channels between the parallel-aligned network of protein fibres. The size and number of these fat-serum channels are dependent on fat content rather than moisture content; with total moisture content of the cheese being a combination of water holding capacity of the protein matrix plus water that is retained in the fat-serum channels (McMahon and Oberg 2017). With fewer fat-serum channels, a lower-fat *Mozzarella* cheese has less water that can be expressible by centrifugation (McMahon *et al.* 1999).

During storage of *Mozzarella* cheese, moisture in the fat-serum channels becomes absorbed into the protein matrix as it expands with new protein matrix material completely filling the spaces between the fat globules (Figure 9.8) (Oberg *et al.* 1993; McMahon *et al.* 1999; McMahon and Oberg 2017). So not only does fat act to interrupt fusion of the protein matrix in *Mozzarella* cheese, it also provides a space to retain moisture in addition to that contained within the protein matrix.

Another impact of having multiple fat-serum channels in *Mozzarella* cheese is that it introduces parallel weak points that allow fractures to easily propagate as the cheese is pulled apart. This is the basis for making string cheese. If fat content is lowered, there are fewer fat-serum channels, more protein-protein interactions and very little stringiness (Mulvaney *et al.* 1997). Adding xanthan gum to hot low-fat *Mozzarella* cheese before it is extruded mimics the fat-serum channels by preventing protein strand coalescence and allowing the cheese to be pulled apart into strings (Oberg *et al.* 2015).

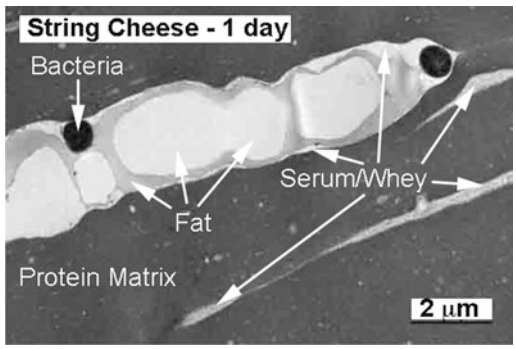

Figure 9.7. Transmission electron micrograph of 1-day-old string cheese (Courtesy of Dr. Almut Vollmer, Western Dairy Center, Utah State University, Logan, UT, USA).

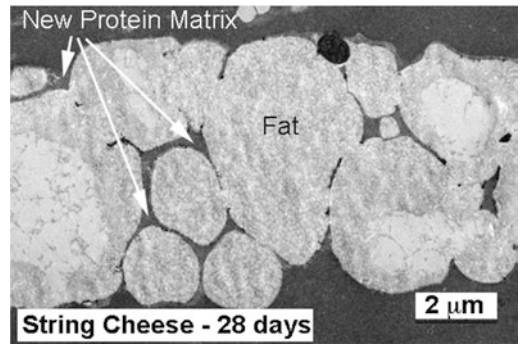

Figure 9.8. Transmission electron micrograph of string cheese after 28-day cold storage (Courtesy of Dr. Almut Vollmer, Western Dairy Center, Utah State University, Logan, UT, USA).

9.3.5 Milk Fat and Cheese Melting

Melting of cheese is a combination of softening of fat and the balance between protein/protein and protein/water interactions (McMahon *et al.* 1999). Fat plays an initial role by melting as the cheese is heated to 40 °C. The liquified fat does not affect further softening and flow as the cheese is heated to 60 to 70 °C (Lucey *et al.* 2003). Fat is important in baking applications, especially when *Mozzarella* is baked on a pizza in a hot-air convection oven. Low-fat cheese lacks melting and stretching characteristics when baked on a pizza because fat contributes to pliability and flowability (Lucey *et al.* 2003; Johnson *et al.* 2009).

Extensive lowering of fat content increases the amount of scorching during convection baking at 250 °C to 300 °C. During baking, cheese exposed on top of a pizza can lose up to 50% of its moisture (Rudan and Barbano 1998). This can be mediated by spraying the cheese shreds with a thin layer of oil (Rudan and Barbano 1998) or by making the cheese so that most of the fat is easily expressed as the cheese shreds soften (Wadhwani *et al.* 2011).

Normally there is ready release of fat from within the cheese, and this free oil lubricates the cheese surface and prevents excessive dehydration of the cheese shreds, so they can flow together to form a mass of melted cheese covering the pizza (Rudan and Barbano 1998; Wadhwani *et al.* 2011). If there is not enough oil covering the shreds, there is too much moisture loss before melting and the surface chars and blisters, preventing flow and leaving dry hard cheese shreds to remain on the pizza surface.

During curd manufacture, the *pasta filata* process, and during storage, the fat globules in the cheese can experience physical stress causing breakage of the milk fat globule membrane (Oberg *et al.* 1993; Lopez, *et al.* 2006; McMahon *et al.* 2009) allowing for easy release of liquified fat during heating. In contrast, fat droplets in low-fat cheeses tend to be smaller and more finely dispersed and trapped within the protein matrix, with fewer fat-serum channels. Hence, there is not enough expressed oil to prevent excessive dehydration, skin formation and charring of the cheese (Wadhwani *et al.* 2011).

9.4 Role of Milk Fat in Ice Cream

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9.4.1 Overview of Ice Cream Ingredients and Manufacture

Fat and fat structure development in ice cream and related frozen dairy desserts are critical to optimal structure and physical properties, stability, flavour and texture. A brief review of the functionality of fat in ice cream follows, with citations to the most recent and most pertinent references. Readers are referred to Clarke (2012), Goff (2002, 2016) and Goff and Hartel (2013) for more detailed information.

The term "ice cream" in its broadest sense includes most whipped dairy products that are manufactured by freezing and are consumed in the frozen state, including ice cream that contains either dairy or non-dairy fats; premium, higher-fat versions; "light", lower-fat versions; ice milk; and frozen yogurt. Ice cream mix formulations specify the content of fat, milk solids-not-fat, sweeteners, stabilizers, emulsifiers and water that are desired (Figure 9.9). Dairy and other ingredients used to supply these components are chosen on the basis of availability, cost, legislation and desired quality. Common ingredients include cream, butter or vegetable fats, as the main sources of fat; condensed skim or whole milk, skim or whole milk powder, milk protein concentrates and/or whey powder or whey protein products, as the sources of concentrated milk solids-not-fat (Goff 2016); sucrose and corn starch hydrolysates, or sugar alcohols, as the sweeteners; polysaccharides, such locust bean gum, guar gum, carboxymethyl cellulose and/or carrageenan, as the stabilizers; egg yolk or mono- and diglycer-

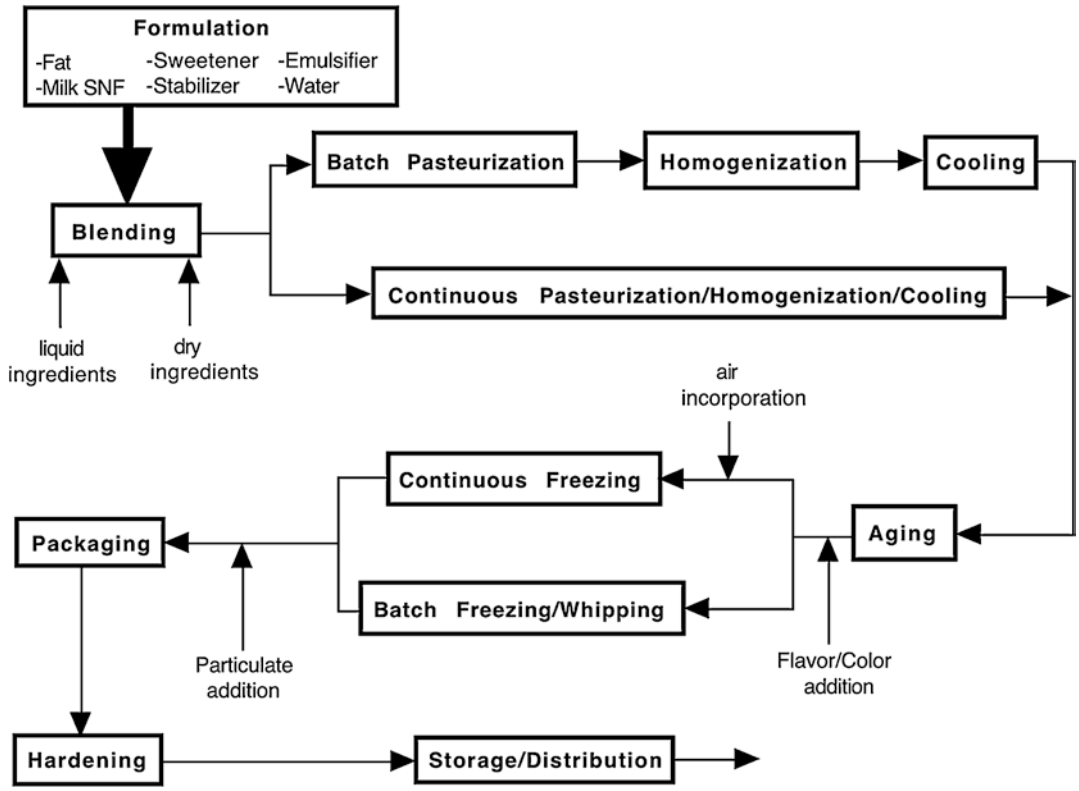

Figure 9.9. Flow diagram for the production of ice cream.

ides, as the emulsifiers; and milk or water, as the main sources of water in the formulation to balance the components (Goff and Hartel 2013). Usually one mix is used for the production of a variety of flavours.

The manufacturing process for most of these products is similar and involves the following steps (Figure 9.9): preparation of a liquid mix by blending of ingredients, pasteurization (65 °C for 30 min or 80 °C for 25 s), homogenization, cooling to 4 °C and ageing of the cold, liquid mix for 4–24 h; adding flavouring; concomitantly whipping and freezing this mix dynamically under high shear to a soft, semi-frozen slurry with an air phase volume of 30–55% (overrun, defined as air/mix ratio, of 40 to 120%) and a draw temperature of about −5 °C; incorporation of discrete flavouring ingredients to this partially frozen mix; packaging the product; and further quiescent freezing (hardening) of the product in blast air to below −25 °C (Goff and Hartel 2013). Homogenization is responsible for the formation of the fat emulsion by forcing the hot mix through a small orifice under a pressure of 14 to 18 MPa, perhaps with a second stage of 3–4 MPa. Ageing allows for hydration of milk proteins and stabilizers (some increase in viscosity occurs during the ageing period), crystallization of the fat globules and a membrane rearrangement due to competitive displacement of adsorbed proteins by small-molecule surfactants. The concomitant aeration and freezing process involves numerous physical changes, including the action of proteins and surfactants in forming and stabilizing the foam phase; partial coalescence of the fat emulsion, causing both adsorption of fat at the air interface and the formation of fat globule clusters that stabilize the lamellae between air bubbles; and freeze concentration of the premix by the removal of water from solution in the form of ice.

9.4.2 Sources of Fat in Ice Cream

The fat component of frozen dairy dessert mixes increases the richness of flavour, is a good carrier and synergist for added flavour compounds, produces a characteristic smooth texture by lubricating the palate, helps to give structure through the process of partial coalescence and foam stabilization and aids in producing desirable melting properties (Goff and Hartel 2013). The fat content can be used as an indicator of the perceived quality and/or value of ice cream. Ice cream must have a minimum fat content of 10% in many legal jurisdictions. Premium ice creams generally have a fat content of 14 to 18%. However, many high-quality ice cream products are also in the market with <10% fat (carrying such descriptors as reduced-fat, light, low-fat and non-fat or fat-free, as appropriate for the legal jurisdiction), where both structure and flavour considerations have been satisfied by other means, e.g. high in protein (Daw and Hartel 2015) or through processing (Tekin *et al.* 2017).

The use of milk fat as a fat source for ice cream formulations is widespread in North America, Australia, New Zealand and much of Europe. The triglycerides in milk fat have a wide melting range (+40° to −40 °C). The crystallization pattern of milk fat is also very complex, due in part to the large variation in fatty acids and a large number of different triglycerides present. Consequently, there is always a combination of liquid and crystalline fat at refrigeration and subzero temperatures, which is critical for structure formation, as will be discussed subsequently. The volatile, short-chain fatty acids also contribute to the unique flavour of milk fat. The best source of milk fat in ice cream for high-quality flavour is fresh cream. Other sources of milk fat include sweet (unsalted) butter, anhydrous milk fat (butter oil), frozen cream or condensed milk blends.

Vegetable fats are used extensively as fat sources in ice cream in the UK, parts of Europe, the Far East and Latin America and to a small but increasing extent North America. Five factors of great interest in the selection of fat sources are (1) the crystal structure of the fat; (2) the rate at which the fat crystallizes during dynamic temperature conditions; (3) the temperature dependence of the melting profile of the fat, especially at chilled and freezer temperatures; (4) the content of high melting point triglycerides (which can cause a waxy, greasy mouthfeel); and (5) the flavour and purity of the oil. It is important that the fat droplet contains an intermediate ratio of liquid/solid fat at the time of freezing. It is difficult to quantify this ratio as it is dependent on a number of composition and manufacturing factors; however, 50–67% crystalline fat at 4–5 °C is a good working rule (Sung and Goff 2010; Mendez-Velasco and Goff 2011). Palm kernel, coconut or palm fats, or fractions thereof, or blends of these fats and other oils are often used in ice cream manufacture, selected to take into account physical characteristics, flavour, availability, stability during storage and cost. Mendez-Velasco and Goff (2011) and Zulim Botega *et al.* (2013a, b) explored options for enhancing the unsaturated fat content, including through oil gelation.

9.4.3 Contribution of Fat to the Structure of Ice Cream

The texture of ice cream is one of its most important quality attributes. Texture is the sensory manifestation of structure; thus, establishment and maintenance of optimal ice cream structure are critical to maximal textural quality. The colloidal structure of ice cream begins with the mix as a simple emulsion, with a discrete phase of partially crystalline fat globules surrounded by an interfacial layer comprised of proteins and surfactants (Figure 9.10a). The continuous serum phase consists of unadsorbed casein micelles in suspension in a solution of sugars, unadsorbed whey proteins, salts and high-molecular-weight polysaccharides. During the dynamic freezing stage of manufacture, the mix emulsion is foamed, creating a dispersed phase of air bubbles (Figure 9.10b), and is partially frozen, forming another dispersed phase of ice crystals. Air bubbles and ice crystals usually range in size from 20 to 50 μm and are surrounded by a temperature-dependent unfrozen continuous matrix of sugars,

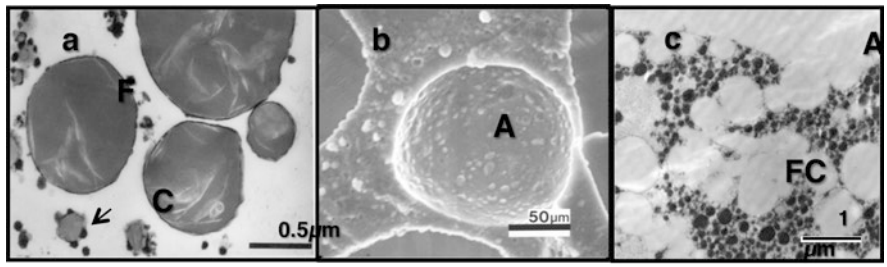

Figure 9.10. The structure of ice cream mix and ice cream. (**a**) Fat globules (F) in mix with crystalline fat within the globule and adsorbed casein micelles (C), as viewed by thin-section transmission electron microscopy. (**b**) Close-up of an air bubble (A) with adsorbed fat, as viewed by low-temperature scanning electron microscopy. (**c**) Air bubble (A) with adsorbed fat cluster (FC) that extends into the unfrozen phase, as viewed by thin-section transmission electron microscopy with freeze substitution and low-temperature embedding.

proteins, salts, polysaccharides and water (Goff 2002; Xinyi *et al.* 2010; Clarke 2012; Goff and Hartel 2013). In addition, the partially crystalline fat phase in the mix at refrigeration temperatures undergoes partial coalescence during the concomitant whipping and freezing process, resulting in a network of agglomerated fat (Mendez-Velasco and Goff 2012a), which adsorbs to the air bubbles and extends into the unfrozen phase, producing a fat network structure throughout the product (Figure 9.10c).

The development of structure and texture in ice cream is sequential, basically following the manufacturing steps. To describe the role of fat in the structure thoroughly, it is necessary to begin with the formation of the emulsion at the time of homogenization and the role of the ingredients present at the time of homogenization, with particular reference to the fat, proteins and emulsifiers. After preheating or pasteurization, the mix is at a temperature sufficient to have melted all the fat present, and the fat is passed through one or two homogenizing valves. The creation of a large population of small, discrete droplets is a prerequisite for the development of structure during dynamic freezing, utilizing these droplets. Thus, homogenization conditions can have a large impact on ice cream structure (Koxholt *et al.* 2001; Ruger *et al.* 2002; Hayes *et al.* 2003; Biasutti *et al.* 2013). Immediately following homogenization, the newly formed fat globules are practically devoid of membranous material and readily adsorb amphiphilic molecules from solution, including casein micelles, non-micellar β-casein, whey proteins, phospholipids, lipoprotein molecules, components of the original milk fat globule membrane and any added surfactants. These species all compete for space at the fat surface. The membrane formed during homogenization continues to develop during the ageing step, and rearrangement occurs until the lowest possible energy state is reached. The transit time through a homogenization valve is in the order of 10–5 to 10–6 s. Protein adsorption or unfolding at the newly formed interface may take minutes or even hours to complete. It is clear, therefore, that the membrane immediately formed upon homogenization is a function of the microenvironment at the time of its creation and that the recombined membrane of the fat globule in the aged mix is not fully developed until well into the ageing process.

Small molecular weight surfactants are not needed in an ice cream mix to stabilize the fat emulsion, due to an excess of protein and other amphiphilic molecules in solution. If a mix is homogenized without added surfactants, both the whey proteins and the caseins will form this new fat globule membrane, with the caseins contributing much more than the whey proteins to the bulk of the adsorbed protein (Zhang and Goff 2004). However, if added surfactants, such as monoglycerides or sorbitan esters or phospholipids, are present, they have the ability to reduce the interfacial tension between the fat and the water phases to a lower value than do the proteins. Thus they become preferentially adsorbed to the surface of the fat, and the mixed membrane of

surfactant and protein gives rise to the appropriate membrane for subsequent partial coalescence of the fat globules (Davies *et al.* 2000, 2001; Sourdet *et al.* 2002, 2003; Mendez-Velasco and Goff 2012b; Warren and Hartel 2018). As the interfacial tension is lowered and proteins are eliminated from the surface of the fat, reducing the surface excess (quantity of adsorbed material, mg m^{-2}), the actual membrane becomes weaker to subsequent destabilization due to this reduction of steric stabilization, although the emulsion is thermodynamically favoured due to the lowering of the interfacial tension and the net free energy of the system. Fat globules with reduced steric stabilization also adsorb to air interfaces, enhancing foam stability (Goff *et al.* 1999; Zhang and Goff 2004).

Crystallization of fat also occurs during ageing, creating a highly intricate structure of needle-like crystals within the globule. The triglycerides with high melting points crystallize first and continue to be surrounded by liquid oil of those with lower melting points. Crystallization of emulsified milk fat at refrigeration temperature reaches equilibrium within 1.5 h (Adleman and Hartel 2002; Relkin *et al.* 2003). A partially crystalline fat droplet is necessary for optimal fat structure formation to occur during freezing (Davies *et al.* 2000, 2001). This has been attributed to the protrusion of crystals into the aqueous phase, which causes a surface distortion of the globule. These protrusions can pierce the film between two globules upon close approach. As the crystals are preferentially wetted by the lipid phase, clumping is inevitable.

The next stage of structure development occurs during the concomitant whipping and freezing step. Air either is incorporated through a lengthy whipping process (batch freezers) or is injected under pressure (continuous freezers). The air bubbles are formed through a combination of comminution and interfacial adsorption (Sofjan and Hartel 2004; Xinyi *et al.* 2010). If the fat globules are sufficiently unstable to shear, as a result of reduction in membrane surface excess and steric stabilization due to added small-molecule surfactants, the aeration and ice crystallization processes cause the emulsion to undergo partial coalescence or fat destabilization, during which clusters of the fat globules form and build an internal fat structure or network in the frozen product. Bolliger *et al.* (2000a) showed a direct relationship between protein content (mg m^{-2}), resulting from displacement by emulsifiers, and partial coalescence. The incorporation of air alone, or shearing action alone, independent of freezing, is not sufficient to cause the high degree of fat destabilization that occurs when ice crystallization and air incorporation occur simultaneously (Kokubo *et al.* 1996, 1998).

Fat destabilization results in the beneficial properties of dryness (shape retention) upon extrusion during the manufacturing stages (which facilitates packaging and novelty moulding, for example); a smooth, creamy texture in the frozen dessert; and resistance to melt-down or good stand-up properties (necessary for soft-serve operations) (Bolliger *et al.* 2000a; Daw and Hartel 2015; Warren and Hartel 2018). The clusters of fat globules formed during the process of partial coalescence are responsible for adsorbing to and stabilizing the air cells (Goff *et al.* 1999; Barfod 2001; Zhang and Goff 2004, 2005) and creating a semi-continuous network or matrix of fat throughout the product that crosses the lamellae between the air cells (Koxholt *et al.* 2001; Muse and Hartel 2004). Hence, a finer distribution of air bubbles, resulting in thinner lamellae, also helps to produce optimal shape retention during extrusion and melting (Bolliger *et al.* 2000b). Optimal formation of fat structure and air bubble size may also help to slow down ice recrystallization (Barfod 2001; Soukoulis and Fisk 2016). If an ice cream mix is subjected to excessive shearing action or contains too much emulsifier, the formation of objectionable butter particles can occur as the emulsion is churned beyond the optimum level.

9.4.4 Contribution of Fat to Ice Cream Texture and Flavour

Fat contributes greatly to the flavour and texture of ice cream. Several recent papers have dis-

cussed flavour and textural aspects of various fat sources, non-fat sources to provide fat-like properties and the effect of fat on sensory properties and flavour perception of ice cream (Roland *et al.* 1999; Hyvonen *et al.* 2003; Amador *et al.* 2017; Rolon *et al.* 2017; Tekin *et al.* 2017).

In addition to positive aspects, numerous flavour and textural defects may be associated with the fat phase of ice cream. Such flavour defects are usually related to either autoxidation of the fat resulting in oxidized flavours (cardboardy, painty, metallic) or, especially in the case of milk fat, lipolysis of free fatty acids from triglycerides by the action of lipases (known in the dairy industry as rancidity). A significant content of free butyric acid gives rise to very undesirable, rancid flavours. These defects tend to be present in the raw ingredients used in ice cream manufacture, rather than promoted by the ice cream manufacturing process itself. However, processing problems can also occur during ice cream mix manufacture, e.g. rapid agitation/foaming of raw milk or cream, that can give rise to these fat flavour defects (Goff and Hartel 2013).

The fat phase can also account for textural defects associated with the fat content (too high or too low) or degree of partial coalescence. Fat contributes smoothness to the finished product. Low-fat mixes must therefore compensate for this lack of inherent smoothness by altering the ratio of other components, particularly the protein, polysaccharide stabilizer and emulsifier components. On the other hand, mixes high in fat, such as the premium products, typically have a heavier, dense texture, related to both high fat and lower-than-normal air contents. Partial coalescence of the fat emulsion modifies the textural perception, giving ice cream a creamier texture, in addition to its role in structure. If too much destabilization has occurred, the ice cream will taste greasy, and a defect known as "does not melt" may occur (Goff and Hartel 2013). This results from a network of fat that gives sufficient structure to the product to hold its shape without collapse in the absence of the ice phase, after warming to a temperature sufficient to melt the ice.

9.5 Role of Milk Fat in Dairy Powders

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9.5.1 Introduction

The fat content of liquid milk fluctuates due to physiological and environmental factors (e.g. lactation, seasonality), but milk can be considered to contain an average of ~3.5% fat (Fox and McSweeney 1998). Liquid milk is often processed into various types of powder, which are less perishable and more efficient to transport, in addition to being highly flexible to use. Lipid levels can vary greatly depending on the type of dairy powder and the procedures involved in their processing. This sub-chapter covers the role of milk fat in dairy powders, with a particular focus on those with functional properties affected markedly by surface fat. Much of the discussion will thus relate to commodity-type dairy powders like whole milk powder (WMP) and cream powder (CP), in addition to milk-derived ingredients such as whey protein concentrate (WPC) and milk protein concentrate (MPC).

WMP contains ~26% fat, making this its second most abundant constituent after lactose and arguably the most critical from a functionality perspective (Buma 1971a,b,c,d). Although CP has over double the fat content (55–70%) of WMP, both have similar levels of fat at particle surfaces (>98% of surface composition), which has a marked negative influence on their functional properties (Kim *et al.* 2005). The comparatively trace level of fat (≤1%) in skim milk powder (SMP) is not known to cause significant technical challenges, which is also the case for other low-fat dairy powders (e.g. caseins, caseinates). High protein (i.e. ≥80%) varieties of WPC and MPC can contain as much as 2–5% fat, which although low on a mass basis can lead to a host of undesirable changes when overrepresented at

particle surfaces (Gaiani *et al.* 2009; Kim *et al.* 2005). Thus, the influence of milk fat on important functional properties of dairy powders, including oxidative stability, flowability and wettability, is now generally agreed to be due to the fact that it is spatially localized at particle surfaces. What follows is a discussion of dairy powders that have been found to be affected by this issue. Powders in which milk fat has been replaced by vegetable oils to create "fat-filled milk powders" (Kelly *et al.* 2014; Vignolles *et al.* 2007) will not be discussed.

9.5.2 Commodity Dairy Powders

Liquid milk that has been standardized to a specific fat content can be processed into a milk powder, typically (though not always) by evaporation followed by spray drying. Additional processing steps are necessary in the production of WMP compared to SMP, namely, homogenization and lecithination. For SMP, agglomeration is sufficient in most cases to ensure instant-like properties; however, WMP typically requires a combination of agglomeration and lecithination (Skanderby *et al.* 2009). Due to the high fat content of WMP, it has a greater tendency to float, as fat is hydrophobic and has a lower density than water. Such issues may be further exacerbated in less common milk powders with higher fat contents, such as CP. Lecithin, a surface-active agent, is mixed with butteroil at <0.5% (w/w) prior to dosing of the WMP during lecithination (Skanderby *et al.*, 2009).

In a series of papers (Buma 1971a, b, c, d), T. J. Buma investigated the factors responsible for the levels of free fat in spray-dried WMP and the relationship between free fat and powder functionality. The work led Buma to develop a physical model of WMP particles that distinguished four types of fat, namely, *surface fat*, *outer layer fat*, *capillary fat* and *dissolution fat* (Buma 1971d). While all forms are extractable using a suitable apolar solvent, the first two, and particularly *surface fat*, were considered to have the most important consequences for powder functionality. Increasing total fat to 20% reduced the flowability of the milk powders, due to a corresponding increase in surface fat, though further increases had a limited effect (Buma 1971b); Buma thus concluded that the coverage of a critical surface area is sufficient to cause maximal cohesion between particles, which has been supported by later studies (Fitzpatrick *et al.* 2004; Kim *et al.* 2005). Particle structuration during atomization was considered by Buma to have the greatest impact on fat distribution in milk powders (Buma 1971c). Buma demonstrated that a high fat content and even a high free fat level are not necessary conditions for a powder to exhibit defects (e.g. poor flowability, solubility); on the other hand, if a powder had a high quantity of fat pooled at its particle surfaces, then such negative properties may be observed. As the major solid component, amorphous lactose plays an important role in encapsulating fat; however, storage conditions need to be carefully controlled and monitored to prevent lactose crystallization, which can lead to the release of fat (Vega and Roos 2006).

In the manufacture of milk chocolate, interactions between milk fat and cocoa butter are desirable. To promote these interactions, it is preferable that WMP has a high free fat content, which helps promote the development of optimal hardness or "snap" (Liang and Hartel 2004). Conventional spray drying results in a very low free fat content, while roller drying promotes high free fat, yielding powders with some desirable chocolate-making properties (Keogh and Twomey 2002); however, as demonstrated by Liang and Hartel (2004), roller-dried WMP has other characteristics (e.g. high particle density) that can promote undesirable phenomena in chocolate, such as bloom. Procedures to prepare WMP with high levels of free fat using spray drying are also available (Skanderby *et al.* 2009).

The localization of fat at particle surfaces commences during spray drying, although surface fat may accumulate further during storage. A high free fat level is associated with the formation of caked material in cyclones. With the exception of homogenization, processing steps prior to drying seem to have a limited impact on free fat. Homogenized concentrates have been

shown to yield WMPs with lower free fat levels (Buma 1971d). In single droplet drying experiments, Foerster *et al.* (2016) suggested that disruption of the lipid-water interface during atomization is primarily responsible for the over-representation of fat on the surface of spray-dried milk powder particles; however, the authors could not prevent these effects by adjusting the atomization conditions. In a later study, the authors reported a successful reduction in surface fat levels through the incorporation of a hydrocolloid into the milk feed before drying (Foerster *et al.* 2017).

9.5.3 Milk- and Whey-Derived Ingredients

Surface fat has recently been found to play a significant role in the functional properties of protein ingredients. For the purpose of this sub-chapter, emphasis will be placed on ingredients made using membrane filtration, which is known to concentrate protein and fat simultaneously. "MPC" and "WPC" will be used broadly to refer to any protein-based ingredients made by ultrafiltration of skim milk or whey, respectively; thus, no distinction is made between "concentrates" and "isolates" for this purpose.

The feed material for membrane processes used to make MPC and WPC is low in fat (pretreated skim milk and whey, respectively); however, the membranes used to concentrate protein are not permeable to residual fat, which is also concentrated as a result. For example, as the protein content of MPC ingredients increases, so does the fat content (Crowley *et al.* 2014). Despite this, the concentration of protein far exceeds that of fat, such that an 85% protein MPC will contain ~2% fat. In protein ingredients such as MPC and WPC, such a small quantity of fat can be overrepresented at particle surfaces, which can influence powder characteristics. Kim *et al.* (2005) found that particle surfaces in a 6% fat WPC consisted of 53% fat, compared to only 18% surface fat in a 1% fat SMP. This overrepresentation of fat (relative to bulk composition) is common in dairy protein ingredients.

Some evidence suggests that fat migrates towards the surface during storage, particularly at elevated temperatures. Gaiani *et al.* (2009) found that the presence of fat at the surface of micellar casein powder particles (which can be considered similar to an MPC) increased during storage at both 20 °C and 50 °C, although more rapidly at the elevated temperature. The increase in surface fat observed corresponded to a decrease in powder wettability. While milk fat is completely liquid at 50 °C, it is only partially so at 20 °C, suggesting that lipids are likely to be more easily "mobilized" at high temperatures. In laboratory studies, it is now common to store MPC and MCC at refrigeration temperature to prevent such effects.

9.5.4 Impact of Fat on Powder Functionality

Even when fat is present at low levels in dairy powders, it can have significant effects on product functionality. The impact of fat is most pronounced in lipid oxidation, powder flowability and rehydration.

Although the fatty acids in milk fat are predominately saturated, the presence of polyunsaturated fatty acids renders products such as WMP susceptible to oxidation (Mahmoodani *et al.* 2018). Oxygen molecules react with free radicals formed during this chemical reaction, which is catalysed by UV light and the presence of metals. Taking these factors into account, it is clear that packaging and storage conditions are important in efforts to prevent oxidation. Thus, flushing packages with nitrogen, the use of opaque materials and limiting the exposure to metals are often successful.

Several studies have shown that the lipids have an important role in determining the flowability of dairy powders (Fitzpatrick *et al.* 2004; Kim *et al.* 2005). In general, the higher the level of fat, the poorer the flow behaviour; thus, WMP is more cohesive than SMP, but WMP is slightly less cohesive than cream powder (Kim *et al.* 2005). As mentioned already, however, it is not total fat *per se* that causes these issues but, rather, that fat which is present at particle surfaces. The

resultant cohesive interactions were found to increase as temperature was elevated, presumably as the lipids become more liquid-like (Vignolles *et al.* 2007).

It has long been known that high-fat dairy powders can be difficult to solubilize effectively. This issue pertains to the early (wetting and sinking) not the later (dispersion and dissolution) stages of the rehydration process. This problem can be related to the hydrophobicity of fat and its low density; hence, powder particles coated in fat have a tendency to penetrate liquid surfaces very slowly. This is why most WMPs, and many WPC powders, are now lecithinated. The mechanism by which lecithin aids the rehydration of powders can be understood as follows: lecithin, an amphiphilic molecule, reduces the surface tension of the liquid in which the powder is to be dispersed, thereby increasing the rate at which the powder penetrates the surface. However, this form of explanation is challenged by a recent study (Mitchell *et al.* 2019), in which it was demonstrated that a reduction in the surface tension of water using a surfactant negatively affected the wetting/sinking of WMP; the authors argue that their finding is in accordance with the Washburn equation, in which a slower capillary penetration rate is predicted for systems with decreased surface tension. Nevertheless, the application of lecithin to improve the rehydration of WMPs and WPCs has achieved practical success, though further studies are required to validate the mechanisms responsible.

9.5.5 Conclusion

Lipids are a major constituent of WMP on a mass basis. In such high-fat dairy powders, lipids are a critical consideration with respect to functionality. However, it is now well documented that only a small proportion of this fat is necessary to cause product defects. Thus, issues associated with WMP, including slow wetting and poor flowability, also arise in MPCs and WPCs with far lower fat contents. In both cases, the spatial distribution of fat seems to be the key, with the presence of fat at particle surfaces being a critical factor.

Dairy materials are dehydrated to extend their life and facilitate their transit. Surface fat can accelerate product deterioration, particularly when powders are exposed to high ambient temperatures during transport and storage. Investigations of the processing factors that enable the creation of particle structures that inhcf future research.

9.6 The Role of Lipids in Infant Milk Formula

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9.6.1 Infant Formula: A Brief History

Human milk is the ideal source of nutrition for infants, providing for all of the dietary requirements of the neonate. When a mother cannot or chooses not to breastfeed, infant formula is a suitable replacement for milk. For millennia, young children have been fed with animal milks – either alone or in combination with other sources of nutrition, such as honey – as an alternative to breastmilk. However, the first formulations to resemble modern infant formula emerged in the nineteenth century, which were made possible due to the advances in analytical methodology that allowed food chemists to determine the compositions of different milks (Stevens *et al.* 2009). In 1865, Justus von Liebig patented a formulation consisting of bovine milk, wheat and malt flours and potassium bicarbonate, and in 1867, Henri Nestlé produced an infant cereal that built upon von Liebig's concept (Stevens *et al.* 2009). Since the pioneering nutritional developments of von Liebig and Nestlé, infant milk formula has advanced exponentially, but the research inspira-

tion and focus have always remained the same: to make a formulation as close as possible to that of human milk.

9.6.2 Human Milk Lipids and Their Influence on Infant Formula Design

Human milk is composed of water, lactose and oligosaccharides, lipids, proteins and various micronutrients. The vast majority of lipids are dispersed throughout milk as part of spherical fat globules consisting of a neutral lipid core surrounded by the milk fat globule membrane (MFGM), which includes polar lipids and cholesterol as part of its composition and prevents the fat globules from coalescing (Garcia and Innis 2013). Carotenoids and other fat-soluble nutrients are also typically dissolved within these fat globules. Human and bovine milk contain similar levels of total lipids (3–4%), which, in both cases, is composed of >97.5% as triacylglycerols (TAGs). However, their lipid profiles have key differences that make bovine milk fat unsuitable as a sole source of lipids in infant formula – particularly the deficit of unsaturated fatty acids in bovine milk – compared to human milk. Until the 1970s, bovine milk was often used as a lipid source in infant formula, before being largely replaced by vegetable oils due to their high levels of unsaturated fatty acids, such as linoleic acid (Innis 2011). Progressive efforts to replicate the lipid profile of human milk have been behind the evolution of the lipid profile of modern infant formula, which will be discussed below for the different classes of lipids.

Table 9.1. Comparison of lipid profiles of human and bovine milk)

Component	Human milk	Bovine milk
Total lipid, g/L	38	37
Lipid class, % w/w of total lipids		
Monoacylglycerols	Trace	0.03
Diacylglycerols	0.70	0.3
Triacylglycerols	98.2	98.3
Free fatty acids	0.40	0.1
Cholesterol	0.25	0.3
Polar lipids	0.56	0.55
Fatty acids, %w/w of total lipids		
$C_{4:0}$ (butyric acid)	0.2	3.9
$C_{6:0}$ (caproic acid)	0.2	2.5
$C_{8:0}$ (caprylic acid)	0.5	1.5
$C_{10:0}$ (capric acid)	1.0	3.2
$C_{12:0}$ (lauric acid)	4.4	3.6
$C_{14:0}$ (myristic acid)	6.3	11.1
$C_{16:0}$ (palmitic acid)	22.0	27.9
$C_{16:1}$ (palmitoleic acid)	3.7	1.5
$C_{18:0}$ (stearic acid)	8.1	12.2
$C_{18:1}$ (oleic acid)	34.0	21.1
$C_{18:2}$ (linoleic acid)	10.9	1.4
$C_{18:3}$ (linolenic acid)	0.3	1.0
$C_{20:4}$ (arachidonic acid)	0.5	–
$C_{22:6}$ (docosahexaenoic acid)	0.32	–

Adapted from MacGibbon and Taylor (2006), Tayor and MacGibbon (2011), Garcia *et al.* (2012).

9.6.2.1 Neutral Milk Lipids

Fatty Acid Composition and the Use of Vegetable Oils

The primary role of lipids in milk is to provide energy – human milk provides 50–60% of the calorific requirements of the neonate (Jensen 1999) – and the vast majority of this energy is provided by neutral lipids, mostly in the form of TAG. These TAGs are composed of a diverse array of fatty acids; human milk contains approximately 200 different fatty acids, compared to ~400 in bovine milk, though only ~15 of these fatty acids are present at more than trace levels (Lindmark Månsson 2008; Hageman *et al.* 2019), and their typical profiles are compared in Table 9.1. Although the composition of human milk is well understood, the exact lipid profile can be influenced by factors such as genetics and diet, with some regional differences observed; for example, the milk of Chinese mothers can vary geographically even within the same country (Giuffrida *et al.* 2016). In general, as well as a relatively low level of small- and medium-chain fatty acids (i.e. shorter than 14 carbons), human milk is characterized by high levels of saturated palmitic acid (16:0), monounsaturated oleic acid (18:1 n-9) and polyunsaturated linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3). These long-

chain polyunsaturated fatty acids (LC-PUFAs) are important nutritional components of milk, serving as precursors for the biosynthesis of other n-6 and n-3 LC-PUFAs, including arachidonic acid (ARA, 20:4 n-6) and docosahexaenoic acid (DHA, 22:6 n-3), which are key in the brain development and function of infants (Hadley *et al.* 2016; Lauritzen *et al.* 2016). It had previously been thought that infants could synthesize adequate amounts of ARA and DHA from their respective precursors, linoleic acid and α-linolenic acid. However, it has been shown that human milk also contains ARA and DHA primarily deriving from the diet of the mother; and there is evidence that infants may benefit from formulae that contain ARA and DHA, in addition to their precursors (Carver 2003).

In order to mimic the human milk profile as closely as possible, the lipid fraction of infant formula is typically composed of a blend of different vegetable fats and oils; the typical sources are described in Table 9.2. An infant formula devised and labelled SMA, "simulated milk adapted", is thought to have been the first such infant nutritional product to contain a fat blend derived from animal and vegetable fats in order to better approximate the human milk profile (Gerstenberger and Ruh 1919). In order to mimic the fatty acid profile of human milk, the vegetable oils used tend to be rich in palmitic, oleic, linoleic and α-linolenic acids. Although currently no single oil can provide a fatty acid composition identical to that of human milk, when combined in the correct proportions, a good distribution of the fatty acid profile can be achieved. Most vegetable oils, with the exception of palm oil, do not contain high levels of palmitic acid; therefore, palm oil is typically required to achieve palmitic acid levels similar to those of human milk (Hageman *et al.* 2019). However, re-emerging milk fat sources such as anhydrous milk fat (AMF) represent a potential alternative source of palmitic acid, which may in time reduce or eliminate this dependency on palm oil.

Due to the importance of PUFAs in the infant diet, infant formula is often fortified with ARA and DHA, typically at ratio ranging from 1:1 to 2:1. Fish oil is probably the most available source of DHA and is suitable to manufacture infant formulae if the level of eicosapentaenoic acid (EPA, 20:5 n-3) is low. Indeed, EPA is a polyunsaturated fatty acid that is present only in traces (<0.3%; Yuhas *et al.* 2006) in human milk and is known to be antagonistic to the functions of ARA (Carlson *et al.* 1992). The technology to isolate and refine significant quantities of ARA and DHA from microalgae and fungal oils, egg yolk derived-lipids and marine oils has been developed and commercialized, and these form the basis of most ARA and DHA ingredients used to fortify infant formula.

There is no consensus on how much ARA and DHA should be supplied to the infant diet from formula; the Food and Agriculture Organization recommend that ARA should supply 0.2–0.3% of

Table 9.2. Typical fatty acid profiles of vegetable oils commonly used in infant formula manufacture

	%w/w of total fatty acids											
Lipid source	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Canola oil	–	–	–	–	–	–	5	–	2	58	20	8
Canola oil, high oleic	–	–	–	–	–	–	4	–	2	70	15	3
Coconut oil	–	–	8	6	47	18	9	–	3	7	2	–
Palm oil	–	–	–	–	–	1	43	–	5	39	10	–
Palm olein	–	–	–	–	–	1	39	–	4	42	11	–
Palm oil, *sn-2* enriched	–	–	–	–	–	1	42[a]	–	3	44	7	–
Safflower oil	–	–	–	–	–	–	6	–	2	16	72	–
Soybean oil	–	–	–	–	–	–	11	–	4	22	53	6
Sunflower oil	–	–	–	–	–	–	6	–	3	27	60	–
Sunflower oil, high oleic	–	–	–	–	–	–	4	–	3	79	11	–

[a]~45% of the $C_{16:0}$ is found in the *sn-2* position.

total energy, with DHA comprising 0.10–0.18% of energy, and that, within these ranges, the precise ARA:DHA ratio is unimportant (FAO 2010). More recently, the European Food Safety Authority concluded that though there have been some demonstrated benefits to supplementing infant formulae with DHA, there is no practical requirement to add ARA, even in the presence of DHA (European Food Safety Authority 2014). The latest Codex standards dictate that if ARA and DHA are added to infant formula, then the ARA:DHA ratio should be at least 1 (Codex Alimentarius 2016). As further research is completed in this area, the recommended intakes of these key nutrients are likely to continue to evolve.

Fatty Acid Positional Distribution

Although the fatty acid composition of TAG varies widely, human milk TAGs often include palmitic acid at the central position (*sn-2*) on the glycerol backbone; 50–70% of palmitic acid chains are esterified in this position (Bar-Yoseph *et al.* 2013; Garcia and Innis 2013). These are often combined with unsaturated oleic acid chains at the *sn-1* and *sn-3* positions, and this configuration of oleic acid moieties (O) esterified either side of a palmitic acid chain (P) yields a characteristic OPO TAG structure. Palmitic acid is of particular importance in human milk – comprising approximately 10% of the energy supply of the infant by itself (Innis 2016) – and its effective absorption during digestion is crucial. When palmitic acid is found at the *sn-2* positon, it is less susceptible to being cleaved from the glycerol by gastric lipases – only ~22% of *sn-2* fatty acids are hydrolysed (Karupaiah and Sundram 2007). In this case, it remains on the glycerol backbone, and the resulting monoacylglycerol is well-absorbed by the infant; this preferential absorption of palmitic acid may improve intestinal comfort, calcium absorption and bone health (Bar-Yoseph *et al.* 2013; Garcia and Innis 2013).

A significant disadvantage of using vegetable oils in infant formula is that the TAGs present tend to contain palmitic acid at the *sn-1* and *sn-3* positions. When long-chain fatty acids such as palmitic acid are released from these positions, they are not absorbed, as well as chains of shorter fatty acids, and instead tend to bind calcium to form poorly absorbed calcium soaps. These soaps can lead to harder stools and constipation, as well as reduced bone mineralization (Nelson *et al.* 1996; Yao *et al.* 2014). To remedy this, ingredients have been developed with increased levels of palmitic acid esterified at the *sn-2* position – one such ingredient is Betapol®, produced by Lipid Nutrition BV, Wormerveer, Netherlands. These ingredients are typically produced by enzymatic interesterification of fractionated palm oil in the presence of an oleic acid source, such as soybean oil or high oleic sunflower oil, resulting in an oil containing high levels of OPO (Zou *et al.* 2016). These OPO-enriched oils, sometimes referred to as *sn-2* palmitate or *sn-2* fat, have allowed the commercialization of infant formula containing OPO levels similar to those of human milk; such formulations have shown clinical benefits in infants, more closely resembling the outcomes observed in breastfed infants (Yao *et al.* 2014; Béghin *et al.* 2018).

The Recent Re-emergence of Milk Fat

With the increasing prevalence of vegetable oils in infant formula throughout the twentieth century, the use of bovine milk fat fell largely out of favour; however, in recent years, fractions rich in milk fat have begun to find increased usage in infant formula as more evidence emerges of the potential benefits of bovine milk fat in the infant diet. Formulations containing a combination of vegetable and bovine milk lipids have been shown to be tolerated as well as those containing vegetable oils alone, with no impact on growth (Gianni *et al.* 2018), and in animal models, bovine milk lipids have been shown to retard beneficially the digestion of β-lactoglobulin while increasing the abundance of peptides from β-casein digestion (Le Huërou-Luron *et al.* 2018). Three main types of milk fat addition to infant formula can be considered: (i) through the direct addition of whole milk or cream; (ii) as complex milk lipids, which include polar lipids (PLs) and other MFGM components; and (iii) as anhydrous milk fat, which is composed mainly of TAG and cholesterol and depleted in PLs. AMF is

produced from either cream or butter that is concentrated, homogenized and separated to remove residual proteins and as much water as possible to result in a material that is almost entirely composed of lipids – by definition, a minimum of 99.8% fat and a maximum of 0.1% water (Mortensen 2011) – and the fatty acid profile of AMF is similar to that of bovine milk. Bovine milk contains a relatively high level of palmitic acid (~30% of the total fatty acids), approximately 40–45% of which is found at the *sn-2* position (Tzompa-Sosa *et al.* 2014; Lindmark Månsson 2008); therefore, the increasing trend of AMF addition to infant formula also represents a means of naturally increasing the amount of *sn-2* palmitate present in the diet of the infant.

9.6.2.2 Polar Lipids

The PL fraction comprises 0.2–2.0% (typically ~0.5–0.8%) of human milk lipids and consists predominantly of phospholipids and sphingolipids found in the MFGM (Garcia *et al.* 2012; Jensen 1999). Although phospholipids and sphingolipids are structurally distinct, they are sometimes collectively referred to as phospholipids in the literature. True phospholipids are structurally composed of a glycerol backbone combined with fatty acids, a phosphoric acid group and a hydroxy compound (Contarini and Povolo 2013). The most commonly occurring hydroxy compounds in phospholipids are ethanolamine, choline, serine and inositol, and, thus, the primary milk phospholipids are phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylserine (PS).

Sphingolipids consist of a long-chain, sphingoid base, usually combined with a fatty acid to form a ceramide, along with a phosphoric acid head, though sugars or alcohols may in some cases be attached. Sphingomyelin (SM) is the most common type of sphingolipid found in milk and consists of a sphingosine base bonded to a fatty acid chain of varying length (most commonly 16:0) and a phosphocholine head group (Byrdwell and Perry 2007; Contarini and Povolo 2013). Glycosphingolipids are a glycosylated family of sphingolipids and may be acidic – for example, gangliosides, which are sialylated sphingolipids – or neutral, such as cerebrosides, which are ceramides with single sugar moieties attached (Jensen 1996; Liu *et al.* 2018). The dominant gangliosides are mono-sialylated (GM_3) or di-sialylated (GD_3), with the most common cerebrosides being galactocerebrosides and glucocerebrosides (Giuffrida *et al.* 2014; Jensen 1996).

Despite their relatively low abundance, PLs are critical in the early development of infants as they play important roles in processes such as brain myelination (Tanaka *et al.* 2013) and lipid and cholesterol digestion, absorption and transport (Nilsson 2016) and are important in lipid membrane integrity (van Meer *et al.* 2008). Gangliosides may have immune and anti-infection properties by promoting the growth of *Bifidobacterium* spp. and binding to pathogenic targets (Rueda 2007).

Owing to the benefits associated with PLs, their levels in infant formula have become a major recent research focus. Conventional infant formula generally has two main sources of PLs: (i) dairy-derived protein ingredients (e.g. skim milk powder and whey protein concentrate), which naturally contain MFGM fragments released during various dairy processes such as separation and homogenization, and (ii) soybean lecithin, which is sometimes used as an emulsifier in infant formula. However, while soybean lecithin is a source of phospholipids, it does not contain SM, which is a particularly abundant and important PL in human milk. This means that effective enhancement of the PL profile of infant formula must come through the enrichment of MFGM components rather than the addition of soybean lecithin or other non-milk-derived PL sources. Several such MFGM-enriched "complex lipid" ingredients are already commercially available for inclusion in infant formula, including the SureStart™ range (Fonterra, Palmerston North, New Zealand).

Although the PL content of bovine milk is similar to that of human milk, the relative proportions of the five major PLs differ, with SM more abundant in human milk (Table 9.3). The PL content of conventional infant formula is typically lower than that of human or bovine milk, as ingredients low in milk fat are typically used in formulating (e.g. whey protein concentrates and isolates). The emerging trend towards the addition

Table 9.3. Comparison of the relative levels of the five major polar lipids in human and bovine milk

Polar lipid	Human milk mg/L	Bovine milk mg/L
Phospholipids		
Phosphatidylcholine	24.5	28.7
Phosphatidylethanolamine	18.3	31.8
Phosphatidylserine	8.1	10.0
Phosphatidylinositol	3.8	3.7
Sphingolipids		
Sphingomyelin	29.7	20.0

Adapted from Garcia *et al.* (2012).

of AMF in infant formula will not impact upon PL content, as AMF contains neutral lipids almost exclusively. However, the emergence of MFGM-enriched dairy ingredients has allowed for the investigation of the efficacy of MFGM supplementation of infant formula as a means of bringing increased benefit to the infant. A limited number of relatively small-scale clinical trials have suggested that supplementation of infant formula with MFGM or SM may lead to better outcomes compared to conventional formulations in terms of normal growth and cognitive function and development (Tanaka *et al.* 2013; Gurnida *et al.* 2012; Timby *et al.* 2014a) as well as reduced incidence of infection (Timby *et al.* 2015) and cardiovascular risk markers (Timby *et al.* 2014b). While some benefits can be linked to individual MFGM components – e.g. increasing SM content leading to better cognitive development (Tanaka *et al.* 2013) – many of the benefits associated with MFGM are likely as a result of the combination of factors present as part of its composition.

These innovations are made possible by technological advances that allow commercial-scale production of safe, high-quality ingredients at a production volume required by infant formula manufacturers. MFGM can be isolated from cream by various means, including centrifugation, churning or using mild detergents (Mather 2011); however, rather than relatively pure MFGM isolates, MFGM-enriched whey or lipid fractions are more commonly used in infant formula, the manufacturing processes of which are often proprietary. Some milk protein fractions may also be selectively enriched in MFGM components due to the fractionation processes used to enrich the desired protein components. One such ingredient, α-lactalbumin-enriched whey protein concentrate, has been shown to provide SM levels in infant formula similar to human milk levels, without the need for the addition of a specific MFGM-enriched ingredient (Moloney *et al.* 2018). This is made possible by a novel production process that combines specific pH and temperature to precipitate α-lactalbumin, which can then be separated by membrane filtration, which also serves largely to retain lipids and MFGM components. A combination of MFGM-enriched fractions and novel dual-purpose ingredients, such as the aforementioned α-lactalbumin-enriched whey protein concentrate, is likely to be employed in the near future to meet the desire to enhance PL levels in infant formula.

9.6.2.3 Other Lipid Components: Carotenoids and Cholesterol

Cholesterol is mostly found as part of the MFGM and is a vital component of lipid membranes, as well as a precursor of bile acids and steroid hormones (Ohlsson 2010). It is the most abundant sterol in milk, and a systematic review established that breastfeeding was associated with lower total blood cholesterol concentrations later in life compared with formula feeding suggesting that the level of cholesterol in human milk may contribute to homeostasis of the sterol in adults who were breastfed as infants (Pfrieger 2003). Bovine milk (300 mg/L) can contain more than double the concentration of cholesterol found in human milk (90–150 mg/L), and infant formula typically contains low levels (<5 mg/L) due to the lack of animal fat (Hageman *et al.* 2019). While no known efforts have been made to increase the cholesterol concentration of infant formula to levels similar to those of human milk, the addition of MFGM components is expected to lead to a concomitant increase in cholesterol, making this likely an area of increased focus for research in the near future.

Carotenoids are not synthesized in the human body but are usually present in human milk as a result of a mother's diet that includes carotenoid-rich food sources. These com-

pounds – including lutein, α- and β-carotene, zeaxanthin and β-cryptoxanthin – are involved in promoting vitamin A function, cognitive performance and eye development (Eggersdorfer and Wyss 2018). Though there is no regulatory requirement to do so, infant formula is sometimes fortified with lutein and β-carotene, the two most abundant carotenoids in human milk (Lipkie *et al.* 2015). Failure to fortify infant formula with key carotenoids may lead to much lower plasma carotenoid levels in the infant, sometimes below detectable levels (Zielińska *et al.* 2017). Feeding with infant formula supplemented with carotenoids has been shown to increase plasma β-carotene, lutein and lycopene concentrations in infants, compared to infants fed with unfortified formula; higher levels of fortification corresponded with higher plasma levels of these nutrients, which may fall within the range of infants fed with human milk through such supplementation (Mackey *et al.* 2012). Though β-cryptoxanthin and zeaxanthin are sometimes detected in infant formula, these components are not typically fortified, and their presence is due to their natural occurrence in some of the ingredients used in infant formula manufacture.

9.6.3 Future Perspectives

As research continues into the benefits of LC-PUFAs, it is possible that the minimum recommended levels of ARA and DHA will be increased in order to maximize any associated benefit. This will likely pose a technical issue to infant formula manufacturers as these lipids are easily oxidized and, depending on the source, can have a "fishy" flavour note. These two factors can combine to cause undesirable sensory characteristics, and increasing LC-PUFA content will exacerbate this issue. Possible solutions to this problem will lie in the use of encapsulated lipids or alternative processing technologies to segregate and protect LC-PUFA until they reach the gastrointestinal tract, thereby maintaining consumer acceptance while enhancing nutritional benefits.

Palm oil is an important component of infant formula and particularly as a substrate for the production of OPO-enriched oils. Concern around the environmental impact of palm oil production is likely to continue, driving manufacturers towards more sustainable sourcing and production practices. This will also encourage ingredient manufacturers to accelerate the development of technologies to generate OPO-enriched oils from alternative substrates, including vegetable and animal fat sources.

Differences in the structure and physical attributes of the fat globules represent a major difference between human milk and infant formula; fat droplets in human milk are larger than in infant formula (Baumgartner *et al.* 2017), and this may influence the lipolysis rate and particle, in turn affecting postprandial responses (Armand *et al.* 1999; Michalski *et al.* 2006; Baumgartner *et al.* 2017) which is believed to impact metabolic health later in life (Oosting *et al.* 2012; Baars *et al.* 2016). Mimicking the physical structure of human milk will require further research to fill this gap and deliver optimal nutrition to infants who cannot be breastfed. However, achieving this may introduce technological complications, as larger fat globules will have a greater tendency to undergo creaming and coalescence in accordance with Stokes' law (Wilbey 2011). Considering the strict regulations governing infant formula composition, the use of traditional stabilizers may not be feasible, which may potentially require the development of novel techniques and technologies to successfully stabilize these larger, more human-like fat globules.

9.7 Milk Fat and Chocolate

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9.7.1 Introduction

Chocolate is a complex suspension of cocoa solids, sugar crystals and milk powder, accounting for 70% of the total mass, dispersed in a fat con-

Table 9.4. Typical ingredient breakdown composition for milk, dark and white chocolate recipes

Chocolate type	Cocoa liquor or mass (%)	Sugar (%)	Cocoa butter (%)	Vegetable fats (%)	Non-fat milk solids[a] (%)	Milk fat (%)	Lecithin (%)	Flavour (%)	Total fat content (%)
Milk chocolate	8–12	34–58	18–25	0–5	12–18	3.5–6.5	0.3–0.5	0–0.5	26–38
White chocolate	0	37–50	22–35	0–5	18–24	4–8	0.2–0.5	0–0.5	29–40
Dark chocolate	45–80	20–55	0–5	0–5	0	0–5	0–0.5	0–0.5	29–49

[a]Dry solids obtained from dehydrated milk, i.e. milk powder, cream, buttermilk powder or milk fat.

tinuous matrix primarily consisting of cocoa butter, emulsifiers and often other fat fractions, e.g. milk fat, cocoa butter equivalents (CBEs).

Cocoa solid is the major ingredient in chocolate and originates from the beans of cocoa tree (*Theobroma cacao* L.) found originally in Central and South America but now cultivated in other geographical regions, such as Africa and Asia. There are four main cultivars of this tree used to produce cocoa beans for chocolate: Forastero, Trinitario, Criollo and Nacional, which differ in their composition and contribute to the flavour and quality of the final product (Fowler and Coutel 2017).

Initially, cocoa was consumed as a beverage, as early as 600 BC in Mesoamerican civilizations. This consumption continued through the centuries, when Aztecs of Mexico drank a cocoa-spiced beverage that was believed to provide them with strength and to act as an aphrodisiac (Grivetti *et al.* 2000). Such a drink was only reserved for high society ranks, such as the emperor, great warriors and priests (Lippi 2013). Cacao was first brought to Spain by Hernán Cortés in the sixteenth century, and its consumption spread afterwards throughout Europe. The use of chocolate in the semisolid emulsion form known today is believed to date back to 1875, when Daniel Peter of Vevey (Switzerland) created an economical way of removing water from milk (Beckett 2017) to obtain a new texture that is nowadays familiar to consumers.

9.7.1.1 Milk Fat and Legislation of Chocolate

Chocolate is categorized into three types: dark, milk and white, depending on the content of cocoa solids, milk fat and cocoa butter (Afoakwa *et al.* 2007). There is a fourth type of chocolate mass, called chocolate equivalent, which is an inexpensive replacement for chocolate, generally made with cocoa powder and vegetable fat, e.g. palm kernel oil, instead of cocoa liquor and cocoa butter. The differences in recipe of these four types of masses result in a variety of textures and sensory perception of the final chocolate, to accommodate consumer preference. Typical composition of different chocolate types is shown in Table 9.4.

The Codex Alimentarius Commission establishes the standards for chocolate and cocoa products to facilitate trade across countries. Today, the World Trade Organization (WTO) and the European Union (EU) participate in Codex work, and they may choose to accept Codex standards in full or with concrete deviations (Wood 2017). As a result, the legal minimum of milk solids and milk fat differs between countries, and this needs to be closely monitored by companies when exporting their chocolate products to other countries so as to abide to local regulations. In many countries, besides cocoa butter, milk fat is the only type of fat that is permitted in chocolate.

As an example, Codex standards (Codex 2003) require a minimum milk solid content of 12–14% and 2.5–3.5% of milk fat in milk chocolate, whereas the European Union indicates a minimum of 14% dry milk solids and at least 3.5% milk fat in the recipe (EU 2000). On the contrary, the USA requires a minimum content of milk solids of 12% and 3.39% milk fat (FDA 2018). Similarly, white chocolate needs to have a minimum of 14% milk solids and 2.5% milk fat

Table 9.5. Approximate composition of ingredients containing milk fat that are used in chocolate

Milk ingredient	Protein (%) as total N × 6.38 in d.m[a].	Fat (%)	Lactose (%)	Moisture (%)	Minerals (%)	Other components [b](%)
Anhydrous milk fat	0.0	99.8	0.0	0.1	0.0	0.0
Anhydrous butter oil	0.0	99.5	0.0	0.3	0.0	0.0
Whole milk powder	24.7	26.3	40.0	3.2	5.8	0.0
Skim milk powder	34.0	0.5	54.0	3.5	8.0	0.0
High-fat cream powder	15.0	55.0	24.6	1.9	3.5	0.0
Crumb	7.0	10.0	12.0	1.0	0.0	70.0
Buttermilk powder	30.8	8.0	50.4	3.8	7.0	0.0

[a]d.m. = dry matter.
[b]Sucrose, cocoa butter, etc.

according to Codex standards. In the EU, legislation for milk content in white chocolate is as for milk chocolate, whereas other components such as defatted cocoa solids or cocoa butter requirements will differ. Finally, the different regulation bodies for dark chocolate mainly focus on cocoa solid content, and neither the Codex standards nor the EU establishes a minimum of milk fat to be present in the recipe. In general, in those countries where regulations permit, up to 5% of the cocoa butter can be replaced with cocoa butter equivalents (CBEs), which are compatible with cocoa butter. Based on European chocolate legislation, CBEs that are made from palm, shea, mango, kokum, Illipe and sal are permitted to be used in chocolate (EU 2000). White chocolate does not contain any non-fat cocoa solids, i.e. from cocoa liquor or powder, and only milk ingredients, cocoa butter and/or vegetable fats are present.

9.7.1.2 Milk Fat Ingredients in Chocolate

Milk fat influences the flavour, texture and quality of chocolate, contributing to a smooth texture and glossy appearance. Traditionally, it can be added to chocolate using whole milk powder (WMP, 26% fat), through incorporation of anhydrous milk fat (AMF, >99.8% fat) or as milk crumb (a cooked mixture of liquid milk, sugar and cocoa liquor) (Minifie 1989). Some manufacturers also use high-fat cream powders (42–75% fat) for the production of special high-fat chocolates (Bolenz *et al.* 2003). Buttermilk powder (8% fat) is another dairy ingredient that contains milk fat and that may be used in chocolate. Skimmed milk powder with less than 1% fat is always used in combination with AMF. The reason for choosing one milk fat-containing ingredient or another depends not only on the nutritional, texture and sensory requirements of the chocolate but also on the cost of the final product. The composition of typical dairy ingredients used for milk fat addition in chocolate is shown in Table 9.5.

Traditionally, milk fat is used in the confectionery industry as AMF or anhydrous butteroil (Hartel 1996) due to the low moisture that is required for chocolate manufacture. AMF is obtained from fresh cream or butter that is not expected to sell within the time to maintain acceptable quality and was developed as a method to store milk fat (Sichien *et al.* 2009; Early 2012). The process involves the removal of water, proteins and other minor components to obtain a product with a minimum fat content of 99.8%, of which 98.8% are triglycerides (TAGs) and 0.1% water (Codex 1999). This allows milk fat to be used over a longer shelf-life (up to 12 months) without a loss of quality. Besides AMF, anhydrous butter oil has the same minimum 99.8% fat content, but it is manufactured from cream and butter of different ages (Rønholt *et al.* 2013). AMF is the preferred option for confectionery applications, due to its higher quality and less susceptibility to oxidation.

Crumb is an ingredient widely used in the manufacture of milk chocolate in the UK, Australia and USA and provides a characteristic caramel flavour to chocolate (Stewart and Timms 2002; Beckett 2003). The chocolate crumb process was developed in the UK in the 1930s to enhance the shelf-life of milk used for chocolate manufacture. Despite the numerous methods to manufacture crumb, the process starts by mixing and heating liquid milk and sugar to remove moisture and guarantee a low water activity in the final crumb. Then, cocoa liquor is added, and the mixture is dried at a high temperature in the range from 75 to 105 °C to reach a final moisture content of 0.8–1.5%.

Although many variants exist, i.e. full milk crumb, white crumb, etc., the main ingredients of crumb are liquid milk, sugar and cocoa liquor, which are heated at high temperatures and dried to a moisture content of about 1%. This intermediate raw material can then be added to cocoa butter and other ingredients to create the final milk chocolate (Edwards 1984). The milk fat content of crumb can vary from 8 to 14%, which, in combination with its cocoa butter content, results in better flow properties than when ingredients with lower free fat contents are used to manufacture chocolate (Skytte and Kaylegian 2017).

9.7.1.3 Free Versus Bound Milk Fat

The form that fat adopts in each of these milk ingredients, namely, whether free or bound, is of paramount importance to the processing and sensory properties of the chocolate. Fat that can be extracted by organic fat solvents under standardized conditions is defined a “free fat. This type of fat is typically the most preferred because it is available to be incorporated to the fat continuous phase of chocolate, making it easier for the mass to flow and later crystallize.

With regard to WMP, the structure of its particles varies depending on its manufacture, i.e. roller- or spray-dried. Particles from roller-dried WMP are more compact, with sharp edges, and typically have >90% free fat content due to the shearing and scraping action of the knives on the drum roll as the film dries (Liang and Hartel 2004; Dewettinck *et al.* 1996). Roller-dried WMP is preferred by chocolate manufacturers, due to its high free fat content, which means that less fat is needed to be added during processing of chocolate (refining and conching) to coat particles and improve its flow. In addition, the higher heat load given to powders in the drum rolls during roller drying is believed to provide an improved flavour and taste to milk chocolate. In contrast, spray-dried WMP contains <10% free fat (Aguilar and Ziegler 1994), the rest of the fat being entrapped in vacuoles and a lactose-protein matrix. This makes spray-dried WMP less efficient for chocolate manufacture, requiring up to 2.5% extra cocoa butter to be added during processing to provide the same flow properties compared to roller-dried WMP (Verhey 1986). Although the use of roller-dried WMP may be preferred from a processing point of view, its high free fat content renders it more prone to oxidation, resulting in a shorter shelf-life.

Dairy manufacturers use different techniques to increase the free fat content of high free fat (HFF) milk powders or in specialized spray-dried milk powders. Pre-treating the milk powder with high shear and elevated temperatures (75 °C) has shown to help crystallize lactose just above its glass transition temperature and induce immobilized fat in the powder particle to be part of the continuous phase of the chocolate emulsion (Franke and Heinzelmann 2008; Koc *et al.* 2003; Twomey and Keogh 1998). Another way to increase free fat is to use high homogenization pressure before evaporation (Skytte and Kaylegian 2017), or controlling the protein and solid fat content of the raw milk (Twomey *et al.* 2000). Despite all the pretreatment methods that are reported in literature, the most common way manufacturers ensure milk fat is 100% free in the chocolate recipe is by addition of AMF in combination with skimmed milk powder (SMP), since SMP has very low fat content (0.5% fat). This mixture of dairy ingredients provides manufacturers an economical alternative to roller-dried WMP and HFF. However, the anhydrous milk fat is still susceptible to oxidative rancidity, leading to off-flavour development.

9.7.1.4 Manufacture of Chocolate

Traditionally, chocolate is manufactured following a set of common steps consisting of mixing, refining, conching and tempering, followed by moulding, demoulding, packaging and storage (Figure 9.11). During the initial mixing, a suspension is formed between the dry ingredients, i.e. sugar and milk powder, and the liquid ingredients, i.e. cocoa liquor, cocoa butter, milk fat and surfactants. Milk is always added to chocolate in a dehydrated form, either as a powder ingredient or as crumb, since moisture is detrimental for the quality and processing of chocolate, as it reduces flow properties and modifies the mouthfeel of chocolate. The final chocolate has a very low moisture content (less than 1.5%) that ensures microbiological stability.

The fat content at the mixing step is key to achieve the desired particle size and throughput during refining. However, the optimal fat content at the refining step is recipe-dependant. As is shown in Figure 9.12, recipes with spray-dried WMP (left) generally need a higher total fat content at the refining step, since it is expected that the free fat content will not be as high as when a combination of AMF and SMP is used (right).

The particle size of the sugar and milk particles present in the agglomerated mass is then reduced to less than 30 μm by grinding. Final particle size influences the rheological and sensory properties of the chocolate (Afoakwa *et al.* 2007). The preferred grinding method for high throughputs of chocolate is roll refining, where chocolate is forced to pass a series of gaps that decrease in width (2- and 5-roll refiners) (Ziegler and Hogg 2017). Minor chocolate manufacturers often use ball mill refining, where agglomerated chocolate is forced towards grinding balls and the mass is recirculated until the desired particle size is achieved (Alamprese *et al.* 2007). The stress applied to the chocolate mass during refining leads to the breakdown of agglomerates and particles, creating smaller particles with varied morphology and distribution within the fat continuous phase. The creation of such particles is also depending on the chocolate recipe, mostly the ratio of crystalline (sugar) to amorphous (milk) material and the amount of free fat available to coat the increased surface area of particles. As previously mentioned, availability of milk fat is an important parameter at the refining step, where the less free fat available will result in drier chocolate masses and that can result in problems achieving the desired particle size. The new particles created during refining will ultimately affect the flow properties of chocolate.

After refining, the chocolate mass is conched, a step with two equally important aims: first, to develop flavour and, second, to convert the crumbly paste, flake or powder that is obtained during refining to a flowable liquid that can be poured into a mould (Beckett *et al.* 2017). The desired viscosity of chocolate is obtained through three conching phases, dry, pasty and liquid phases, although not all phases occur in all types of recipes. During the dry phase, the moisture content of the chocolate mass needs to be reduced from the initial 1.6% in milk chocolates to <1%, through mixing and heating with temperatures as high as 80 ° C. The moisture is released slowly while trying to avoid the formation of agglomerates. During this evaporation, unwanted flavour compounds are also eliminated (Beckett *et al.* 2017). In the following pasty phase, the viscosity of the chocolate mass starts to fall due to the moisture removal and solid particles becoming now coated with fat and surface-active ingredients, such as lecithin, with the aim to modify viscosity. As a result, a pasty mass is obtained, and flavour will continue to be developed through heating. Finally, the last additions of fat and emulsifier are done in the liquid phase, in order to mix the added ingredients in a short time. The final viscosity of the chocolate will depend on both the shearing intensity in the conche and the final additions of fat and emulsifier (Beckett *et al.* 2017). The desired outcome is a product that has as low viscosity as possible. Nevertheless, this usually means higher amount of fat to cover the surface of the particles, resulting in more expensive chocolate.

After conching, the chocolate is ready to be tempered, a thermal treatment within the range of 27–29 °C (depending on the recipe) to ensure the formation of βV fat crystals with correct size. During tempering, these stable crystals will be

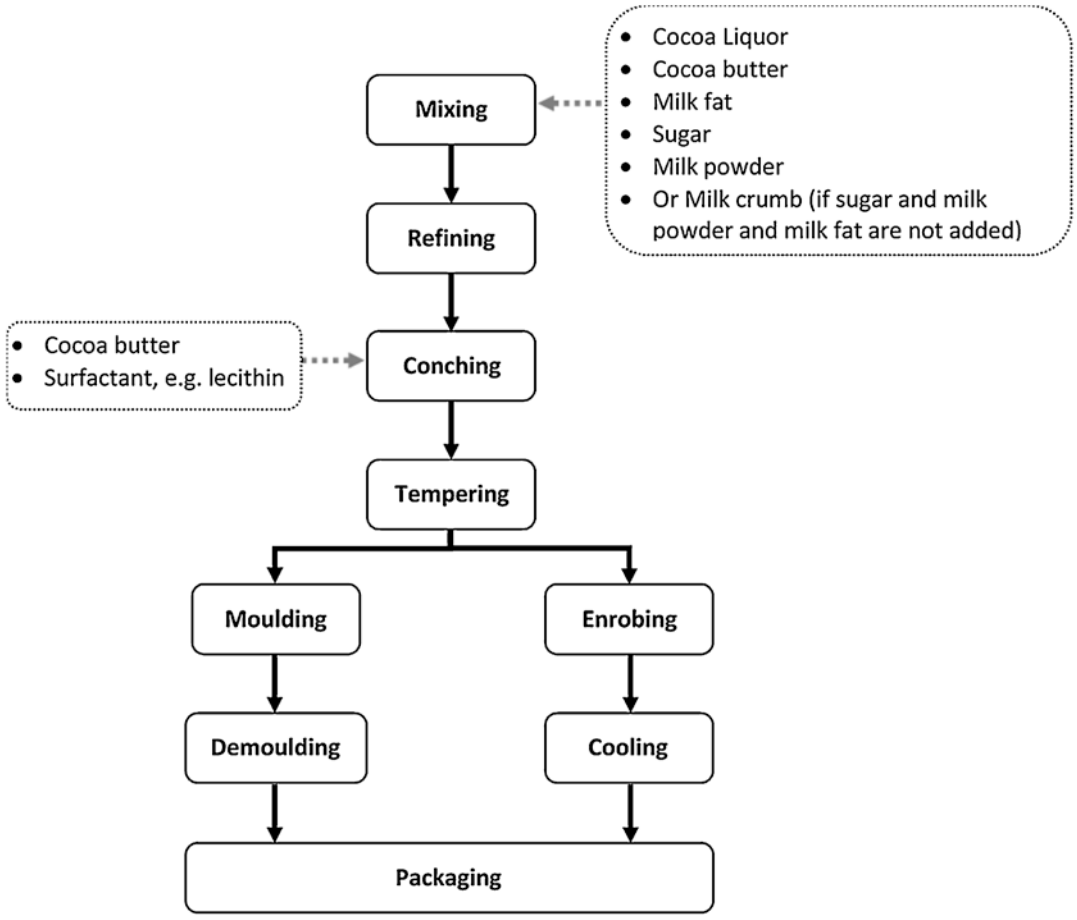

Figure 9.11. Chocolate processing scheme for chocolate manufacture.

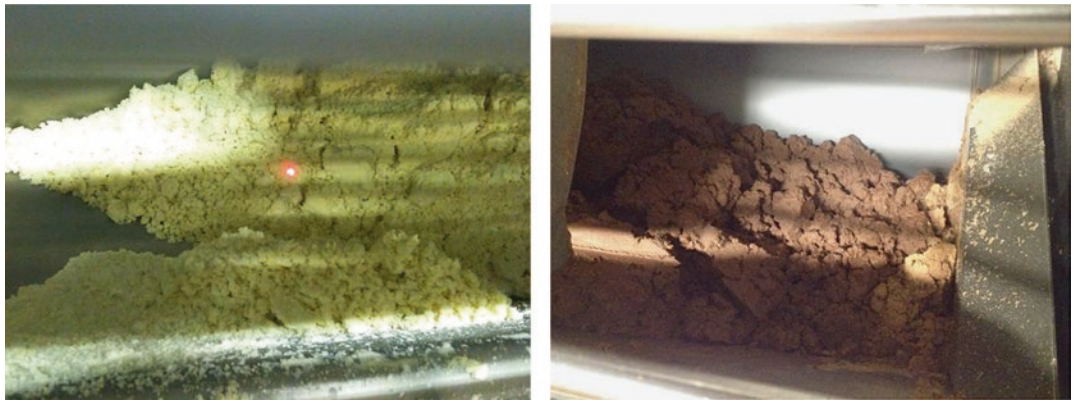

Figure 9.12. On the left, a white chocolate recipe made using spray-dried WMP (29.3% total fat at refining). On the right, a milk chocolate recipe made using AMF and SMP (24.5% total fat at refining).

subsequently used as seeds to obtain a homogenous fat crystal network during the cooling stage (Windhab 2017). At this time, the tempered chocolate mass can be used in moulding or enrobing (coating of products).

9.7.2 The Role of Milk Fat in Chocolate

Milk fat is used in a wide variety of confectionery products, such as milk, dark and white chocolate, as well as in fillings, e.g. caramel, toffee and cream. Besides cocoa butter, milk fat is the only fat that is permitted to be used globally in chocolate, as compared to other vegetable fats that cannot be added with the resulting product being called "chocolate". Not only it imparts a distinctive buttery flavour to chocolate applications but also reduces hardness, affecting therefore consumer satisfaction. Moreover, addition of milk fat into chocolate reduces the incidence of fat bloom, a defect of chocolate that results in a white or grey appearance and crumbly texture (Marangoni. 2002). Despite these advantages, milk fat has a high cost compared to other fats and oils, and its use needs to be always assessed for rheological suitability in the chocolate product (Hartel 1996).

In general, milk fat is compatible with cocoa butter, allowing a maximum addition of 30% of milk fat, calculated on the basis of total fat, without notable effect on the cocoa butter polymorphism (Metin and Hartel 1996). However, a eutectic effect in solid state is observed when mixing milk fat into chocolate (Marangoni. 2002). The mixture of TAGs from both milk fat and cocoa butter decreases the melting point of the fat mixture below the melting point of either components. Another factor changing the melting properties of milk fat-cocoa butter blend is the shifts in polymorphism. Changes in the crystal structure and stability of TAGs from both fats will cause crystallization as separate entities rather than as a mixture. All these factors will ultimately have a detrimental effect on the rheological behaviour of the chocolate mass, as well as on the hardness of the chocolate. Therefore, the total amount of milk fat that is often added into chocolate will depend on the chocolate recipe and tempering conditions (i.e. time and temperature).

9.7.2.1 Effect of Milk Fat Composition Variation in Chocolate

Composition of milk is very complex, and it is affected by herd (genetic variation), stage of lactation, seasonal variation and feeding regime of cows (Palmquist *et al.* 1993). Apart from the changes in the composition of protein, lactose and minerals of the milk, characteristics of milk fat are also greatly affected. Consequently, not only the fat content will vary but also the fatty acid and triglyceride composition, impacting, therefore, the solid fat content of the milk, which is particularly affected by the ratio of oleic acid to palmitic acid. The proportion of short-chain fatty acids in milk fat is low at the beginning of the cow's lactation, and it gradually increases during lactation (Palmquist *et al.* 1993). Since short-chain fatty acids play an important role in butter-like flavour, the use of milk fat obtained during early stage of lactation may also have a detrimental impact on chocolate flavour. However, the dietary regime of cows has been shown to have a greater influence on solid fat content of milk fat than stage of lactation (Twomey *et al.* 2000; Rowney and Christian 1996). Dairy cow diets are often composed of fresh forages (grass), conserved forages (silage, hay) and concentrates (plant seeds), in different ratios depending on season and geographical area (Elgersma *et al.* 2006). The proportion of fresh grass in the feed seems to be linearly correlated with a decreasing amount of milk fat content. In addition, if the proportion of grass in the forage is higher than 30% of the total cow's feed, the amount of unsaturated fatty acids increases (Couvreur *et al.* 2006). This level of unsaturated fatty acids can cause flavour defects, such as oxidation that ultimately may influence the flavour delivery in chocolate. Nowadays, it is possible to standardize the feeding regimes of cows to avoid seasonal variation in the fatty acid composition.

9.7.2.2 Use of Milk Fat Fractions in Chocolate

Over the years, milk fat fractionation has offered opportunities for creative use of this important dairy ingredient. From the complex composition of milk fat, and its broad melting range from −40 to +40 °C (Sichien *et al.* 2009), three main triglyceride groups can be generated via fractionation: low (LMFs), medium (MMFs) and high (HMFs) melting fractions. Two extra milk fractions are added by some authors, which include a very high melting fraction (VHMF), melting above 45 °C, and a very low melting fractions (VLMF), melting below 10 °C (Kaylegian and Lindsay 1995). Table 9.6 summarizes the potential benefit of using milk fat fractions in confectionery applications. HMFs can be used in milk and dark chocolate because they improve texture, hardness, gloss, snap and mouthfeel (Early 2012). Other potential applications for MMFs and LMFs could be confectionery fillings, e.g. caramels or toffees, due to their colour, flavour and softness.

Though several studies have been carried out exploring the structural benefits of using milk fat fractions in chocolate (Barna *et al.* 1992; Hartel 1996; Schmelzer and Hartel 2001; Kaylegian *et al.* 1993), added cost is still a primary concern. Therefore, besides the more traditional contribution of milk fat for bloom retardation, texture modification and flavour, the use of milk fat fractions in chocolate has been rather limited.

Table 9.6. Potential benefits of using milk fat fractions in confectionery applications

Milk fat fractions[a]	Benefit	Application
HMF	Improved texture	Milk chocolate, dark chocolate, confectionery fillings, coatings
	Improved firmness	
	Antibloom	
	Gloss	
	Snap	
	Flavour and mouthfeel	
MMF	Softness	Confectionery fillings
	Flavour	
	Colour enhancement	
LMF	Softness	Chocolate used for enrobing, confectionery fillings
	Flavour	
	Colour enhancement	

[a]HMF = high melting fraction, MMF = middle melting fraction, LMF = low melting fraction.

The most common industrial way to fractionate milk fat is dry crystallization, separating TAGs based on differences in their melting points (Kaylegian 1999). This is the fractionation method preferred by chocolate manufacturers, due to the growing consumer trend towards natural and clean label as solvent fractionation would not yield clean label fractions and there is a very limited choice of solvents suitable for extraction. As it can be seen in Figure 9.13, the three milk fat fractions melt at different temperatures: LMF melts below 10 °C, MMF between 10 and 21 °C and HMF above 21 °C (Vanhoutte *et al.* 2002). Timms (1980) similarly studied three fractions from untampered native milk fat using differential scanning calorimetry (DSC). His results showed that HMF melts at >50 °C and MMF in the range of 35 to 40 °C and LMF is melting at <15 °C. These melting ranges differed quite considerably from those obtained by Vanhoutte *et al.* (2002), highlighting the variability in melting behaviour that one can expect from milk fat fractions.

With regard to fractionation, Timms (1980) calculated the yield of HMF to be around 5%, 25% the yield of MMF and 70% the yield of LMF. On the other hand, Marangoni and Lencki (1998) reported 12% of HMF, 33% MMF and 55% LMF using solvent fractionation. These fractions are chemically distinct; generally long-chain saturated fatty acids are found in HMF, while TAGs of MMF consist of two long-chain saturated fatty acids and one short-chain or *cis*-unsaturated fatty acid, and one long-chain saturated fatty acid and two short-chain or *cis*-unsaturated fatty acids are found in TAGs of the LMF (Marangoni and Lencki 1998; Timms 1980).

As described before, these fractions are known to improve performance of milk fat in chocolate and confectionery applications, due to their different physical and chemical properties, melting point and solid fat content (SFC) (Bystrom and

Hartel 1994; Dimick *et al.* 1996). From these differences, the three fractions have very diverse melting characteristic as featured by the solid fat content profiles in Figure 9.14. Equally the fractions exhibit unique crystallization performance producing unique polymorphic forms. The use of these milk fat fractions, individually or as a blend, may allow for changing the rheological characteristics of the final chocolate. However, their suitability needs to be carefully evaluated to ensure the final product has the desired quality attributes, without incurring into a considerable cost increase.

9.7.3 Contribution of Milk Fat to Microstructure and Texture of Chocolate

Functional attributes that are commonly associated with milk fat include flavour and physical properties, such as structure formation, hardness, spreadability, layering, shortening and lubricity. However, these attributes are very dependent on the type of food system in which milk fat is used. For example, butter is used for its shortening properties in cookies to yield a tender crumb but is used in pastries for its layering properties, which promotes the characteristic flakiness of croissants and puff pastry. In chocolate, the inclusion of milk fat is done to modify the processing and subsequently impart the desired texture to the finished products, i.e. mouthfeel and melting behaviour.

9.7.3.1 Factors Affecting Crystallization of Milk Fat in Cocoa Butter

Traditionally, the addition of milk fat to chocolate to impart a creamy texture and desired milk flavour has notable structural implications. This is attributed to the fact that the composition of milk fat is among the most complex of fats and oils, comprising no less than 100 fatty acid types and 28 triglyceride species (Lopez *et al.* 2006; Shi *et al.* 2001; Van Aken and Visser 2000). This undoubtedly accounts for the highly variable crystallization behaviour of milk fat. The solidification of chocolate with milk fat added is dependent on the co-crystallization of the cocoa butter-milk fat phases, which ultimately affects the appearance and physical properties of chocolate (Koyano *et al.* 1990). In general, any crystallization of fat involves two stages: nucleation followed by crystal growth. Without considering tempering, which is necessary to induce the formation of the desired polymorphic form in cocoa butter, the crystallization of milk fat within a blend with cocoa butter remains sensitive to factors like temperature (undercooling), agitation, cooling rate and minor lipids. The impact of minor lipids is driven by concentration, which can fluctuate drastically, and this is discussed below. With regard to the temperature used, very low temperatures are normally avoided as they promote the formation of unstable polymorphs. Simultaneously, the rapid cooling of the fat after melting generates small regular crystals. On the contrary, slow cooling to warmer temperatures results in fewer but larger crystals. Therefore, using high agitation for recipes containing milk fat can help in breaking large crystals formed into smaller ones (a form of secondary nucleation). Low agitation rate conversely will result in slow crystal growth rates as both heat and mass transfer become less efficient.

9.7.3.2 Microstructure in Relation to Processing Conditions

Milk fat is partially solid at temperatures between approximately 5 and 25 °C, and its consistency is due to the presence of a network of fat crystals in liquid fat (Precht 1988; De Man and Wood 1959). In this network, the fat crystals are connected by solid connections, i.e. primary bonds. During crystallization, these bonds are formed by sintering, either when growing crystals come into contact with each other or via flocculation of small crystal nuclei between two fat crystals (Johansson and Bergenståhl 1995). Rupture of primary bonds by mechanical treatment leads to a softening of the fat (Heertje 1993). However, the firmness will quickly increase again due to a reorganization of the fat crystals into a relatively weak network held together by van der Waals

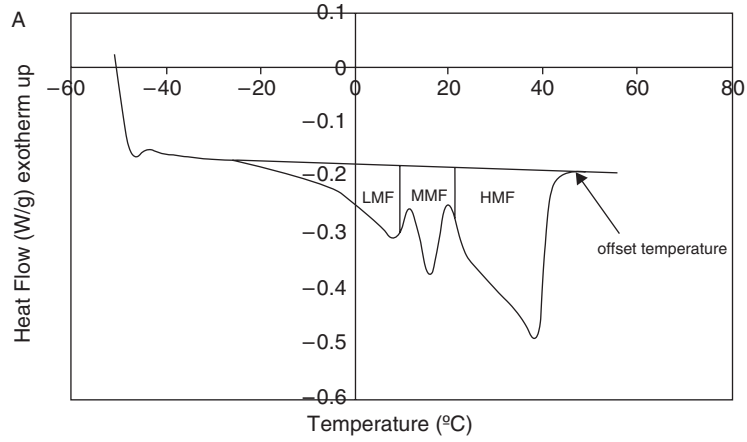

Figure 9.13. Differential scanning calorimetry (DSC) melting profile of milk fat showing different fractional components. Scanning rate: 5 °C/min (from Vanhoutte *et al.* 2002).

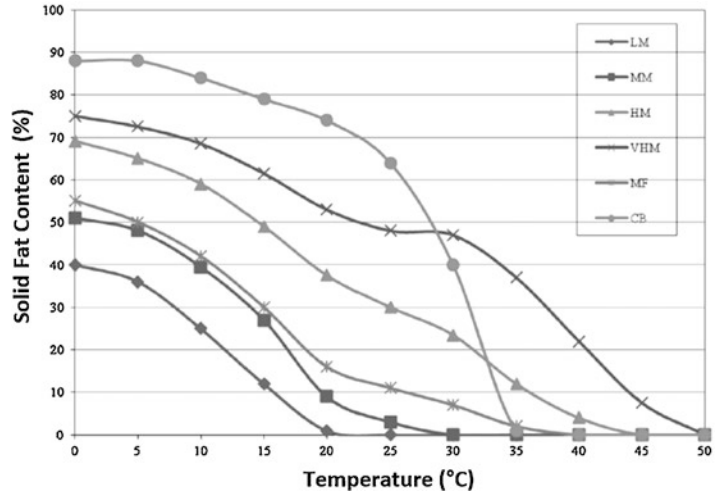

Figure 9.14. Solid fat content curves for a range of milk fat fractions. CB, cocoa butter; MF, milk fat; VHMF, very high melting fraction; HMF, high melting fraction, MMF, middle melting fraction; LMF, low melting fraction (from Metin and Hartel 2012).

forces, i.e. secondary bonds. This step is followed by the slow ongoing recrystallization processes, which ultimately leads to the formation of new primary bonds (Pedersen 1991; Van Aken and Visser 2000). Compared with most other fats, milk fat sets into its final firmness relatively slowly, mainly due to the very large number of triglyceride components with very large differences in fatty acid composition. This would lead to structural incompatibilities, which obstruct the incorporation of TAGs in the growing crystals. As milk fat may constitute a maximum of 20% in the total fat content in chocolate, the more crucial consideration in terms of structure will be its eutectic effect upon mixing with cocoa butter.

9.7.3.3 Eutectic Effect in Milk Fat–Cocoa Butter Blend

The occurrence of a eutectic effect when adding milk fat (or some of its fractions) is caused by the incompatibility of TAGs of milk fat and cocoa butter, the key fat components in chocolate. Hence, the use of non-fractionated milk fat in chocolate is limited to around 5% (Beckett 2008), before undesirable softening of the product is observed. This behaviour is primarily attributed to the LMF and MMF of milk fat, which interact with the TAGs of cocoa butter (Timms and Parekh 1980). The LMF of milk fat can act as a solvent dissolving the cocoa butter depending on the storage temperature of chocolate. On the other

hand, the MMF forms a eutectic mix with the cocoa butter TAGs, combining with other low melting polymorphic cocoa butter crystal to form a semi-solid fraction. While a severe eutectic effect exhibited by HMF was previously reported by Hartel (1996) (Figure 9.15), Marangoni (2002) attributed this occurrence to residual MMF. Likewise, no eutectic effect with cocoa butter was previously observed for HMF by Kaylegian *et al.* (1993).

Hydrogenated milk fats are also good bloom inhibitors, although they still have been reported to soften the chocolate at ambient temperatures (Campbell *et al.* 1969; Timms and Parekh 1980). Hydrogenated milk fat exhibits lower solubility effects caused by the LMF. However, this advantage is compromised by the corresponding increase in the eutectic effects of the MMF that contributes to softening (Timms and Parekh 1980). With growing concern of hydrogenated fat ingredients by consumers, the industry is gradually moving away from this process.

9.7.3.4 Minor Components in Milk Fat

The TAGs and minor lipid components in milk fat such as diglycerides (DAGs), monoglycerides (MAGs), cholesterol, individual fatty acid components and phospholipids exhibit a range of characteristics that can be used to provide unique functional properties for applications with added milk fat. Wright *et al.* (2000a, b) and Herrera *et al.* (1999) verified that the minor lipids in milk fat slowed the nucleation period at temperatures above 25 °C. However, differences in the composition of the milk fat samples used have led to contradictory results between these studies. Mazzanti *et al.* (2004) found that the minor lipids present in milk fat can slow the early stages of crystallization, reducing the rate of crystal growth and generating unstable β′ form.

To understand the impact of minor lipids on the crystallization behaviour and physical properties of chocolate recipes, a study was conducted by Tietz and Hartel (2000) using a blend of cocoa butter and milk fat (10%). Evaluations were made with (a) complete removal, (b) normal level (2.5% w/w) and (c) double level (5.0% w/w) of minor lipids in the milk fat. Results showed that removing the minor lipids from milk fat leads to delayed nucleation. In addition, irregularly shaped primary and secondary crystals were observed trapping a significant amount of liquid fat. When the blend was then used in a chocolate recipe, it led to rapid bloom formation. In contrast, normal levels of minor lipids in milk fat resulted in quick onset of nucleation, generating spherical and uniform crystals that led to denser packing of the fat crystal network. Similar to the effects seen with the removal of minor lipids, doubling the level of minor lipids slowed nucleation, reduced the crystallization rate and led to a rapid development of bloom in chocolate. From this study, it is suggested that minor lipids may be acting as catalytic sites of nucleation at low levels but, when present in higher concentrations, may interfere with crystallization.

Based on the latest research studies, there is a consensus that the presence of milk fat minor lipids in chocolate at natural or slightly higher concentrations would give rise to uniform crystals and help reduce fat bloom. On the contrary, the exact impact of minor lipids on the crystallization and polymorphic habit remains unclear, as there is limited data on the model system containing milk fat, minor lipids and cocoa butter (Metin and Hartel 2005).

9.7.4 Contribution of Milk Fat to Flavour of Chocolate

Chocolate has a complex flavour profile composed of numerous volatile and partly odour-active compounds. Although these compounds are not unique to chocolate, their concentration and combination in different ratios result in the main flavour notes associated with chocolate: fruity, spicy, floral, cocoa, acidity, bitterness, astringency, woody and also some off-flavours (Engeseth *et al.* 2018). Flavour in chocolate is the outcome of the combination of individual raw materials, i.e. cocoa, milk ingredients, sugar, etc., and the processing steps involved in the manufacture of chocolate. Likewise, each of these ingredients has been subjected to specific processing

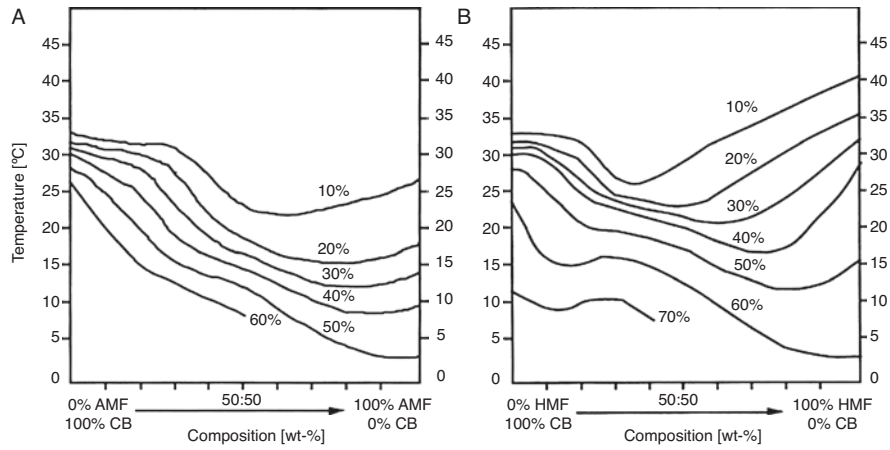

Figure 9.15. Isosolid phase diagrams of mixtures of cocoa butter (CB) with (A) anhydrous milk fat (AMF) and (B) high melting fraction (HMF) of milk fat (from Hartel 1996).

steps, e.g. roasting of cocoa beans, which are likely to affect the final flavour of chocolate. To develop a desirable flavour in chocolate, understanding of the key aroma and taste-active compounds in the finished product is important, as well as those in the raw materials. For a long time, industry and researchers considered conching of chocolate as the critical step where flavour development occurs. Nowadays, it is generally recognized that during conching, the flavour components in the chocolate mass are redistributed (Ziegleder 2017), and this can help to modulate the final flavour of the conched mass. As a result, a deep knowledge on the composition of sensorially active components in raw materials in combination with changes in conching conditions can help manufacturers to achieve the desired flavour in chocolate.

The most important raw materials for flavour development in chocolate are cocoa beans and milk ingredients (milk powder and milk fat). Fermentation, drying and roasting of cocoa beans are considered as some of the most important processing steps for chocolate flavour (Ziegleder 2017). Likewise, the flavour of milk fat ingredients is largely dependent on the cream used for its preparation. Key odorants of milk fat ingredients that contribute to butter-like flavour are diacetyl, δ-decalactone (coconut, peach) and butanoic acid (sweaty) (Schieberle *et al.* 1993). Their concentration in the final chocolate may promote differences in the overall flavour of chocolate and could act as an indicator of buttery flavour note.

In the case of milk chocolate, flavour is due to a balance between the primary flavour compounds originating from the cocoa mass and the volatiles found in milk ingredients. The flavour of milk ingredients used in chocolate, i.e. milk powders, AMF, or anhydrous butteroil, depends not only on the composition of such ingredients but also on how they have been manufactured. The most important aroma compounds derived from milk fat origin in milk chocolate have been identified as γ- and particularly δ-lactones (sweet, coconut) (Schlutt *et al.* 2007; Schieberle *et al.* 1993). In general, chocolates made with larger amounts of free fat are better perceived in terms of flavour by consumers than those with bound fat, e.g. using spray-dried WMP (Bolenz *et al.* 2003). In addition, free fatty acids present in milk ingredients, as well as the lactones (milky, sweet, coconut), seem to be fundamental contributors to flavour. The components formed from β-hydroxy fatty acids released from the TAGs when milk fat is heated (Skytte and Kaylegian 2017) are lactones and are responsible for milky, buttery or creamy odour (Shiratsuchi *et al.* 1994b). Moreover, aldehydes, aromatic hydrocarbons and some heterocyclic compounds are likely to par-

ticipate indirectly in milk flavour (Shiratsuchi *et al.* 1994b).

The use of milk crumb in the manufacture of milk chocolate provides a characteristic caramel flavour to chocolate, widely used in the UK, in Australia and in the USA (Stewart and Timms 2002; Beckett 2003). This specific flavour is difficult to develop using other processing conditions. The process relies on the Maillard reaction occurring between proteins found in milk and cocoa and reducing sugars found in milk (lactose) in a high moisture environment. The crumb heating process will result in key flavour compounds such as furfural (sweet, woody, baked bread), maltol (sweet caramel, toffee), lactones and methyl ketones (Ziegleder 2017). The final crumb flavour will depend on the moisture content, the temperature and the time spent at each stage. Since most of the flavour of the chocolate is developed during the crumb process, conching in this type of chocolate is not needed for flavour purposes, and only a liquefaction is required to achieve the correct viscosity of the chocolate mass. In addition to the role of cocoa liquor for flavour formation in crumb processing, it is worth mentioning that the high polyphenol content in cocoa liquor acts as an antioxidant to prevent milk fat from becoming rancid and contributing a sour or cheesy flavour in the final chocolate (Beckett 2003). For milk chocolates where crumb is not used as an ingredient, it is not possible to develop the same caramel and flavour notes during conching as those obtained during crumb processing. This is due to the low moisture content present in the chocolate mass during conching. In milk chocolate, milk notes are usually represented by δ-lactones, 2,3-butanedione, 1-octen-3-one and 2,4-decadienal (Schnermann and Schieberle 1997).

With regard to novel milk fat ingredients, there is extensive research on the use of milk fat fractions to enhance butter flavour in milk chocolate (Hartel 1996). LMF tends to increase milk flavour, whereas higher melting ones seem to improve bloom stability (Full *et al.* 1996). On the contrary, chemical or enzymatic modification performed on milk fat to improve its rheological behaviour, e.g. interesterification, often leads to a decreased flavour perception in the products in which it is incorporated (Weihe 1961; Rousseau and Marangoni 1998).

9.7.4.1 Off-Flavours from Milk Fat Ingredients

Care must be taken on production and storage of dairy ingredients used in chocolate, since off-flavours generated from cow's diet, environment, heat treatment, bacterial spoilage, oxidation of lipids, etc. (Shiratsuchi *et al.* 1994a) could be transferred to the final chocolate, affecting its sensory perception. This was observed in stale milk chocolates, where an increase in short-chain free fatty acids and volatile lipid oxidation products, 3,5-octadien-2-ones, was found. These are formed by residual enzymatic activity of lipases and lipoxidases, despite the low water activity of chocolate (Ziegleder 2017).

Factors such as herd, stage of lactation and dietary feed affect the composition of milk fat, and this will finally influence its flavour and stability. Dietary fat fed to cows was shown to affect the flavour of milk fat, as well as its oxidative stability (Palmquist *et al.* 1993). High-fat diets decrease the content of components exhibiting coconut flavour (δ-octalactone and δ-decalactone), peach flavour (δ-dodecalactone) and blue cheese (methyl ketones), whereas these components increase with low-fat diets, e.g. alfalfa (Urbach 1990). Off-flavours originating from animal feeding can cause aroma defects, e.g. fermented silage, musty silage and alfalfa (Mallia *et al.* 2008). In addition, oxidative flavours can occur easily, depending on cows' feed. Thus, milk from cows fed pasture is less susceptible to oxidation than milk from cows on dry stored feed. Likewise, milk fat with more than 20% of linoleic acid (C18:2), originating from low-fat feed, results in oxidized off-flavours (Edmondson *et al.* 1974).

Among the several off-flavour compounds that can occur in chocolate, those described as cardboardy or fishy are related to oxidation of milk fat (Skytte and Kaylegian 2017). The main factor needed to start the oxidative chemical reaction is the availability of oxygen.

Nonetheless, other variables such as storage temperature, light, α-tocopherol level and metallic contamination, especially copper, will also speed the rate of the reaction (Keogh and Higgins 1986). In general, lipid oxidation can be promoted in two ways: firstly, when double bonds of unsaturated fatty acids uptake oxygen yielding hydroperoxides that are then further converted into off-flavour components such as aldehydes and ketones and, secondly, by the action of lipolytic enzymes on TAGs that can produce DAGs, MAGs and particularly free fatty acids (FFA), which are more prone to oxidation. Some of the shorter FFA may contribute to off-flavours, e.g. butanoic acid (sweaty), in products such as AMF (Keogh and Higgins 1986; Schieberle *et al.* 1993). Lipolysis can also be beneficial in milk chocolate, and it is characteristic of American-type chocolates, such as Hershey's, where lipolysed milk fat is used to obtain a chocolate with cheesy, sour or tangy or butyric flavour (Hayes *et al.* 2016; Martin Jr. 1988).

Oxidation in AMF is measured by the peroxide value, where intermediate compounds related to oxidative status of the fat are detected (Keogh and Higgins 1986). Peroxide values below 0.2 meq O_2/kg fat guarantee a high-quality AMF product with no risk of oxidation, although the maximum allowed value is 0.3 meq O_2/kg (Codex 1999). Considering that the use of antioxidants in AMF is not permitted by Codex standards (Codex 1999), mechanisms that are used to reduce the rate of oxidation and contribute to longer shelf-life include:

- Removal of oxygen by flushing AMF with nitrogen
- Using oxygen barrier packaging
- Storage at chilled temperatures, i.e. 4 °C
- Avoiding the presence of oxidation catalysts (Skytte and Kaylegian 2017)

Therefore, only high-quality premium AMF is generally used in confectionery in order to avoid changes in colour, flavour, aroma or nutritive value of the product.

9.7.5 Bloom-Retarding Effect of Milk Fat in Chocolate

A common quality defect that is observed in chocolate industry is development of bloom. Several factors have to date been identified that are known to contribute to occurrence of bloom in chocolate. These factors are (a) improper processing conditions, (b) use of incompatible ingredients and (c) excessive heat exposure. Any of these factors can itself lead to bloom, as well as their interaction can result in more complex situations that generate the following bloom types: (a) heat damage bloom, (b) migration bloom and (c) storage bloom. It is to rectify the latter two forms of bloom that milk fat has been widely used by chocolatiers and manufacturers.

9.7.5.1 Cause and Mechanism of Migration Bloom?

A good overview on the interplay of the various factors that contribute to fat bloom was systematically discussed by Ziegler (2009). Three mechanisms are discussed relative to fat bloom: polymorphic transformations, liquid-mediated recrystallization (thermally induced) and oil migration. The contribution of each mechanism to bloom in a given recipe may vary, and as reported, the occurrence of any one of these phenomena does not necessarily lead to bloom formation.

The mechanism that drives the appearance of migration bloom first stems from the presence of significantly increased semi-liquid fat fraction in chocolate recipes. This increase can be brought by incorporation of nuts, soft indulgent filled chocolate or contaminants containing incompatible fats, such as lauric-based fats. As the semi-liquid fraction tends to melt readily at around 20 °C (typical ambient temperature in temperate climates), when recipes with these components are made and exposed to temperature cycling where the maximum temperature does not exceed 25 °C, liquefaction of this semi-liquid fraction will occur. As a consequence, the mobile liquid fat will gradually move through the matrix of the chocolate.

Several hypothesized mechanisms have been put forward and examined through the years to explain the kinetics of oil migration in filled and solid chocolates, but the exact mechanisms have not been completely elucidated yet. The hypothesis of Fickian diffusion has been widely proposed as the key driver of oil migration (Galdámez *et al.* 2009; Lee *et al.* 2010; McCarthy and McCarthy 2008; De Clercq *et al.* 2014; Dahlenborg *et al.* 2015b; Maleky *et al.* 2012; Ghosh *et al.* 2002; Guiheneuf *et al.* 1997). On the contrary, there is evidence suggesting the capillary flow model (Aguilera *et al.* 2004; Choi *et al.* 2005), correlating with observations made on porosity in chocolate (Rousseau 2006; Smith and Dahlman 2005). Other groups suggest a combination of both hypotheses (Rousseau and Smith 2008; Deka *et al.* 2006; Reinke *et al.* 2015), and most recent studies have even pointed at a third hypothesis known as pressure-driven convective flow (Dahlenborg *et al.* 2011, 2015a; Dahlenborg 2014; Altimiras *et al.* 2007). Increasingly, it has been accepted that most likely an interaction of all these different hypotheses provides the best fit to experimental data.

In confectionery, the use of nuts and indulgent low melting fats is unavoidable, as these ingredients impart unique sensory delight critical to the product. Some work has been invested to understand the optimized usage level of these ingredients acceptable for product quality (Rothkopf and Danzl 2015). Comparatively more work has been done on understanding the nature of migration with the aim of developing solutions in immobilizing liquid fat or oil within recipes. Further challenges come with the expanding footprint of chocolate into tropical markets, and this is gradually shifting the focus to addressing migration bloom at tropical ambient temperatures (>25 °C). Nevertheless, formulating shelf-stable filled shelf-stable filled chocolate products under tropical conditions will unquestionably restrict the type of fat-rich ingredients, not to mention the stability of the chocolate itself. It has been observed that exposing praline and other filled chocolates to temperatures between 25 and 30 °C can confer unusual bloom resistance (Walter and Cornillon 2001; Juul 2010). On the contrary, increasing the incubation temperature beyond 30 °C will see the likelihood of heat damage bloom depending on the duration of exposure.

As molten fat moves to the surfaces of the product, dissolution of cocoa butter crystal within the chocolate matrix can happen. Particularly vulnerable are the small crystals that exhibit higher solubility in liquid fat/oil. Following the drop in surrounding temperatures, the dissolved cocoa butter can come out of solution growing onto larger crystal (acting as seed) as explained by Ostwald ripening process (Ziegler *et al.* 2004). As this liquid-mediated recrystallization occurs uncontrollably on the chocolate surface, these growing enlarged crystals will appear as light powdery dusting on the chocolate surface as illustrated in Figure 9.16. Adding to this random crystal growths would be minute crystals that were carried up to the surface with the molten fat before depositing there contributing to more nucleating centres.

Though Ziegleder (1996) has shown a clear correlation between the onset of fat bloom resulting from oil migration, additional factors seem to influence the recrystallization that subsequently lead to the appearance of bloom. The key driver influencing recrystallization lies in the composition of the liquid fat phase that is usually a mixture of filling fats, cocoa butter and nut oil. An important physical property which is affected by the composition of the migrated liquid fat phase is the solubility of the solid fat components therein. In the presence of high amount of nut oil or low melting eutectic blend, fat bloom is usually being suppressed since it is difficult for the cocoa butter to crystallize (Ziegler *et al.* 2004). Hence this observation has led to the proposal of saturation of filling fat with cocoa butter to reduce the dissolution of the cocoa butter by the migrated liquid fat phase. It is noted that though blooming has been retarded, the softening of the chocolate undoubtedly indicates that bulk transfer of the soft filling fat or nut nevertheless has occurred.

Conversely, limited presence of nut oil in the recipe tends to promote polymorphic transformation. Smith *et al.* (2007) demonstrated that hazel-

nut oil promotes the transition from βV to βVI in cocoa butter. Even small amounts of hazelnut oil (1%) can accelerate the polymorphic transition. Transformation from low polymorphic form to higher form crystal is similarly observed by Stewart (2017) where β' to βV cocoa butter crystal occurs in the presence of hazelnut oil. Similarly, the solution will be further enhanced if this is coupled with a mean to block cocoa butter recrystallization or polymorphic transformation.

9.7.5.2 Function of Milk Fat in Retarding Migration Bloom and Storage Bloom

The addition of milk fat to chocolate formulations to improve bloom resistance is now a common industrial practice The addition of 2 to 3% AMF has been recommended to combat migration bloom in dark chocolate particularly in the recipes with nut inclusion (Minifie 1989). The mechanism through which milk fat can retard bloom formation has been studied using two different approaches, (a) by slowing down the rate of cocoa butter recrystallization (Timms 2003) and (b) by retarding the form V to VI transition, as milk fat TAGs are primarily β-stable (Lohman and Hartel 1994).

The ability of milk fat to prevent the recrystallization of cocoa butter from solution derives primarily from the eutectic effect when mixed with cocoa butter. However, excessive addition of milk fat to the recipe would in turn lead to increased liquid fat phase when exposed to elevated temperature. For example, a 10% milk fat addition to milk chocolate leads to a higher migration rate compared to chocolates containing no milk fat (Choi *et al.* 2005).

The inhibitory effect of milk fat on cocoa butter crystallization would also affect the processability of chocolate recipe requiring the latter to be tempered at different conditions. A rule of thumb is to temper milk chocolate at slightly lower temperatures than dark chocolate to offer more undercooling to allow more cocoa butter crystallization (Metin and Hartel 2012). Generally it is well accepted that a reduction of 1 °C in the cooling or crystallization zone will be adequate (Manning and Dimick 1985).

9.7.5.3 Impact of Milk Fat in Processing Conditions

By tempering chocolate containing milk fat, a more densely packed crystal network can be achieved into which the added milk fat can be incorporated. This network can serve to immobilize or delay any liquid fat phase comprising of oil or any low melting fat migrating through the chocolate matrix (Ghosh *et al.* 2002; Svanberg *et al.* 2011; Dibildox-Alvarado *et al.* 2004). A good temper can also ensure a substantial amount of the cocoa butter is crystallized in the stable βV form. This will significantly improve the structural integrity of the chocolate making liquefaction of the fat network less likely to occur when the chocolate is exposed to warmer conditions.

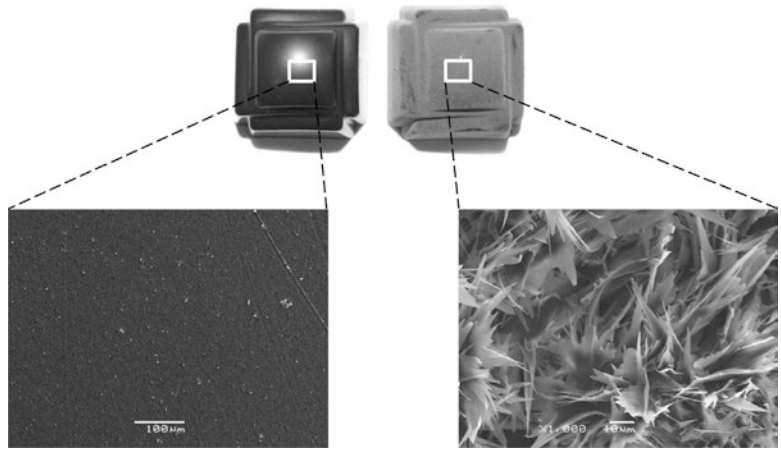

Figure 9.16. Pictures and scanning electron micrographs of the surface of dark chocolate pralines: unbloomed sample stored at 20 °C (left) and bloomed sample stored at 23 °C for 3 months (right) (from Delbaere *et al.* 2016).

However, optimization of tempering conditions to counter migration should never be overlooked in formulation of any chocolate involving the use of milk fat, as this is a critical step in compensating the risk of decreased temperability of the chocolate caused by the eutectic effect of mixing milk fat and cocoa butter (Reddy *et al.* 1996; Liang and Hartel 2004; Hartel 1996). However, inconsistent results have been found when assessing the impact of milk fat on bloom development in chocolate (Bricknell and Hartel 1998; Lohman and Hartel 1994) due to the variability in the tempered status of chocolate used by different authors. This points to the fact that the processing conditions of the chocolate are an essential factor in developing the full antibloom potential of any AMF or milk fat fractions used.

Between the two key processes of tempering and cooling that directly impact on creating the desired structure in chocolate, considerable focus has been placed on tempering either by conventional tempering or through seeding (Barna *et al.* 1992; Reddy *et al.* 1996; Svanberg *et al.* 2011).

Barna *et al.* (1992) found that tempering procedures had to be altered to produce good chocolate and that these alterations were based more on the replacement level of milk fat than on the type of fraction used. As the milk fat content was increased, the tempering temperatures had to be decreased to overcome the inhibition of cocoa butter crystallization caused by the milk fat. However, as is widely known, rapid cooling at low temperature will cause the formation of metastable form. This can lead to lower quantity of βV crystal formation since the bulk of the cocoa butter can be locked in the less stable form which tend to nucleate more rapidly. Under this circumstance, even with the reheating of the chocolate mass to remove the less stable crystal, there will be inadequate βV crystals to achieve the critical level for seeding the chocolate. In other words, there is always a limit below which the crystallization temperature within the temperer cannot be reduced in order to generate adequate βV crystals. Hence, Barna *et al.* (1992) reported tempering difficulties while working on recipes with 20% replacement of cocoa butter by HMF. For other fractions and AMF, 30% replacement level was observed by Barna *et al.* (1992) as the limit. However, other findings set the limit at 20% (Sabariah *et al.* 1998) to 25% of AMF (Brown 2009), at which the crystallization efficiency of a more complex fat blend fat is considered such as involving the use of cocoa butter equivalents (CBEs).

As discussed earlier, the need to lower the crystallization temperature when using conventional tempering to process recipes with high milk fat can severely limit the formation of stable βV crystals. However, this limitation can be circumvented by using seeding technology. The advantage of this technology is the generation of the seed is independent on the crystallization efficiency of the fat system. With availability of sufficient seed, a stable fat crystal network can then be generated during cooling, providing a binding matrix for any liquid fat, as illustrated in Figure 9.17 (Svanberg *et al.* 2011).

With adequate seeds generated in the tempering process, cooling is applied to use these crystals to crystallize the bulk liquid cocoa butter. During cooling, the TAGs from the molten phase will be deposited onto the seeds. These growing crystals eventually join to form an interconnecting network (Afoakwa *et al.* 2009). Depending on the temperature and cooling rate, crystallization of the matrix can vary widely. This can give rise to different polymorphic forms and random crystal size (Kamphuis 2009). For example, when cooling is done at low temperatures, this tends to favour the formation of metastable crystal (βIV), resulting in a less compact network. Consequently, any massive nucleation of βIV will naturally compete for the bulk liquid cocoa butter, limiting the latter from growing onto the βV seeds.

In order to reap the benefits of milk fat in bloom retarding while lessening the adverse effect on the crystallization of cocoa butter, researchers are increasingly looking into milk fat fractions (Kaylegian *et al.* 1993; Lohman and Hartel 1994; Dimick *et al.* 1996) to bridge the gap as previously discussed. The use of HMF and MMF with modified tempering condition is reported to give better resistance to bloom than AMF as compared to LMF (Reddy *et al.* 1996);

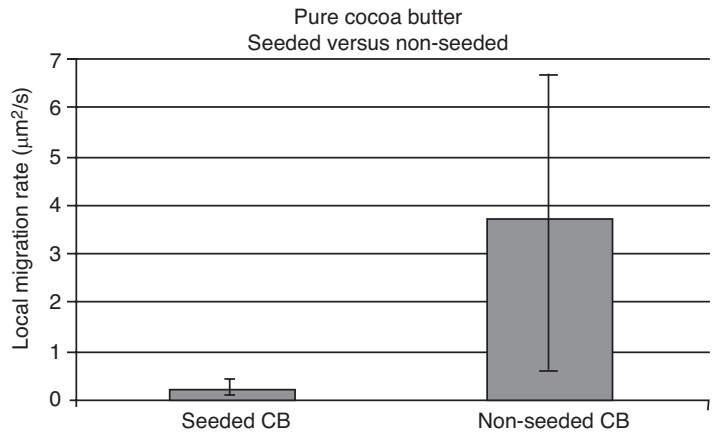

Figure 9.17. Calculated local diffusion rate of boron-dipyrromethene (BODIPY) dye (μm²/s) in seeded versus non-seeded cocoa butter immediately after cooling. Error bars represent standard deviation of fluorescence recovery after photobleaching (FRAP) measurements of three sample replicates (from Svanberg *et al.* 2011).

the eutectic effect of HMF and MMF with cocoa butter (Hartel 1996; Bystrom and Hartel 1994) remains a key challenge in using these milk fat fractions. Studies by other teams however show no eutectic effect for HMF (Kaylegian *et al.* 1993; Marangoni and Lencki 1998) showing little or no reduction in hardness of the chocolate while conferring observable bloom resistance (Lohman and Hartel 1994).

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