11

Significance of Milk Fat in Cheese

T.P. Guinee and P.L.H. McSweeney

11.1. Introduction

Fat is a major component in most cheese types, but its level and importance differ markedly with variety. Inter- and intra-variety differences in fat content are affected by a number of factors, including milk composition (particularly ratio of protein to fat), and the cheesemaking process (recipe, manufacturing procedure and technology), which control the levels of milk fat and moisture retained in the cheese curd and the moisture content of the cheese. The ratio of protein-to-fat in the cheese milk is probably the principal factor influencing fat content, as it controls the relative proportions of two of the three major compositional components in cheese, namely protein and fat; the third major component is moisture. Owing to the inverse relationship between the percentage of moisture and fat in cheese, as discussed in Section 11.2.1, differences in moisture content can lead to relatively large differences in the fat content of cheese varieties of similar fat-in dry matter (FDM) content [e.g., Comte cheese (30% fat, 33.5% moisture, 45% FDM), Danish Havarti (~26.5% fat, 43.5% moisture, 46.9% FDM), Tilsiter cheese (26.5% fat, 42.3% moisture, 46% FDM) and Coulommiers (22% fat; 53% moisture, 46.8% FDM)]. Therefore, it may be more meaningful, to express fat content as a % of dry matter (% FDM) rather than as fat on a total weight basis (%, w/w).

The FDM content is between 42–56% for most of the rennet-curd cheese varieties (e.g., Cheddar, Gouda, Blue, Brie), but varies from ~33% in Grana Padano and low-moisture part-skim Mozzarella to ~70% in Cambazola (see USDA, 1976; Holland *et al.*, 1991; Robinson, 1995; Kosikowski

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and Mistry, 1997); the FDM of the reduced-fat versions of these cheeses is, of course, lower [e.g., ~52 and 31% for full-fat (33%, w/w, fat) and half-fat (17%, w/w, fat) Cheddar cheese, respectively]. Usually, the FDM of fresh acid-curd cheeses is relatively low (< 40% FDM), apart from Double Cream cheese (~73% FDM) and Mascarpone (~89% FDM) (Schulz-Collins and Senge, 2004).

The level of fat influences several aspects of cheese, including composition, biochemistry, microstructure, yield, rheological and textural properties, and cooking properties (Bryant *et al.*, 1995; IDF, 1991; Mistry and Anderson, 1993; Tunick *et al.*, 1993a,b; Merill *et al.*, 1994; Olson and Bogenrief, 1995; Fife *et al.*, 1996; Rudan and Barbano, 1998; Rudan *et al.*, 1999; McMahon *et al.*, 1999; Guinee *et al.*, 1998, 2000a; Fenelon and Guinee, 1999, 2000; Fenelon *et al.*, 2000b; Metzger *et al.*, 2001a,b; Gwartney *et al.*, 2002; Michaelidou *et al.*, 2003a,b). Moreover, for a given fat content, the type of fat (melting point) and the state of the fat (non-globular, free fat, homogenized, globule size distribution, solid-to-liquid ratio) has a major impact on the rheology, flavor and cooking properties of cheese (Lelievere *et al.*, 1990; Metzger and Mistry, 1995; Poudaval and Mistry, 1999; Tunick *et al.*, 1993a,b; Rudan *et al.*, 1998b; Wijesundra *et al.*, 1998; Nair *et al.*, 2000; Oommen *et al.*, 2000; Guinee *et al.*, 2000a). Fat also contributes to flavor directly and indirectly *via* lipolysis (Balcao and Malcata, 1998; Gripon, 1997; Guinee and Law, 2002). Moreover, in some varieties, free fatty acids (FFAs) serve as precursors for other flavor compounds, (e.g., in Blue cheeses) in which FFAs are oxidised to methyl ketones, which in turn may be reduced to secondary alcohols (Collins *et al.*, 2004).

The increased affluence in western society has resulted in excessive calorie intake, and expert panels have recommended reduction in the intake of both total and saturated fat (see O'Brien and O'Connor, 2004). Dietary fat has been shown to be associated with an increased risk of obesity, atheroosclerosis, coronary heart disease, elevated blood pressure, and tissue injury diseases associated with the oxidation of unsaturated fats (Hayes, *et al.*, 1991; Hodis *et al.*, 1991; Doyle and Pariza, 2002; McNamara, 1995; Simon *et al.*, 1996;). This has created increased consumer concern over the implications of dietary fat on health, and large increases in the supply, and demand for, low-fat foods, including cheese (Shank and Carson, 1990; Dexheimer, 1992). However, it is generally acknowledged by market experts that the consumption of low- and reduced-fat cheeses remained relatively low (e.g., 8% of total cheese in the UK) throughout the 1990s, though some current commercial information indicates large increases in the market for low-fat cheese varieties (e.g., see www.arlafoods.com). The relatively low consumption of reduced-fat cheese has been attributed to poor consumer perception of the products based on taste and texture (Olson and Johnson, 1990; Bullens, 1994; Anonymous, 1996). Textural defects include increased firmness, rubberiness, elasticity, hardness, dryness, and graininess. The negative flavor attributes of reduced-fat Cheddar include bitterness (Ardö and Mansson, 1990) and a low intensity of typical Cheddar cheese aroma and flavor (Banks *et al.*, 1989; Jameson, 1990). Approaches used to improve the quality of reduced-fat cheese include:

(1) Alterations to the cheese making procedure to reduce the calcium-to-casein ratio, increase the moisture-to-protein ratio and reduce the extent of *para*-casein aggregation [e.g., by high pasteurization temperature, high pressure treatment of milk, reducing the pH at setting and whey drainage, and/or increasing gel firmness at cutting (e.g., Banks *et al.*, 1989; McGregor and White, 1990; Metzger and Mistry, 1994; 1995; Guinee *et al.*, 1998; Fenelon *et al.*, 1999; Rudan *et al.*, 1998b; Poduval and Mistry, 1999; Molina *et al.*, 2000; Nair *et al.*, 2000)]
(2) The use of specialized starter cultures and starter culture adjuncts, and/or exogenous enzymes (e.g., Ardö *et al.*, 1989; Skeie *et al.*, 1995; Midje *et al.*, 2000; Tungjaroenchai *et al.*, 2001; Broadbent *et al.*, 2002; Fenelon *et al.*, 2002; Katsiari *et al.*, 2002)
(3) The addition of fat mimetics to the milk (e.g., Desai and Nolting, 1995; Kucukoner and Haque, 1995; McMahon *et al.*, 1996; Fenelon and Guinee, 1997; Rudan *et al.*, 1998a; Bhaskaracharya and Shah, 2001; El-Sheikh, *et al.*, 2001).

These approaches have been reviewed extensively (Jameson, 1990; Ardö, 1997; Fenelon and Guinee, 1997; Fenelon, 2000). Various recommendations for the manufacture of reduced-fat cheeses with improved sensory and textural properties (Mistry *et al.*, 1996; Johnson *et al.*, 1998), (e.g., half-fat Cheddar prepared by: homogenization of cream used to standardize the cheese milk) (Nair *et al.*, 2000); the combined effects of increases in milk pasteurization temperature and pH at curd milling, and the use of selected starters and starter culture adjuncts (Guinee *et al.*, 1999; Fenelon *et al.*, 2002);

The focus of this chapter is on the generic effects of fat on the composition, structure, yield, flavor, rheology and functionality of hard and semi-hard cheeses and pasteurized processed cheese products.

11.2. Effect of Fat on Cheese Composition

11.2.1. Fat Content

Fat is a major compositional component of most cheese varieties and major changes in its level result in concomitant changes in the levels of moisture and protein, and in cheese yield (Drake *et al.*, 1995a, b; Fenelon and Guinee, 1999; Melilli *et al.*, 2002).

Numerous workers have investigated the effects of the fat content of milk on the composition of several cheese types, including Cheddar and Mozzarella (Gilles and Lawrence, 1985; Tunick *et al.*, 1991, 1993a; Bryant *et al.*, 1995; Nauth and Ruffie, 1995; Drake *et al.*, 1996b; Fenelon and Guinee, 1999; Rudan *et al*, 1999). In studies where cheese making conditions are held constant, reduction of the fat content of cheese is paralleled by

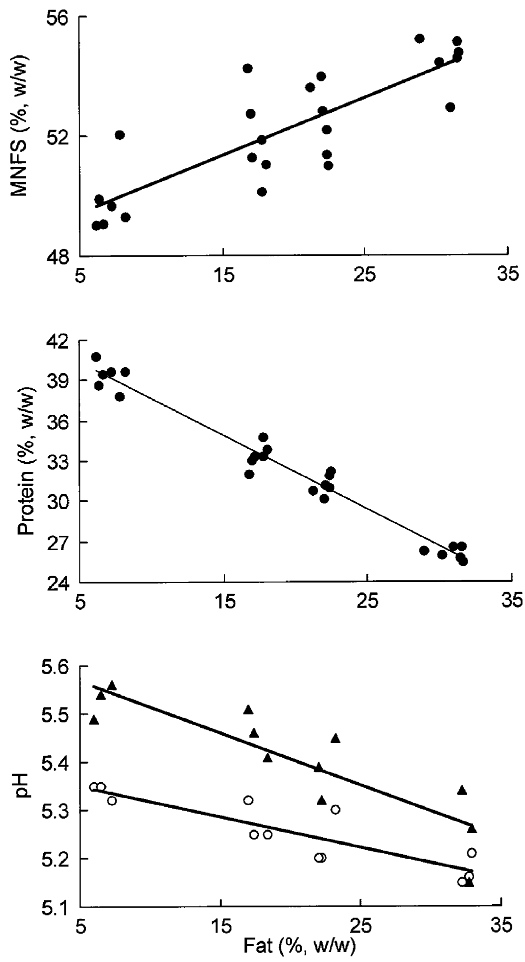

Figure 11.1. Effect of fat content on the composition of Cheddar cheese; pH is shown for the 60 (○) and 180 (▲)-day old cheeses (redrawn from data of Fenelon and Guinee, 1999; Guinee *et al.*, 2000a).

Table 11.1. Gross Composition of Some Cheese Varieties

Cheese type	Fat (%, w/w)	Moisture (%, w/w)	Protein (%, w/w)	MNFS[a] (%, w/w)	Calcium (mg/100g)	Phosphorus (mg/100g)	pH
Cheddar[b]							
low-fat	7.2	46.1	38.5	49.6	1097	839	5.52
half-fat	17.2	43.0	33.3	51.9	937	680	5.45
reduced-fat	21.9	40.9	31.0	52.4	872	639	5.37
full-fat	30.4	37.8	26.4	57.0	742	533	5.25
Feta[c]	21.9	56.4	15.9	72.2	n.a.[a]	n.a.	4.57
	6.5	66.8	20.1	71.4	n.a.	n.a.	4.68
Kefalograviera-type[d]	9.8	48.4	33.4	53.6	n.a.	n.a.	5.40
	30.6	37.8	26.1	54.6	n.a.	n.a.	5.49
Mozzarella[e]							
reduced-fat	12.3	48.5	32.8	55.3	n.a.	n.a.	n.a.
reference	21.2	47.0	25.5	59.6	n.a.	n.a.	n.a.
Mozzarella[f]							
low-fat	2.2	51.2	24.6	64.5	n.a.	n.a.	n.a.
low-fat	5.0	62.5	30.4	64.5	n.a.	n.a.	n.a.
part-skim	19.3	63.6	30.1	65.0	n.a.	n.a.	n.a.
Mozzarella[g]							
low-fat	9.9	54.0	n.a.	60.0	n.a.	n.a.	n.a.
high-fat	24.4	48.5	n.a.	64.5	n.a.	n.a.	n.a.

[a] Abbreviations: MNFS, moisture in non-fat cheese substances; n.a., not available
[b] From Guinee *et al.* (2000a); pH measured at 120 d.
[c] From Michaelidou *et al.* (2003a); pH measured at 120 d.
[d] From Michaelidou *et al.* (2003a); pH measured at 90 d.
[e] From Puduval and Mistry (1999).
[f] From Fife *et al.* (1996).
[g] From Tunick *et al.* (1995).

increases in the concentrations of moisture and protein and reductions in the levels of fat-in-dry matter (FDM), moisture-in-non-fat substance (MNFS), and pH (Figure 11.1; Table 11.1). The unit changes in the latter compositional parameters on reducing the fat content in the range 33 to 6% (w/w) for Cheddar cheese were: +0.36 g moisture/g fat, +0.55 g protein/g fat, +0.05 g ash /g fat, –0.2 g MNFS/g fat, and –1.5 g FDM/g fat (Fenelon and Guinee, 1999). The increase in cheese pH as the fat content is reduced may be attributed to the concomitant decrease in the level of MNFS and, hence, lactate-to-protein ratio (Fenelon and Guinee, 2000). A similar effect was observed in cheeses with a similar level of protein but with different levels of lactic acid, as affected by altering the lactose concentration in the cheese milk (Shakeel-Ur-Rehman *et al.*, 2004).

A small increase in MNFS (2–4%, w/w) leads to a relatively large increase in free, available water, which in turn leads to increases in the activity of microorganisms and enzymes and the degree of proteolysis in cheese (Creamer, 1971; Pearce and Gilles, 1979; Lawrence and Gilles, 1980; Lawrence *et al.*, 1987). Hence, normalization of the MNFS is considered especially important to improve the quality of reduced-fat cheeses. Consequently, in commercial cheese manufacture and in many studies relating to the improvement of the quality of reduced-fat cheese, cheese making procedures are frequently altered so as to give reduced-fat cheese with a level of MNFS similar to that of the full-fat equivalent (Banks *et al.*, 1989; Ardö, 1993; Drake *et al.*, 1995b). Hence, the fat content of retail Cheddar cheese is inversely correlated with the levels of moisture and protein but it does not significantly affect the level of MNFS (Banks *et al.*, 1992; Fenelon *et al.*, 2000b).

11.2.2. Effect of Degree of Fat Emulsification as Influenced by Homogenization of Milk, Cream and/or Curd

It is generally accepted that homogenization of milk, or the cream used to standardize the cheese milk, at a combined first-stage and second-stage pressures of 5–20 MPa, reduces the degree of curd syneresis (Pearse and MacKinlay, 1989) and thereby increases the levels of moisture and MNFS in cheese (Table 11.2). Cheeses, for which increases in moisture or MNFS have been reported, include Cheddar of different fat content (Emmons *et al.*, 1980; Metzger and Mistry, 1994; Nair *et al.*, 2000), Edam (Amer *et al*, 1977), Gouda (Versteeg *et al.*, 1998) and Mozzarella (Jana and Upadhyay 1991, 1992, 1993; Tunick *et al.*, 1993a; Rudan *et al.*, 1998b). A similar trend was reported for the effect of high-pressure (~100 MPa) homogenization of milk for goats' milk cheese (Guerzoni *et al.*, 1999) and Cheddar (O'Mahony, Hayes, McSweeney and Kelly, unpublished results). The extent of the increase in moisture varies depending on homogenization pressure and cheese making practices (Jana and Upadhyay 1991; 1992; Table 11.2). Oommen *et al.* (2000) reported an interactive effect between the protein content of cheese milk (as varied by ultrafiltration) and homogenization of cream (at first and second stage pressures of 6.9 and 3.5 MPa, respectively) on the levels of moisture and MNFS in Cheddar cheese, with the magnitude of the increase decreasing as the milk protein level was increased from 3.2 to 6.0% (w/w). Hence, homogenization of milk may be a convenient means to offset the reductions in moisture (~3%, w/w) and MNFS in Cheddar when the protein content of milk is increased from 3 to 5%, w/w, by ultrafiltration (Guinee *et al.*, 1996, 2004b; Oommen *et al.*, 2000). Microfluidization of milk at 7 MPa or cream at 14 or 69 MPa also increases the moisture content in Cheddar cheese (Lemay *et al.*, 1994) (Table 11.2).

Table 11.2. Effect of Homogenization or Microfluidization of Milk, or Cream, on the Composition of Cheese

Treatment	Treated	Cheese	Composition (%, w/w)					Reference
			Fat	FDM[a]	Protein	Moisture	MNFS[a]	
Microfluidisation[b] pressure (MPa)		Cheddar						
0	Milk		34.4	52.9	25.5	35.0	53.4	Lemay *et al*. (1994)
7	Milk		34.0	54.5	23.0	37.6	57.0	
14	Cream		33.4	54.3	22.6	38.4	57.7	
69	Cream		33.0	36.6	22.1	39.3	58.6	
Homogenization[c] pressure (MPa)		Cheddar						
0	Milk		29.9	49.2	25.3	39.2	55.9	Guinee *et al*.(2000c)
30	Milk		30.6	51.2	23.9	40.2	57.9	
Homogenization[d] pressure (MPa)		Cheddar						
0	Cream		33.1	53.3	24.7	37.9	56.7	Nair *et al*. (2000)
7	Cream		32.9	54.1	23.9	39.2	58.4	
10.4	Cream		32.8	53.7	23.6	38.9	57.9	
13.7	Cream		32.1	52.8	23.5	39.3	57.8	
Homogenisation[e] pressure (MPa)		Reduced-fat Cheddar						
0	Cream		17.7	32.9	30.5	46.0	55.9	Metzger and Mistry (1995)
20.7	Cream		17.8	33.6	29.6	47.1	57.3	
Homogenization[f] pressure (MPa)		Half-fat Cheddar						
0	Milk		18.2	31.8	33.5	42.7	52.2	Fenelon (2000)
15	Milk		18.0	32.1	32.6	44.0	53.6	
Homogenization[b] pressure (MPa)		Mozzarella						

(*Continued*)

Table 11.2. (*Continued*)

			Composition (%, w/w)					
Treatment	Treated	Cheese	Fat	FDM[a]	Protein	Moisture	MNFS[a]	Reference
0	Milk		25.8	47.8	21.5	46.0	62.0	Jana and Upadhyay (1991)
4.5	Milk		26.5	54.6	19.0	51.4	69.9	
Homogenization[b] pressure (MPa)		Mozzarella						
0			25.9	48.2	n.a.[a]	46.3	62.4	Tunick *et al.* (1993b)
10.3			26.9	51.7	n.a.	47.9	65.5	
17.2			27.8	52.2	n.a.	46.8	64.8	
Homogenization[b] pressure (MPa)		Reduced-fat Mozzarella						
0			11.1	23.3	n.a.	52.5	59.0	Tunick *et al.* (1993b)
10.3			9.4	20.9	n.a.	55.1	60.8	
17.2			10.8	23.5	n.a.	23.9	60.5	

[a] Abbreviations: FDM, fat-in-dry matter; MNFS, moisture-in-non-fat substances; n.a., not available.
[b] Total pressure; first and second stage pressures not specified.
[c] First/second stage pressures of 25/5 MPa, respectively.
[d] The first and second stage pressures (MPa) for the different homogenization treatments were: 3.5/3.5, 6.9/3.5, and 10.4/3.5, respectively.
[e] First/second stage pressures of 17.3/3.4 MPa, respectively.
[f] First/second stage pressures of 10/5 MPa, respectively

The impaired syneresis in curd/cheese made from homogenized milk may be due to the increased interaction between the casein and the fat, which reduces the surface area of the casein micelles available for mutual interaction (Green *et al.*, 1983). Curd syneresis is the physical expulsion of whey, which accompanies contraction of the protein matrix. Matrix contraction may be viewed at the microstructural level as an increased aggregation and joining of adjacent casein strands into larger aggregates, both within curd particles and at curd particle junctions (Kimber *et al.*, 1974). The reduction in casein hydration and the increase in matrix contraction that parallels casein aggregation reduce the ability of the matrix to retain whey. Hence, a lower degree of casein-casein interaction in curds made from homogenized milk would lead to higher moisture content. The increase in the fineness of rennet-induced milk gels that accompanies milk homogenization (Green *et al.*, 1983) may also contribute to the higher moisture content of homogenized milk cheeses; a finer gel has lower porosity, a factor that would be expected to impede moisture expulsion.

11.3. Contribution of Fat to the Microstructure of Cheese

11.3.1. Microstructure of Rennet-Curd Cheese

11.3.1.1. The Protein Phase

Cheese is essentially a concentrated protein gel, which occludes fat, moisture, and other materials. Gelation of the milk is brought about either by :

- Hydrolysis of the casein micelle-stabilizing κ-casein by the action of selected acid proteinases (rennets), and the resultant slow quiescent aggregation of the destabilized micelles in the presence of calcium ions (~3 mM) at ~30–36°C; (e.g., for most rennet-curd cheeses such as Cheddar, Mozzarella and Gouda)
- Acidification to the isolectric pH of casein using lactic acid bacteria or food-grade acids/acidogens, at 20–40°C, and resultant slow quiescent aggregation of the sensitized casein micelles e.g., for cream cheese. [A combination of acidification and rennet-hydrolysis (a smaller quantity of rennet than for rennet-curd cheeses, e.g., 5–100 versus 900–1000 chymosin units per 100 L milk) is normally used for low-fat acid-curd cheeses such as Quark and related varieties (Schulz-Collins and Senge, 2004)]
- Non-quiescent acidification to pH ~5.4–5.6 at a high temperature (80–90°C), [e.g., for Paneer, Ricotta, and some forms of Queso Blanco cheese (Farkye, 2004)].

The microstructure of milk gels and of the resultant cheeses has been studied extensively (Hall and Creamer, 1972; Kimber *et al.*, 1974; de Jong, 1978a; Brooker, 1979; Gavarić *et al.*, 1989; Kiely *et al.*, 1992, 1993; Desai and Nolting, 1995; Bryant *et al.*, 1995; Kaláb, 1995; Mistry and Anderson, 1993; Guinee *et al.*, 1998, Fenelon *et al.*, 1999; Auty *et al.*, 2001). The gelation of milk is characterized by aggregation of the rennet-altered casein micelles into interconnected clusters and forming a network in which fat globules are interspersed as loose inclusions (Gavarić *et al.*, 1989). The protein matrices of both acid-induced and rennet-induced milk gels are particulate (Gavarić *et al.*, 1989), being composed of entangled clusters of partially fused casein or *para*-casein aggregates. Continued aggregation of the casein, or *para*-casein, and expulsion of whey during cheese manufacture leads to the gradual fusion of the *para*-casein micelles and contraction of the protein gel network around the fat globules, which are, consequently, forced into closer proximity. Hence, the matrix changes from being particulate to a highly fused aggregated structure in which the fat globules and protein are microstructurally in contact (Figure 2a).

The integrity of the matrix is maintained by various intra-aggregate and inter-aggregate electrostatic and hydrophobic attractions between amino acid side groups on the caseins, and between calcium ions and organic serine phosphate groups or ionized carboxyl residues (calcium bridges) (Walstra and Van Vliet, 1986). The network is essentially continuous, extending in all directions, although some discontinuities exist at the micro-structural and macro-structural levels. Micro-structural observations made using transmission electron microscopy (TEM) suggest that the hydrolysis of *para*-casein (e.g., by rennet activity) to water-soluble peptides results in parts of the matrix losing contact with the main *para*-casein network, an occurrence that leads to discontinuities or 'breaks' in the *para*-casein matrix at the microstructural level (de Jong, 1978a). Hence, it is noteworthy that ageing of Mozzarella for 50 d results in the degradation of 50% of α_{s1}-casein to α_{s1}-CN (f 24–199) and an increase in the porosity of the defatted *para*-casein matrix, as observed using scanning electron microscopy (SEM) (Kiely *et al.*, 1993). Discontinuities at the macrostructural level exist in the form of curd granule junctions and, in Cheddar and related dry-salted cheese varieties, as curd chip junctions. Both kinds of junction are discernible by the naked eye in appropriately prepared sections (Brooker, 1979; Kaláb, 1979; Lowrie *et al.*, 1982; Rüegg and Blanc, 1987). Unlike the interior of the curd particles, which consists of protein and fat at a ratio corresponding closely to that for the overall cheese, the junctions are comprised mainly of casein, being almost devoid of fat. The difference in cheese composition between the interior and the surface of curd particles arises as a result of the cutting or breaking of the coagulum into curd particles, which leads to the loss of fat globules from the freshly cut surfaces into the surrounding whey. As the protein matrix contracts and adjoining curd particles mat through their fat-depleted surface layers, these fat-depleted areas become part of the internal structure of the cheese.

Various physico-chemical changes occur in the structural components of the *para*-casein matrix during maturation; these changes are mediated by the residual rennet, microorganisms and their enzymes, and changes in mineral equilibrium between the serum and *para*-casein matrix. The type and level of the physico-chemical changes depend on the cheese variety, cheese composition and ripening conditions. These may include:

- Hydrolysis of the *para*-casein into peptides and amino acids (Upadhyay *et al*., 2004) and of tryglycerides into fatty acids and various catabolic products such as esters, alcohols and lactones (Collins *et al*., 2004)
- Changes in the equilibrium concentrations of calcium and inorganic phosphate between the matrix and the occluded serum, with the equilibrium being influenced by maturation time, pH and other factors such as the concentration of Na^+ in the moisture phase and soluble Ca (Le Graet *et al*., 1983; Karahadian and Lindsay, 1987; Guo and Kindstedt, 1995; Guo *et al*., 1997; Paulson *et al*., 1998)
- Increase in hydration of the *para*-casein and physical expansion or swelling of the *para*-casein matrix, at least in Mozzarella cheese (Kindstedt, 1995; Guo and Kindstedt, 1995; Guo *et al*., 1997; Thierry *et al*., 1998; Boutrou *et al*., 1999; Guinee *et al*., 2000a, 2002)

The hydration and swelling of the casein matrix has a major influence on the structure of the fat phase and the cooking properties of the cheese, as discussed in Sections 11.3.1.2 and 11.9.

11.3.1.2. Microstructure of the Fat Phase

The enmeshed fat globules occupy the spaces between the protein strands and may be considered to impede physically the aggregation of the *para*-casein matrix, to a degree dependent on their volume fraction and size distribution. Consequently, a higher fat level leads to slower syneresis during manufacture (Dejmek and Walstra, 2004), and an increase in the level of MNFS in the cheese (Tunick *et al*., 1995; Poudaval and Mistry, 1999; Fenelon and Guinee, 1999); the increase in MNFS has a major impact on cheese yield and quality, as discussed in Sections 11.4, 11.6–11.9.

Some clumping and/or coalescence of fat globules generally occur in most cheese varieties. Evidence for the clumping of fat globules in Cheddar cheese has been demonstrated clearly by both SEM and TEM

(Hall and Creamer, 1972; Kalab, 1979; Mistry and Anderson, 1993; Bryant *et al*., 1995; Metzger and Mistry, 1995) and confocal laser scanning microscopy (CLSM) (Guinee *et al*., 1999; Auty *et al*., 1999). SEM micrographs of cheese samples (e.g., Cheddar) from which the fat globules had been extracted during sample preparation, reveal irregularly shaped voids in the *para*-casein matrix (Mistry and Anderson, 1993; Bryant *et al*., 1995; Figure 11.2a). Similarly, SEM shows coalescence in Mozzarella and String cheeses in which clumped fat globules coexist with moisture as long channels between the *para*-casein fibers (Taneya *et al*., 1992; Kiely *et al*., 1992; McMahon *et al*., 1993, 1999; Tunick *et al*., 1993a; Kalab, 1995; Guinee *et al*., 1999). TEM micrographs taken over the course of Cheddar manufacture clearly show the aggregation of fat globules, which is first notable at maximum scald and increases during cheese making as the protein network shrinks and forces the fat globules into closer contact (Kimber *et al*., 1974; Kalab, 1995; Laloy *et al*., 1996). In contrast to Cheddar and Mozzarella, relatively little clumping and coalescence of fat globules is evident in other cheese types such as Cheshire, Gouda (Hall and Creamer, 1972) or Meshanger cheese (de Jong, 1978a). The relatively high degree of fat globule coalescence in Cheddar and Mozzarella is probably due to the relatively large displacement of neighboring layers of protein matrix, between which fat globules and fat globule clusters are sandwiched during the cheddaring and/or kneading/stretching stages of manufacture. Such displacement can be expected to "stretch" the fat globules and, consequently, shear and disrupt their membranes.

Factors that may contribute to the clumping and coalescence of fat globules in cheese include:

- Shearing of the MFGM during cheese manufacture, as a result of stress and strains inflicted on the curd particles during cutting, stirring and curd handling operations such as dewheying, cheddaring, milling, salting, pressing and/or plasticization.
- Dehydration and contraction of the *para*-casein matrix during manufacture that force the occluded globules into closer contact (Kimber *et al*., 1974).
- Hydration and swelling of the casein matrix during storage, at least in Mozzarella cheese, an event which is expected to shear, and rip, residual membrane from the fat globules/globule clumps (Kindstedt and Guo, 1997; McMahon and Oberg, 1999).
- A possible increase in the permeability of the MFGM during maturation due to storage-related hydrolysis of membrane components by lipoprotein lipase activity (Sugimoto *et al*., 1983; Deeth, 1997).

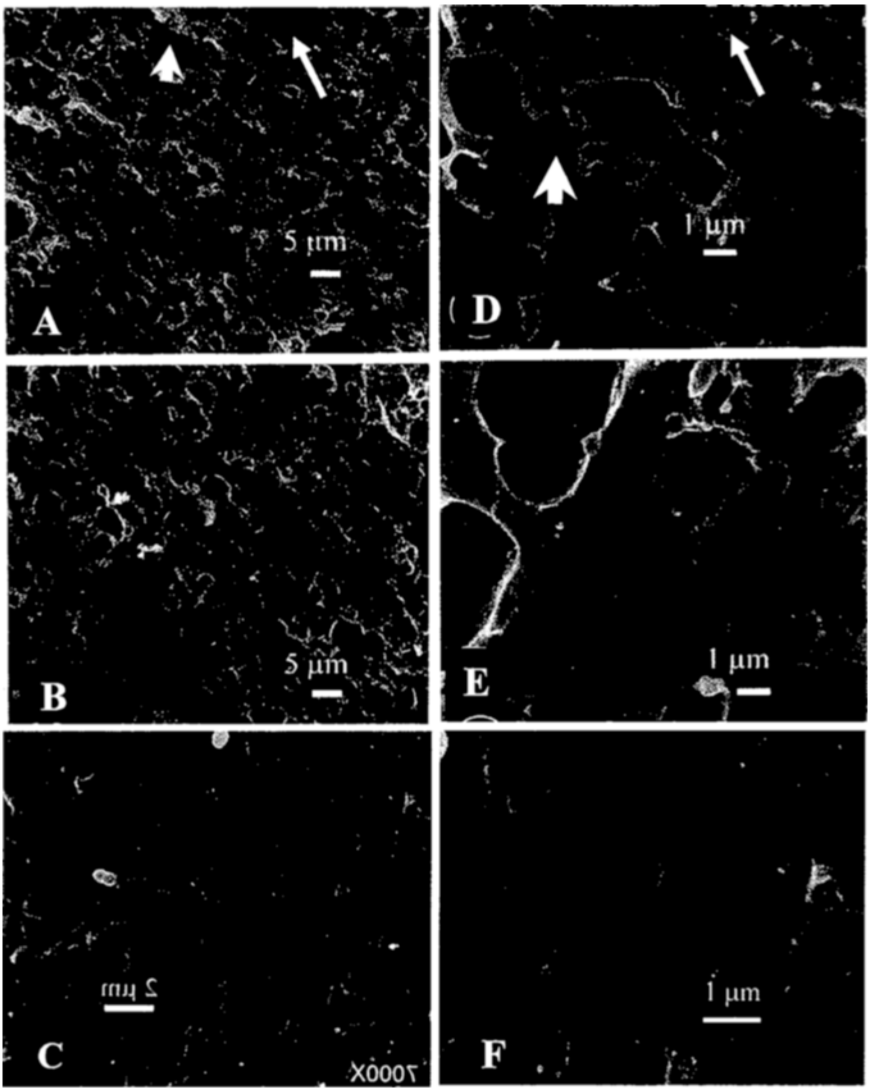

Figure 11.2. Scanning electron micrographs of full-fat (33.0%, w/w: A, D); half-fat (17.0%, w/w; B, E) and low-fat (3.9% w/w; C, F) Cheddar cheese at low (A, B, C) or high (D, E, F) magnifications. The arrows indicate the *para*-casein matrix and the arrowheads the areas occupied by fat and free serum prior to their removal during sample preparation [modified from Guinee *et al*., 1998 (a, b, d, e) and Fenelon *et al*., 1999 (c, f) with permission].

- An increase in the porosity of the *para*-casein matrix, concurrently with the age-related increase in proteolysis (Tunick and Shieh, 1995), which may reduce the impedance of the matrix structure to the movement of fat.

The more extensive degree of coalescence of fat in Mozzarella and String cheeses compared to Cheddar reflects the shearing of the MFGM during plasticization and elongation of the curd. Plasticization that involves kneading and heating of curd to ~57°C in hot water, is conducive to deformation and disruption of the MFGM and aggregation of globular and non-globular fat. Similarly, elongation of plasticized curd in the manufacture of String cheese results in the deformation (and probably coalescence) of the fat globules lying between contiguous layers of the *para*-casein matrix, to a degree that increases with elongation (Taneya *et al.*, 1992).

At the temperatures used in the manufacture of cheese (~30–55°C) much, or all, of milk fat is liquid (Wright *et al.*, 2002) and can therefore flow and aggregate, leading to coalescence on the application of stress. A significant portion of the fat (~20–30% total) may be liquid at the ripening temperatures used for Cheddar or Mozzarella (~4–7°C) (Wright *et al.*, 2002), and will aggregate, leading to coalescence. Thus, increasing the liquid-to-solid fat ratio, by increasing the ratio of low melting point fat fraction (olein) to the high melting point (stearin), results in a higher level of free oil in Mozzarella cheese made from recombined milk prepared by homogenizing skim milk and fat fractions (Rowney *et al.* 2003).

In addition to protein and fat, the matrix occludes moisture and its dissolved solutes and enzymes, and bacteria (Kimber *et al.*, 1974; Laloy *et al.*, 1996). The starter and non-starter bacteria appear to attach, *via* filaments from their cell walls, to the casein matrix (Kimber *et al.*, 1974) and are concentrated near the fat-casein interface (Laloy *et al.*, 1996; Haque *et al.*, 1997). The concentration of bacteria at the fat-casein interface may have several potential consequences (Laloy *et al.*, 1996):

- Concentration of intracellular bacterial peptidases in the vicinity of the protein-fat interface following bacterial lysis
- A more heterogeneous distribution of starter bacteria in cheese at the microstructural scale, as the level of fat is reduced owing to the concomitant reduction in fat/casein surface area, and
- A more uneven distribution of starter cell proteinase/peptidase activity in reduced-fat cheese and possible restricted access of substrates (polypeptides/peptides released by the action of coagulant, etc.) to enzymes

Hence, the location of bacteria in cheese at the fat-casein interface may be important in relation to the growth dynamics of starter and non-starter bacteria in cheese and their effects on cheese maturation (*cf*. Sections 11.5, 11.7–11.8).

11.3.2. Microstructure of Pasteurized Processed Cheese Products (PCPs) and Analogue Cheese Products (ACPs)

Microstructural studies on PCPs or ACPs indicate that the structure is that of a concentrated emulsion of discrete, rounded fat droplets of varying size in a hydrated protein matrix (Kimura *et al*., 1978; Taneya *et al*., 1980; Rayan *et al*., 1980; Heertje *et al*., 1981; Lee *et al*., 1981; Kalab *et al*., 1987; Tamime *et al*., 1990; Savello *et al*., 1989; Kalab, 1995; Guinee *et al*., 1999). The fat and *para*-casein are distributed more homogeneously and the matrix is finer, being less compact and fused, than in natural rennet-curd cheese. High resolution TEM (e.g., 60 000 $\times$) shows that the protein phase consists of varying proportions of individual *para*-caseinate particles and strands, which are probably formed through association of *para*-caseinate particles. The individual particles (20–30 nm in diameter) may correspond to casein sub-micelles released from the *para*-casein micelles in the matrix of the natural cheese as a result of sequestration of calcium by the emulsifying salt (ES). The rheolgy and texture characteristics of the cheese vary with the proportions of strands to individual particles, with hard PCPs containing a high level of long protein strands (e.g., $\sim$100 versus 25 μm), which form a matrix and soft PCPs usually consisting mainly of individual particles.

Compared to natural cheese, there is less clumping or coalescence of fat globules in PCPs and ACPs. Consequently, the mean fat globule size tends to be generally smaller (Sutheerawattananonda and Bastian, 1995), although it varies depending on the type and level of ES, types and levels of milk protein added, processing time and extent of shear (Rayan *et al*., 1980; Kaláb *et al*., 1987, 1991a; Savello *et al*., 1989; Tamime *et al*., 1990). Generally, for most emulsifying salts, the fat globule size decreases as the processing time at a high temperature increases, [e.g., up to 40 min (Kaláb *et al*., 1987; Ryan *et al*., 1980)]. The *para*-caseinate membrane of the emulsified fat globules appears to attach to the matrix strands, thereby contributing to the continuity of the matrix. The incorporation of emulsified *para*-caseinate-coated fat globules, which can be considered as pseudo-protein particles, into the new structural matrix may be considered as increasing the effective protein concentration (van Vliet and Dentener-Kikkert, 1982; Marchesseau *et al*., 1997; Michalski *et al*., 2002).

The degree of fat emulsification and the fat globule size have a major impact on the rheolgy and cooking properties of PCPs and ACPs (Guinee *et al.*, 2004a)

11.3.3. Effect of Fat Level on Microstructure

The effects of fat content on the microstructure of Cheddar (Mistry and Anderson, 1993; Bullens *et al.*, 1994; Baer *et al.*, 1995; Bryant *et al.*, 1995; Desai and Nolting, 1995; Metzger and Mistry, 1995; Drake *et al.*, 1996a,b; Guinee *et al.*, 1998, 2000a) and Mozzarella (McMahon *et al.*, 1996, 1999) have been evaluated in a number of studies but little information is available on effect of fat content on the structure of other rennet-curd cheese varieties.

Increasing the fat content of Cheddar results in a reduction in the volume fraction and continuity of the casein matrix, which becomes more interrupted by fat globules (Figure 11.2). Concomitantly, the fat globules become more numerous and varied in size and shape, and the degree of clumping and coalescence of the fat globules increase. The increased degree of fat globule aggregation is expected because the number of encounters of the fat globules within a given volume of the casein matrix increases as the enveloping protein matrix contracts during manufacture and as the curd particles undergo deformation during the various stages of cheese making. Conversely, as the fat content of cheese is reduced there are longer stretches of uninterrupted casein matrix and the fat globules become more uniformly dispersed and less clumping is evident. Some fat mimetics (e.g., Novagel™, Dairy Lo™, ALACO PALS™) have been found to enhance the uniformity of the fat distribution in reduced-fat Cheddar cheese (Drake *et al.*, 1996a).

Similarly, a comparison of regular (Oberg *et al.*, 1993; McMahon *et al.*, 1999) and low-fat Mozzarella (LFMC) (McMahon *et al.*, 1996) showed that reducing the fat content gives a denser casein matrix and a lower degree of fat coalescence. Indeed, in low-fat Mozzarella (e.g., $< 4\%$, w/w), there are insufficient fat globules to keep the casein strands apart as the curd is forming (McMahon *et al.*, 1993). Owing to the high degree of casein aggregation, the matrix is extremely compact and the number and width of the serum-fat channels between the protein layers is reduced markedly compared to the regular part-skim Mozzarella curd ($\sim$17%, w/w, fat). Consequently, the matrix of low-fat Mozzarella has a low moisture-retaining capability and the cheese has a relatively low level of MNFS. The change in microstructure that accompanies fat reduction adversely affects the heat-induced functionality of Mozzarella (Section 11.9). McMahon *et al.* (1996) reported that the addition of Novagel™ (a blend of microcrystalline cellulose and guar gum with a particle size of 10 to 100 μm) to the cheese milk resulted in the formation of large amorphous particles in low-fat Mozzarella. These large particles had the effect of impeding the degree of *para*-casein aggregation during curd manufacture and plasticization. Consequently, the Novagel™ particles resulted in the formation of relatively large serum channels (e.g., up to 300 μm) between the protein fibers and a higher moisture content, compared to the control low-fat Mozzarella.

11.3.4. Effect of Fat Emulsification on Microstructure

The degree of fat emulsification in natural rennet-curd cheeses may be increased by homogenization or microfluidization of the cheese milk, or of the cream used to standardize the cheese milk. Microfluidization is also a particle size reduction process that is used mainly in the manufacture of products such as antibiotic dispersions, parenteral emulsions, and diagnostics. It is generally accepted that for the application of equivalent pressures to the milk, microfluidization gives a lower mean fat globule diameter (e.g., 0.03–0.3 μm compared to 0.5–1.0 μm) and a narrower size distribution than homogenization (typically 1–2 μm). Moreover, the fat globule membrane in microfluidized milk has a higher proportion of fragmented casein micelles than homogenized milk and has little, or no, whey protein. Additionally, the degree of fat emulsification in acid-curd natural cheeses may be increased by shearing of the curd after whey separation (Mahaut and Korolczuk, 2002).

Homogenization of milk is an integral part of the manufacturing process for soft, high-fat, acid-curd cheeses such as Cream cheese, as it prevents creaming (flocculation of fat globules) during the relatively long gelation time (e.g., >4 h) and contributes to the formation of a homogeneous, viscous, creamy texture in the end product (Mahaut and Korolczuk, 2002). In contrast, milk or cream is not normally homogenized in the manufacture of hard and semi-hard rennet-curd cheeses, as it leads to curds that do not knit/mat well together and to crumbly cheese. The defects are probably associated with the formation of casein-covered emulsified fat particles, which are more stable than the native fat globules to coalescence during manufacture. The absence of free fat that acts as a lubricant on protein surfaces (Marshall, 1990), probably impairs the ability of layers of the casein matrix on the surface of neighboring curd particles to deform and fuse. Moreover, the casein-coated emulsified fat particles, which may be considered to behave as pseudo-protein particles (van Vliet and Dentener-Kikkert, 1982), probably lead to more interactions between the adjoining layers of the casein matrix. This in turn probably impedes the ability of the casein matrix to deform and curd particles to flow together after whey removal and during cheddaring, molding, and/or pressing. In addition to the above, homogenization of milk or cream leads to increased moisture retention by the casein matrix (Guerzoni *et al.*, 1999) and higher moisture cheeses (Jana and Upadhyay, 1992; Oommen *et al.*, 2000; Nair *et al.*, 2000). Homogenization of cheese milk also increases the sensitivity of the fat to lipolysis (Geurts *et al.*, 2003), which alters the flavor balance and for many cheeses would be undesirable.

Homogenization is essential in the manufacture of rennet-curd cheeses from recombined milk (homogenized blend of anhydrous butter oil and reconstituted skim milk powder) and the above defects are minimized *inter alia*, by the use of a very low pressure (e.g., 3–5 MPa) (Jana and Thakar, 1996). Sometimes, homogenization of milk may be used advantageously, for example, to give a whiter color in the resultant cheeses (e.g., Danablu) (Cantor *et al.*, 2004), or to increase the accessibility of the fat to fungal lipases and thereby increase the formation of fatty acids and their derivatives (e.g., methyl ketones) in cheeses where lipolysis is important for flavor development, (e.g., Blue cheese) (Collins *et al.*, 2004). Much work has been undertaken to evaluate the potential of homogenization of milk and/or cream as a technique for improving the texture and heat-induced functionality of reduced-fat Cheddar and Mozzarella cheeses (Oommen *et al.*, 2000). The basis for such potential is that homogenization increases the number of fat particles, which may be considered as spacers between, and to limit interaction between, neighboring layers of the casein matrix. However, this effect may be mitigated by the ability of the casein-covered fat globules to interact with, and thereby strengthen, the casein matrix of the cheese (van Vliet and Dentener-Kikkert, 1982). Moreover, the effectiveness of emulsified fat globules as spacers is undoubtedly dependent on their size distribution and spatial distribution within the casein matrix, and it is conceivable that below a critical mean size they have little, or no, effect. Consequently, there is considerable discrepancy between published studies *vis-à-vis* the results of homogenization on cheese texture and functionality, depending, *inter alia*, on the homogenization conditions, including pressures, number of stages, and temperature used (see Jana and Upadhyay, 1991, 1992, 1993; Jana and Thakar, 1996; Sections 11.8 and 11.9).

Homogenization of cheese milk, at a pressure in the range 2.6–30 MPa, causes a more uniform dispersion of fat globules, and a marked reduction in both the size and the degree of clumping and coalescence of fat globules in Cheddar and Mozzarella (Figure 11.3; Metzger and Mistry, 1995; Baer *et al.*, 1995; Tunick *et al.*, 1997; Rudan *et al.*, 1998b; Guinee *et al.*, 2000c; Rowney *et al.*, 2003); a similar effect is generally observed on homogenization of cream at 21 MPa prior to addition skim milk (Metzger and Mistry, 1995; Poudaval and Mistry, 1999; Oommen *et al.*, 2000), although the effect varies with homogenization pressure (Nair *et al.*, 2000). Moreover, the *para*-casein matrix of full-fat Cheddar made from homogenized milk is more continuous and

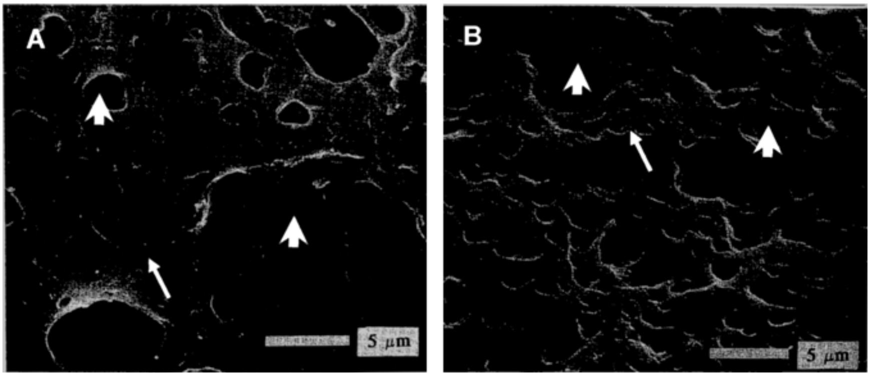

Figure 11.3. Scanning electron micrographs of 26 week-old reduced-fat (~18.0% w/w) Cheddar cheese prepared from non-homogenized milk (A) or from milk consisting of skim milk and cream homogenized at first- and second-stage pressures of 17.3 and 3.4 MPa (B), respectively. The arrows show the *para*-casein matrix and the arrowheads the fat globules. The micrographs show the presence of elongated fat globules of variable size unevenly dispersed in A, and relatively small fat globules evenly dispersed in the *para*-casein matrix in B (reproduced from Metzger and Mistry, 1995, with permission).

occupies a greater volume than, that of the corresponding control, which contains large protein-deficient areas occupied by pools of coalesced fat (Figure 3).

The manufacture of PCPs and ACPs involves the application of high temperature, high shear and the use calcium-chelating salts. These conditions assist in the dispersion of free fat and the conversion of the insoluble calcium *para*-casein to a more hydrated sodium *para*-caseinate, which coats the surface of dispersed free fat droplets and emulsifies them (Guinee *et al.*, 2004a). Consequently, the fat droplets in processed cheese are discrete with little evidence of clumping or coalescence (Guinee *et al.*, 1999). The actual size depends on the formulation and processing conditions, (e.g., addition of emulsifying salts and milk proteins, processing time, and extent of shear) (Rayan *et al.*, 1980; Carić *et al.*, 1985; Savello *et al.*, 1989).

11.3.5. Effect of Fat on Heat-induced Changes in Microstructure

On cooking, cheese is heated to a high temperature (e.g., 80–100°C), which alters its microstructure to a level dependent on the level of fat, degree of fat emulsification, nature of the fat globule membrane, and, hence, cheese type. In some cheese varieties, including full-fat Cheddar and high-fat (28% w/w) and stirred-curd Mozzarella, heating has been found to cause extensive clumping and coalescence of fat globules/pools (Paquet and Kalab, 1988;

Auty *et al.*, 1999; Guinee *et al.*, 2000c) and a concomitant increase in the heterogeneity of the distributions of the fat and *para*-casein phases (Figure 11.4). The *para*-casein matrix of Mozzarella tends to loose its orientation and become more compact on heating; this effect is attributed to the depletion of fat between adjoining layers of the protein matrix owing to fat liquefaction and coalescence, and its seepage from the cheese mass.

Changes in the distribution of fat on baking half-fat Cheddar and reduced-fat (15%, w/w) Mozzarella are similar to those noted for their full-fat counterparts. However, the degree of aggregation of fat globules and the size of the coalesced fat particles in the reduced-fat cheeses are generally smaller than in full-fat cheeses. This trend undoubtedly reflects the lower

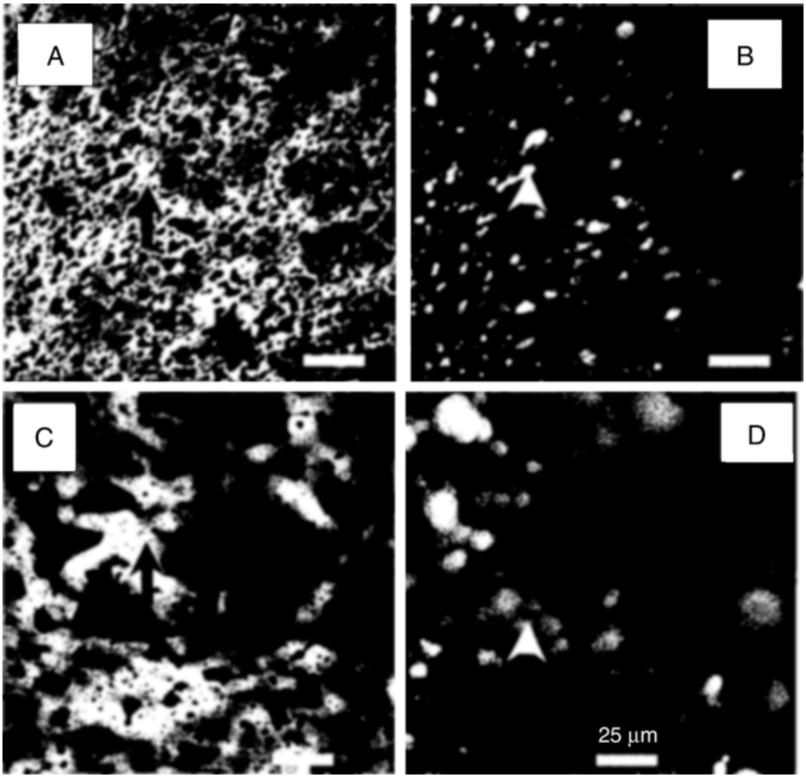

Figure 11.4. Confocal laser scanning micrographs of 5 day-old full-fat Cheddar cheese before heating (A, B) and after heating to 95°C and then cooling to room temperature (C, D). The micrographs show protein (black arrows in A, C) and fat (white arrow heads in B, D) as light areas against a dark background. Bar = 25 μm (modified from Guinee *et al.*, 2000b).

volume fraction of the fat phase in reduced-fat cheese (van Boekel and Walstra, 1995). The heat-induced coalescence of fat in cheese suggests a tendency towards phase separation and is consistent with the increase in the leakage of fat or oiling-off that occurs on baking or grilling cheese.

In contrast to natural cheeses prepared from unhomogenized milk, heating generally has little influence on the microstructure (distribution of fat and protein) of pasteurized PCPs (Paquet and Kalab, 1988) or on that of full-fat Cheddar cheese prepared from homogenized milk (Guinee *et al*., 2000c). The relatively high thermo-stability of these products against fat coalescence is probably due to the higher degree of emulsification prior to heating and a higher heat stability of the artificial fat globule membrane compared to the native fat globule membrane.

11.4. Effect of Fat on Cheese Yield

Milk fat contributes directly and indirectly to cheese yield (Table 11.3). The direct contribution of fat to cheese yield is clearly reflected by prediction equations, which relate cheese yield to the concentrations, and recoveries, of milk fat and protein (Fox *et al*., 2000; Melilli *et al*., 2002). An example of such an equation is the modified van Slyke formula, (Fenelon and Guinee, 1999):

$$Yp = \frac{\left[F\left(\frac{\%\ FRC}{100}\right) + (CN - a) + \left(WPum \times \frac{\%\ WPDpm}{100}\right)\right] \times (1 + SNFP)}{1 - \left(\frac{reference\ moisture\ content}{100}\right)}$$

where, F and CN are the percentages of fat and casein in the cheese milk (with added starter culture), $\%\ FRC$ = percentage fat recovery, a =

Table 11.3. Effect of Fat Content of Milk on the Yield of Cheddar Cheese and Fat Recovered in Cheese[a]

Fat in milk (%, w/w)	Actual yield (kg/100 kg milk)	Predicted yield[b] (kg/100 kg milk)	Dry matteryield (kg/100 kg milk)	Fat recovered to cheese (% of total)
0.54	6.37	6.47	3.43	80.84
1.5	7.49	7.58	4.29	87.16
2.00	8.09	8.21	4.79	89.48
3.33	9.50	9.61	5.92	87.84

[a] Compiled from data of Fenelon and Guinee (1999)
[b] Predicted using modified Van Slyke formula, as described in text.

coefficient for casein loss (typically 4% of total casein); *WPum* = percentage whey protein in the unpasteurized milk; *%WPDpm* = percentage of total whey protein denatured on pasteurization; and *SNFP* = cheese solids non-fat-non-protein (e.g., lactates, ash) as a percentage of cheese dry matter. Actual yield and predicted yield (as determined by the above equation) of Cheddar cheese from milk with fat ranging from ~0.5 to 3.4% (w/w), were closely correlated, with yield increasing at a rate 1.16 kg per 100 kg milk for every 1% increase in the fat content of the milk.

Recovery of fat in Cheddar cheese increases significantly on raising the fat content in the cheese milk from 0.5 to 2.7%, w/w, and thereafter decreases as the fat is increased further to 3.3%, w/w (Table 11.3) (Fenelon and Guinee, 1999). A similar trend was noted by Banks and Tamime (1987) who reported that the recovery of fat in Cheddar cheese manufacture increased to a maximum as the casein-to-fat ratio (CFR) was raised from 0.65 to 0.72, and decreased as the CFR was raised further to 0.75. The increase in fat recovery with milk fat content to 2.7%, w/w, may be due to the associated increase in the extent of clumping and coalescence of fat globules in the cheese milk during gelation and in the curd during cheese making (Section 11.4). The probable consequence of this partial clumping is an increase in the effective size of the fat globules (clumps), which, in effect, impedes their flow and escape through the pores of the surrounding *para*-casein matrix into the whey. A tentative explanation for the reduction in fat recovery at higher fat levels (>2.7%, w/w) is excessive clumping, which leads to coalescence and the formation of free fat that easily permeates the *para*-casein matrix and is lost into the whey.

The level of fat recovered during cheese manufacture is also influenced by cheese variety, which determines the type and intensity of processes to which the curd is subjected, which in turn influence the level of damage to the MFGM. Hence, the percentage for fat recovery reported for Mozzarella is markedly lower than that for Cheddar, (e.g., 80% *versus* 88% in pilot-scale studies) (Fenelon *et al.*, 1999; Guinee *et al.*, 2000b). The lower fat recovery for LMMC compared to Cheddar may be attributed to the high loss of fat during the kneading and stretching of the curd in hot water (plasticization). The high loss of fat during the plasticization process is consistent with the increase in the degree of fat coalescence that accompanies shearing of the curd, as observed by CLSM (see Fox *et al.*, 2000). In contrast, homogenization or microfluidization of cheese milk or cream stabilizes the fat to coalescence during manufacture and thereby reduces the level of fat lost in the whey and in the stretch water (Quarne *et al.*, 1968; Lelievre *et al.*, 1990; Lemay *et al.*, 1994; Lemay *et al.*, 1994; Metzger and Mistry, 1994; Oommen *et al.*, 2000) during the manufacture of plasticized cheeses. The increases in the recovery of fat and moisture (as discussed in Section 11.2) associated with the homogenization of milk or cream lead to an increased yield of cheese, to a degree dependent on milk composition, cheese type, and homogenization conditions (number of stages, pressure, and temperature) (Jana and Upadhyay, 1991, 1992); (e.g., the percentage increase in yield over the control for Cheddar ~32%, w/w, fat) from unhomogenized milk was ~4, 6 and 8% when cream used for milk standardization was homogenized at a total pressure of ~7, 10 or 14 MPa (Oommen *et al.*, 2000). How homogenization of milk increases the retention of moisture in cheese is unclear; however, a tentative explanation may be the increase in the firmness of the rennet-induced gels (at a given time after rennet addition) on homogenization of the milk (Guinee *et al.*, 1997; Nair *et al.*, 2000). The moisture content of cheese increases markedly as the firmness of the gel at cutting is increased (Mayes and Sutherland, 1989; Guinee *et al.*, 2004b). Thus, as the firmness of the gel at cutting was not standardized in most cheese making studies, the expected increase in curd firmness at cutting may contribute, at least partly, to the higher level of moisture in cheese made from homogenized milks. However, in contrast to the above, other investigators have reported that homogenization impairs curd-forming properties, leading to a weaker gel and a longer set-to-cut time (Jana and Upadhyay, 1992, 1993). Other factors that contribute to increased moisture retention in curds from homogenized milk may include associated alterations in the microstructure of the curd (Tunick *et al.*, 2002) and permeability of the matrix to moisture.

Fat also contributes indirectly to cheese yield, as its presence in the *para*-casein curd matrix affects the degree of matrix contraction and hence moisture content and cheese yield. The occluded fat globules physically limit contraction of the surrounding *para*-casein network and therefore reduce the extent of syneresis. Thus, it becomes more difficult to expel moisture as the fat content of the curd is increased. Consequently, the moisture-to-casein ratio generally increases unless the cheese making process is modified to enhance casein aggregation, (e.g., by increasing the scald temperature) (Gilles and Lawrence, 1985; Fenelon and Guinee, 1999). Owing to its negative effect on syneresis, fat indirectly contributes more than its own weight to actual cheese yield, (e.g., Cheddar cheese yield increases by ~1.16 kg/kg milk fat). The greater than *pro rata* increase is due to the concomitant increase in the level of moisture associated with the cheese protein as reflected by the positive relationship between milk fat level and MNFS (Figure 11.1). Hence, while the percentage moisture in Cheddar cheese is inversely related to its fat content, the weight of cheese moisture from a given weight of cheese milk increases as the fat content of the cheese is increased (Figure 11.5). Moreover, the increase in the moisture-to-protein ratio with fat content contributes indirectly to cheese

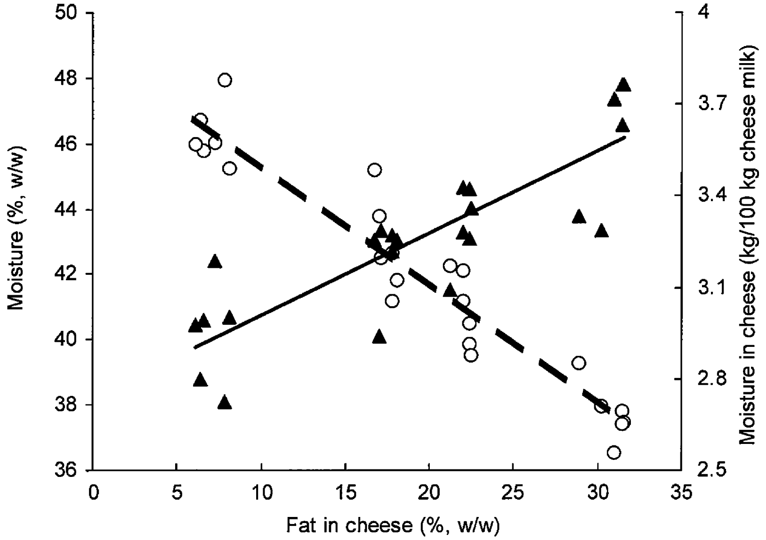

Figure 11.5. Effect of fat content on the percentage moisture in Cheddar cheese (○) and the weight of Cheddar cheese moisture obtained from 100 kg cheesemilk (▲) (drawn from data of Fenelon and Guinee, 1999; Guinee *et al.*, 2000a).

yield due to the presence of dissolved solids, including native whey proteins, κ-casein glycomacropeptide, lactate, and soluble salts. However, if the level of MNFS is maintained constant (e.g., by process modifications), fat contributes less than its own weight to cheese yield (i.e., ~0.9 kg/kg for Cheddar), due to the fact that ~10% of the fat in milk is normally lost in the whey during Cheddar manufacture.

The dry matter cheese yield (Y_{dm}) increases with the level of milk fat but at a lower level than actual yield (Y_a), [i.e., ~0.93 *vs.* 1.16, kg/kg milk fat for Cheddar (Table 11.3; Fenelon and Guinee, 1999)]. The difference between the increase in Y_a and Y_{dm} per unit weight of fat in milk (i.e., 0.23 kg/kg milk fat for Cheddar) is due to the fact that Y_{dm} excludes the effect of milk fat on cheese moisture (i.e., 0.24 kg/kg fat for Cheddar) whereas Y_a incorporates it. However, the increase in Y_{dm} per kg of milk fat is greater than expected based on the corresponding increase in the weight of cheese fat per kg of milk fat (i.e., 0.90 kg/kg; Table 11.3). The difference (i.e., 0.03 kg/kg milk fat) in the extent of the increase between Y_{dm} and weight of fat in cheese per unit weight of fat in the milk, may be attributed to the increased weight of the soluble portion of the SNFP (which forms a major part of the dissolved solids) in the cheese as the fat content increases (Table 11.3). The latter trend in turn is due to the increase in cheese moisture per kg cheese milk as the level of fat in milk increases (Figure 11.5). However, the direct contribution of fat to Y_{dm} is less than its own weight in milk due to the loss of fat in cheese whey (~10% total).

11.5. Effect of Fat on Cheese Microbiology

During cheese ripening, the population of starter bacteria generally decreases while the number of non-starter lactic acid bacteria (NSLAB) generally increases; these changes are well documented for many full-fat rennet-curd cheese varieties, (e.g., Cheddar) (Cromie *et al.*, 1987; Jordan and Cogan, 1993; McSweeney *et al.*, 1993; Lane *et al.*, 1997; Haque *et al.*, 1997; Beresford and Williams, 2004).

However, comparatively little information is available on the effect of fat content on the dynamics of starter and NSLAB populations in cheese. Laloy *et al.* (1996) reported that the number of starter cells in full-fat curd prior to pressing was 4-fold to 10-fold higher than the corresponding population in fat-free Cheddar curd, depending on the starter strain used. The authors suggested that the higher number of starter cells in the full-fat cheese might be attributable to:

- The association between starter lactococci and fat globules, ~90% of which are retained in the curd
- The physical impedance to syneresis by the fat globules, which, in effect, act as 'stoppers' in the pores of the *para*-casein matrix and thereby reduce the loss of starter cells in the whey exuding from the curd; the retained starter cells aggregate around the fat globules

In agreement with the trend noted by Laloy *et al.* (1996), Fenelon *et al.* (2000a) reported that the starter cell count in full-fat Cheddar (FFC; 32.5%, w/w) at 1 d was significantly higher than that in low-fat Cheddar (LFC, 6.3%, w/w). However, the starter population in the FFC declined more rapidly and was significantly lower than that in the LFC at 180 d (Figure 11.6a). In contrast, Haque *et al.* (1997) reported similar populations ($\sim 3.2 \times 10^8$ cfu/g) of starter lactococci in LFC and FFC at 1 d but counts in the LFC decreased more rapidly during maturation; the populations in the LFC and FFC at 180 d were $\sim 6.3 \times 10^3$ and 2.5×10^4 cfu/g, respectively.

The number of NSLAB in Cheddar decreases with fat content (Figure 11.6b), with the count in LFC (5%, w/w) being significantly lower than that in FFC (33%, w/w) (Haque *et al.*, 1997; Fenelon *et al.*, 2000a). The decrease in NSLAB as the fat content of cheese is reduced may be due to a number of factors including:

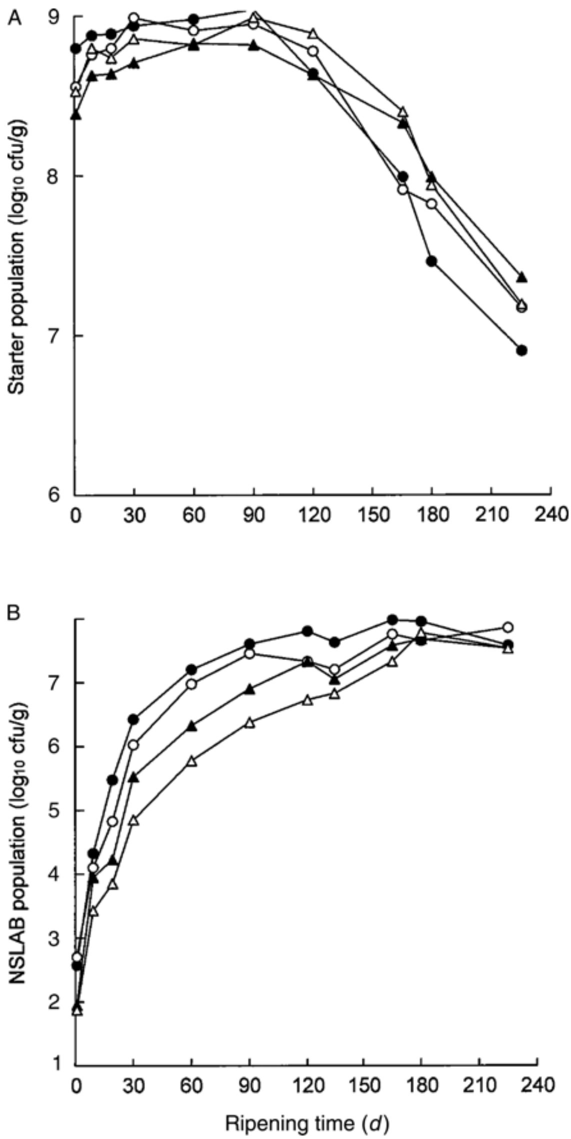

Figure 11.6. Age-related changes in the populations of starter (A) and non-starter bacteria (B) in full-fat (30.4%, w/w; (●); reduced-fat (21.9%, w/w; (○); half-fat (17.2%, w/w; ▲); and low-fat (7.2%, w/w; △) Cheddar cheese. The values presented are the means of three replicate trials (drawn from data of Fenelon *et al.*, 2000a).

- The reduction in the concentration of milk fat globule membrane-associated glycoproteins. Mesophilic lactobacilli possess glycoside hydrolases and may be able to release sugars from the glycomacropeptide of casein and glycoproteins of the fat globule membrane (Beresford and Williams, 2004). The released sugars may be a source of energy for propagation
- The lower level of MNFS (Lane *et al.*, 1997)

11.6. Effect of Fat on Proteolysis

Proteolysis in cheese has been studied extensively and reviewed (Fox and Wallace 1997; McSweeney, 2004; Upadhyay *et al.*, 2004). It contributes directly to flavor, *via* the formation of peptides and free amino acids (FAA), and indirectly *via* the catabolism of free amino acids to various compounds including amines, acids, thiols (Curtin and McSweeney, 2004). Proteolysis directly affects the level of intact casein, which is a major determinant of the fracture and functional properties, and of cheese texture (de Jong, 1978b; Creamer and Olson, 1982; Creamer *et al.*, 1982; Guinee, 2003; Brown *et al.*, 2003).

Most studies on proteolysis have focused on full-fat cheese or half-/reduced-fat cheeses, with little systematic comparison on the effects of incremental fat reduction, especially in cheese varieties other than Cheddar.

11.6.1. Primary Proteolysis

Those studies in which the effect of fat on primary proteolysis has been studied indicate that the effect depends on the level of MNFS in the cheese.

11.6.1.1. Cheese with Similar Levels of MNFS

Rank (1985) investigated the effects of fat level, in the range 13.5–30.6%, w/w, on proteolysis in Colby cheese for which alterations were made to the manufacturing protocol of the low-fat cheese so as to give a level of MNFS similar to that in the full-fat cheese. After 6 to 8 months storage at 4°c, the concentration of α_{SI}-casein in the lowfat cheese (13.5% w/w fat) was only slightly lower than that in the full-fat cheese (30.6%, w/w, fat). Similarly, Michaelidou *et al.* (2003a, b) reported that a large reduction in the fat content of Feta-cheeses (from ~29 to 7%, w/w) or Kefalograviera-cheeses (from ~31 to 10%, w/w) had little effect the type or level of peptides detected by urea-PAGE.

Banks *et al.* (1989) found that a 50% reduction in the fat content of Cheddar cheese only slightly reduced the level of water-soluble N at 2 (9.9 *vs.* 11.8 g/100g N) or 4 (15.3 *vs.* 17.8 g/100g N) months. Likewise, only relatively small decreases (0 to 3 g /100 g N) in the level of pH 4.6-soluble or water-soluble N were reported for Herrgårds and Drabant cheeses (Ardö, 1993), Feta-type cheese (Michaelidou *et al.*, 2003a), or Kefalograviera-type cheese (Michaelidou *et al.*, 2003b) for large reductions in fat content (40–70%) when the level of MNFS in the reduced-fat and full-fat cheeses were similar. Moreover, the use of an adjunct culture in the manufacture of the reduced-fat cheeses eliminated the difference in the level of WSN between the full-fat and reduced-fat Kefalograviera-type and Feta-type cheeses (Michaelidou *et al.*, 2003a, b). Small differences in proteolysis in cheeses of different fat content, despite the large effect of fat on gross composition (i.e., fat, protein, and moisture), indicate the importance of MNFS as a major compositional factor controlling proteolysis in, and quality of, cheese (Creamer, 1971; Thomas and Pearce, 1981; Lawrence *et al.*, 2004). The relatively minor effect of fat on proteolysis may be attributed in part to alterations to the cheese making procedures, which minimized the difference in the level of MNFS between full-fat and reduced-fat cheeses. Hence, studies on retail Cheddar cheeses have shown no significant relationship between the levels of fat and MNFS or between fat content and the levels of primary or secondary proteolysis (Banks *et al.*, 1992; Fenelon *et al.*, 2000b).

11.6.1.2. Cheeses with a Different Level of MNFS

In contrast to the above, other studies (Fenelon and Guinee, 2000; Fenelon *et al.*, 2000a) showed that the level of fat, in the range 6–33%, w/w, had a marked influence on the level of proteolysis in Cheddar cheese manufactured using identical conditions but with a different level of MNFS (Table 11.1). The mean level of primary proteolysis throughout the 225 d ripening period, as measured by the percentage of total N soluble at pH 4.6 (pH 4.6 SN %TN), decreased significantly (from ~30 g/100 g total N for cheese with ~33%, w/w, fat to 15 g/100 g total N for cheese with ~6%, w/w, fat at 225 d) as the fat level was reduced (Figure 11.7a). The decrease in proteolysis was expected owing to the parallel decrease in MNFS (~56–49%, w/w; Figure 11.1) and ratio of residual rennet activity to protein (Fenelon and Guinee, 2000; Fenelon *et al.*, 2000a). Moreover, the higher volume fraction of the protein in the reduced-fat cheese may be conducive to a higher degree of protein interaction, which in turn could restrict the availability of the casein to chymosin and other proteinases. However, the level of pH4.6-soluble N per 100 g cheese (pH4.6 SN %TN) was not significantly influenced by fat content. This suggests that the reduction in pH4.6SN%TN as the fat content decreased was compensated for by the concomitant increase in the protein content of the reduced-fat cheese. Michaelidou *et al.* (2003a, b) reported similar trends for Feta-type and Kefalograviera-type cheeses.

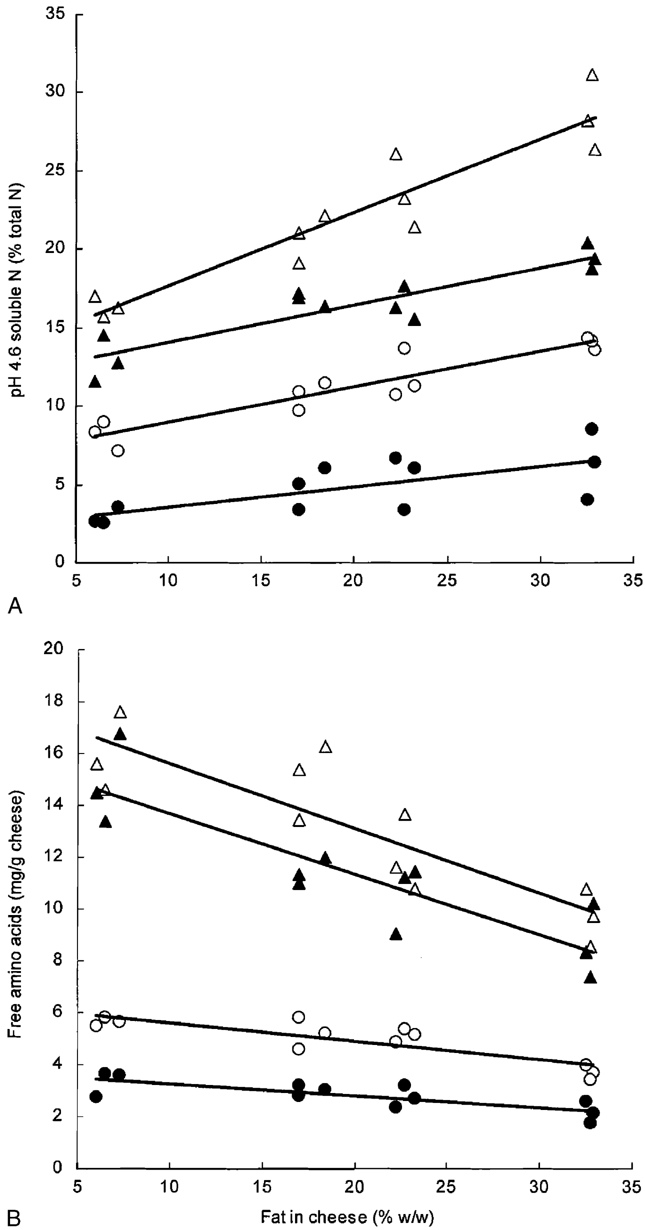

Figure 11.7. Effect of fat content on the levels of pH 4.6-soluble N (A) and free amino acids (B) in Cheddar cheeses aged for 30 (●), 90 (○), 180 (▲), or 225 (△) days (drawn from data of Fenelon and Guinee, 1999; Guinee *et al.*, 2000a).

Variation in the level of fat also leads to differences in the level of primary proteolysis as monitored by PAGE. Reducing the fat content resulted in higher levels of intact α_{s1}-casein and β-casein in Cheddar (Fenelon and Guinee, 2000) and Mozzarella cheese (Tunick *et al.*, 1993b, 1995). This was expected because of the inverse relationship between the levels of fat and protein in cheese. However, for a given protein content, reduction in the level of fat led to more extensive degradation of β-casein and the accumulation of γ-caseins (Fenelon and Guinee, 2000). The increase in the concentration of γ-caseins was attributed to the higher pH in the reduced-fat cheeses (~5.5 at 6%, w/w, fat *versus* ~5.2, at 6%, w/w, fat at 225 d), which would enhance the activity of the indigenous milk proteinase, plasmin (Grufferty and Fox, 1988). The increase in pH as the fat content increased may also affect the degree of hydration and aggregation of the β-casein (Creamer, 1985), which could affect its susceptibility to hydrolysis by chymosin or plasmin. In contrast to the trend noted for β-casein, the degradation of α_{s1}-casein decreased as the fat content was reduced. This effect may be due to a number of associated factors:

- The decrease in the ratio of residual rennet activity-to-protein level (Fenelon and Guinee, 2000)
- The decrease in the level of MNFS (Table 11.1);
- The high pH of low-fat cheeses that is less favorable to the proteolytic activity of residual rennet (Tam and Whitaker, 1972; O'Keeffe *et al.*, 1975).

11.6.2. Secondary Proteolysis

For Herrgårds and Drabant cheeses with similar MNFS, Ardö *et al.* (1993) found that the level of 5% phosphtungstic acid-soluble N (as % total N), which includes low molecular mass peptides (< 0.6 kDa) and free amino acids (FAA) (Jarrett *et al.*, 1982), was scarcely affected by a 40% reduction in fat content. In contrast, reducing the level of fat in Feta-type (from ~22 to 7%, w/w) or Kefalograviera-type (from ~31 to 10%, w/w) cheeses lead to large decreases in the level of 5% phosphotungstic acid soluble N (as % total N, and as % of total cheese), despite similar levels of primary proteolysis in the corresponding full-fat and low-fat cheese types (Michaelidou *et al.*, 2003a, b). The discrepancy between the latter studies (Ardö *et al.*, 1993; Michaelidou *et al.*, 2003a, b) may be due to factors that affect the type of proteolysis, including differences in starter type, cheese making procedure, and cheese composition (Fox and Cogan, 2004; Lawrence *et al.*, 2004; McSweeney, 2004; Upadhyay *et al.*, 2004).

For cheeses with a different level of MNFS, the effect of reducing fat content on the degree of secondary proteolysis in Cheddar cheese is the opposite to that noted for primary proteolysis (Fenelon *et al.*, 2000a). Reducing the level of fat led to a significant increase in the concentration of total FAAs per 100 g cheese (Figure 11.7b) but did not significantly affect the concentration of FFAs per 100 g cheese N. Hence, the increase in FAA as the fat content was reduced is due, at least partly, to the concomitant increase in the total concentration of protein in the cheese. Consistent with the higher level of FAAs in the reduced-fat cheeses, the level of low molecular mass peptides (< 1 kDa) in the pH 4.6-soluble cheese extracts increased as the fat content of the cheese decreased (Altemueller and Rosenberg, 1996; Fenelon *et al.*, 2000a).

11.7. Contribution of Lipolysis and Catabolism of Free Fatty Acids (FFA) to Cheese Flavor

Rennet-coagulated cheeses are ripened (matured) for a period from *ca*. two weeks (e.g., Mozzarella) to two or more years (e.g., Parmigiano-Reggiano or extra-mature Cheddar) during which time the flavor characteristic of the variety develops (McSweeney, 2004). Cheese generally contains several hundred volatile and non-volatile compounds at concentrations greater than their flavor thresholds. Hence, most workers now accept the "component balance" theory of cheese flavor (Mulder, 1952; Kosikowski and Moquot, 1958), which proposes that cheese flavor is caused by the correct balance and concentrations of a wide range of sapid compounds. Thus, differences between the flavors of different varieties are usually due to differences in the *balance and concentration* of flavor compounds rather than to the presence or absence of specific compounds. Indeed, most flavor compounds are present in most varieties but at very different concentrations.

The flavor compounds in cheese are produced during ripening by a complex series of microbiological changes and biochemical events. Biochemical transformations of residual lactose, and lactate and citrate, and proteolysis of the caseins to a large number of peptides and ultimately to free amino acids, which are subsequently catabolized to a range of volatile flavor compounds, are important in most cheeses (for recent reviews see McSweeney, 2004; McSweeney and Fox, 2004; Upadhyay *et al.*, 2004; Curtin and McSweeney, 2004). However, lipids in cheese also play important roles in the development of cheese flavor during ripening by:

- Affecting the rheology and texture of cheese and hence the rate of release of sapid compounds from the cheese matrix
- Acting as a source of fatty acids, which when liberated from triacylglycerols by the action of lipases, contribute directly to cheese flavor

(particularly in the case of short-chain FFAs) or indirectly, through their metabolism to a range of volatile flavor compounds, and

- Perhaps by facilitating reactions which occur at the fat-water interface (Collins *et al.*, 2004)

The role of milk-fat in the development of flavor in cheese during ripening will be discussed below although it should not be forgotten that lipolysis and the metabolism of fatty acids do not occur in isolation from other important biochemical events during ripening.

11.7.1. Lipolysis

In foods in general, fats undergo oxidative and hydrolytic degradation. However, since the oxidation-reduction potential of cheese is low (*ca.* −250 mV) and milk-fat contains low levels of polyunsaturated fatty acids, lipid oxidation does not occur to a significant extent in cheese during ripening (McSweeney and Sousa, 2000; Collins *et al.*, 2003a, 2004). However, the liberation of free fatty acids (FFA) by the action of lipases (lipolysis) is an important biochemical event during cheese ripening and has been studied extensively (see McSweeney and Sousa, 2000; Collins *et al.*, 2003a, 2004; McSweeney, 2004). Lipases in cheese originate from six possible sources:

- Milk
- Rennet paste
- Starter bacteria
- Secondary starter organisms (e.g., *Penicillium roqueforti*, *P. camemberti*, *Propionibacterium* sp., smear bacteria)
- Non-starter lactic acid bacteria (LAB)
- Exogenous addition

The indigenous lipase in bovine milk, lipoprotein lipase (LPL), is a 55 kDa homodimeric protein with catalytic optima at pH ~8.5 and 37°C. Under optimum conditions, there is sufficient LPL activity in milk to cause perceptible rancidity within *ca.* 10 s (Walstra and Jenness, 1984). However, in milk, enzyme and substrate are compartmentalized since *ca.* 90% of the enzyme is associated with the casein micelles and the fat is protected by the milk-fat globule membrane. LPL has a preference for medium-chain fatty acids and for acids esterified at the *sn*-1 or *sn*-3 position of triacylglycerol molecules at which are largely esterified short and medium chain fatty acids (Collins *et al.*, 2004). LPL activity is of most significance in cheese varieties made from raw milk since pasteurization largely inactivates this enzyme, although heating at 78°C × 15 s is needed for complete inactivation (Driessen, 1989).

Most commercial rennet preparations are free from lipase activity. However, the rennet pastes used for the manufacture of certain traditional Italian and Greek varieties (e.g., Provolone, the various Pecorino cheeses and traditional Feta) contain a potent lipase, pregastric esterase (PGE), the action of which contributes to the strong flavor of these cheeses (McSweeney and Sousa, 2000). PGE is produced by glands at the base of the tongue and is washed into the stomach with the milk on suckling. Rennet pastes are produced by macerating the abomasum of the young dairy animal into a paste, which is slurried in milk before use and they contain chymosin and pepsin in addition to PGE.

Although weakly lipolytic, starter and non-starter LAB are important sources of lipolytic enzymes in many varieties, particularly those without a strongly lipolytic secondary flora (e.g., Cheddar and Gouda) since LAB are present at high numbers for long periods of time. A number of lipolytic enzymes have been characterized from LAB most of which have optimal activity at *ca*. 37°C and pH 7.0–8.5. Most were assayed on soluble derivatives of fatty acids and were optimally active on substrates esterified to short-chain fatty acids and hence were esterases, although genuine lipase activity (assayed on emulsified substrates) has also been demonstrated in LAB (see Collins *et al*., 2004). The lipolytic enzymes of LAB appear to be intracellular and thus must be released into the cheese matrix by cell lysis; the relationship between lipolysis and lysis was demonstrated in Cheddar cheese by Collins *et al*. (2003b).

The ripening of some varieties is characterized by the development of a secondary microflora, which often contributes greatly to lipolysis. In Blue cheese, *Penicillium roqueforti* grows in fissures in the cheese and produces two extracellular lipases with an optimum pH of 7.5–8 and 9–9.5, which cause extensive lipolysis in these varieties (McSweeney and Sousa, 2000). The white mold, *P. camemberti*, produces an extracellular lipase, which is optimally active at pH 9 and 35°C and which contributes to lipolysis in Camembert and Brie (Lamberet and Lenoir, 1976). Swiss cheeses are characterized by the growth of *Propionibacterium freudenreichii,* which is about 10–100 times more lipolytic than LAB (Dupuis, 1994) and produces an intracellular lipase with pH and temperature optima of 7.2 and 47°C, respectively (Oterholm *et al*., 1970). Finally, smear-ripened cheeses initially develop the mold, *Geotrichum candidum*, on their surface followed by a complex Gram-positive bacterial flora. However, with the exception of *Brevibacterium linens* and *G. candidum*, lipases from these organisms have not been studied in detail (see Collins *et al*., 2004). The surface flora contributes to the significant levels of lipolysis observed in these cheeses during ripening.

As shown for some examples in Table 11.4, the level of lipolysis in cheese varies considerable from moderate (e.g., Cheddar, Mozzarella and Edam) to very extensive (e.g., Blue cheese). Generally, cheeses that are characterized by a high level of FFAs are manufactured using rennet paste (e.g., Pecorino varieties), have a strongly lipolytic secondary microflora (e.g., Blue cheese or smear-ripened varieties) or have been ripened for a very long period (e.g., Parmigiano-Reggiano). High levels of FFAs in many varieties (e.g., Cheddar and Emmental) lead to cheeses being rejected as rancid (see Collins *et al.*, 2004). FFA, particularly short chain acids, contribute directly to cheese flavor but they also act as substrates for a range of catabolic reactions.

11.7.2. Metabolism of Fatty Acids

In addition to their direct role in cheese flavor, FFAs also act as precursors for a range of other volatile flavor compounds such as *n*-methyl ketones (alkan-2-ones), secondary alcohols, hydroxyacids, lactones, esters, and thioesters (Collins *et al.*, 2003a, 2004; McSweeney, 2004). General pathways though which FFAs are catabolised are shown in Figure 8.

n-Methyl ketones (alkan-2-ones), particularly heptan-2-one and nonan-2-one, are the main compounds responsible for the characteristic pungent flavor of Blue cheese in which they are produced from fatty acids by the action of *P. roqueforti* through a pathway corresponding to the early stages of β-oxidation (Collins *et al.*, 2003a, 2004; McSweeney, 2004). The rate of production of methyl ketones in Blue cheese is affected by temperature, pH, physiological state of the mold and by the concentration of FFAs. Methyl ketones are also found in many other varieties at levels much lower than those found in Blue cheese (see Collins *et al.*, 2004, for references). Methyl ketones may be reduced to the corresponding secondary alcohol (see Figure 11.8).

Lactones are cyclic compounds formed through the intramolecular esterification of a hydroxy fatty acid. γ-Lactones and δ-lactones, with five-sided and six-sided rings, respectively have been found in cheese (Jolly and Kosikowski, 1975; Wong *et al.*, 1975; Collins *et al.*, 2004). The origin of the precursor hydroxy fatty acids has been ascribed to a δ-oxidation system in the mammary gland of ruminants (see Fox *et al.*, 2000), the reduction of keto acids (Wong *et al.*, 1975) and/or the action of lipoxygenases and other enzymes present in members of the rumen microflora (Dufossé *et al.*, 1994). Lactones have low flavor thresholds and while their aromas are not specifically cheese-like (their aromas have been described variously as "peach," "apricot" and "coconut"), they may contribute to the overall flavor of cheese (see Collins *et al.*, 2004).

Table 11.4. Concentration (mg/kg Cheese) of Free Fatty Acids in Some Selected Cheese Varieties (From Mcsweeney, 2004, with Permission)

Cheese Type	$C_{2:0}$	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	TOTAL	Reference
Parmesan		1055	451	243	440	439	1540	3896	1171	3471	123		13697	de la Feunte *et al.* (1993)
Cheddar	476	952	143	175	159	571	952	1556	794	2841	635	238	9492	Kilcawley *et al.* (2001)
	1587	952	191	159	175	619	746	1253	508	1476	413	175	8254	
	1270	794	111	111	48	238	397	619	270	667	206	111	4842	
Swiss		170	90	45	122	208	311	1904	1427*				4277	Woo *et al.* (1984)
		345	21	25	53	88	267	930	1197*				2926	
Edam		60	8	9	14	47	39	122	57*				356	Woo *et al.* (1984)
Mozzarella		54	7	1	120	12	27	76	66*					Woo and Lindsay, 1984
Provolone		782	308	81	172	122	120	199	334*					
Camembert		35	5	14	35	43	69	270	210*				681	Woo *et al.*, 1984
Camembert	208	101	58				448	1028		1421				Lesage *et al.* (1993)
Camembert		361	287	160	225	298	622	1442	303	1043			5066	de la Feunte *et al.* (1993)
Roquefort		961	626	707	2280	1295	3185	6230	2241	6282	896		25969	de la Feunte *et al.* (1993)
Port Salut		41	4	8	54	33	86	275	199*				700	Woo *et al.* (1984)
Limburger		1475	688	24	50	92	602	565	709*					
Münster		163	102	66	154	206	704	2057	833	1412	59	504		de Leon-Gonzalez *et al.* (2000)

*C18:0 congeners

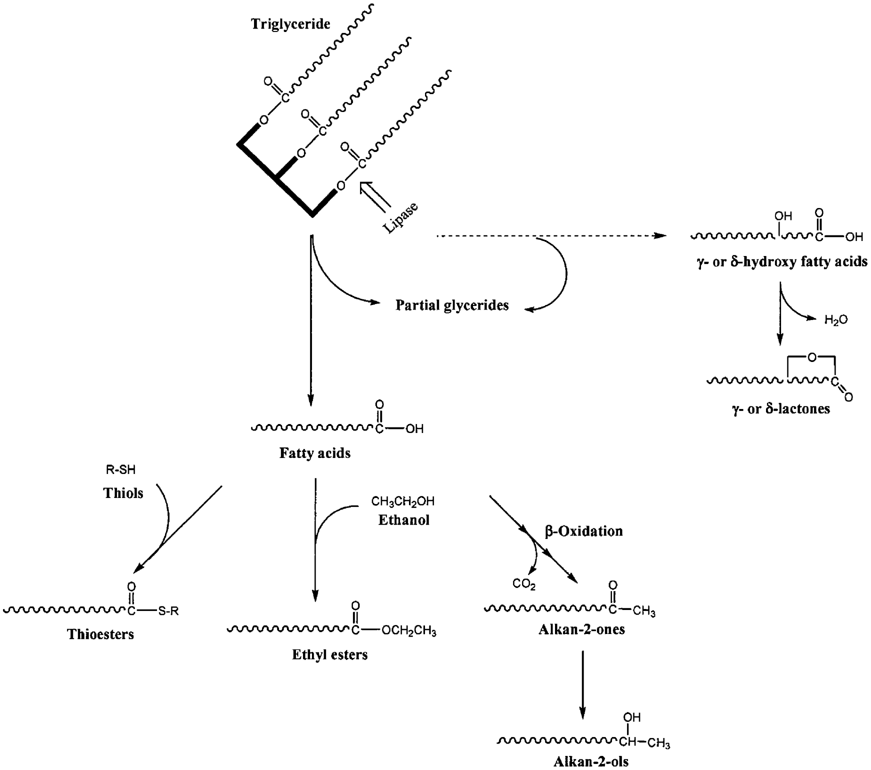

Figure 11.8. Pathways for the catabolism of free fatty acids in cheese during ripening (reprinted from *Cheese: Chemistry, Physics and Microbiology*, 3rd edn P.F. Fox *et al.* (eds.), Collins, Y.F., McSweeney, P.L.H., Wilkinson, M.G., Lipolysis and Catabolism of fatty acids in cheese, pp. 373–379, 2004, with permission from Elsevier).

Esters of short to medium-chain FFAs are commonly found in many cheese varieties (Meinhart and Schreier, 1986; Gonzalez de Llano *et al.*, 1990; Imhof and Bosset, 1994; Arora *et al.*, 1995; McSweeney, 2004). FFAs are most commonly esterified to ethanol, although low levels of methyl esters have also been found in cheese (e.g., Villaseñor *et al.*, 2000). Ethanol that may be produced from lactose metabolism or through the catabolism of FFAs, is usually the limiting reactant in the production of esters (Collins *et al.*, 2004). Although ethyl esters may be produced by direct reaction of an FFA with ethanol, Holland *et al.* (2002) suggested that they may arise during cheese ripening by the transesterification of an FFA from a partial glyceride to ethanol. Most esters have a buttery or fruity aroma

(Arora *et al.*, 1995; Engels *et al.*, 1997). Thioesters are formed by the reaction of an FFA with a sulphydryl compound, particularly methanethiol (CH_3SH; Molimard and Spinnler, 1996; Collins *et al.*, 2003a, 2004). Methylthioesters of short-chain FFAs have been associated with the characteristic aromas of Cheddar and smear-ripened cheeses (Arora *et al.*, 1995; Lamberet *et al.*, 1997).

11.8. Effect of Fat on the Fracture-Related Properties of Unheated Cheese

In most applications, whether as a consumer product or as an ingredient, cheese is subjected to size-reduction operations. Cheese may be portioned (e.g., for consumer packs), sliced, crumbled into irregularly-shaped pieces (e.g., Feta or Stilton for salads), shredded into cylindrical pieces (e.g., 2.5 cm long and 0.4 cm diameter; for sandwiches, pizza), diced into very small cubes (e.g., 0.4 cm, for salads) grated into particles < 1 mm (e.g., for dried parmesan), or comminuted by forcing pre-cut cheese through die plates with narrow apertures (e.g., in preparation of sauces or processed cheese products). Similarly, when eaten, cheese is subjected to a number of forces, which reduce it to a paste before being swallowed; first, the cheese is bitten (cut by the incisors), compressed (by the molars) on chewing, and sheared (between the palate and the tongue, and between the teeth). During the above applications the cheese is subjected to high stresses (e.g., > 200 kPa) and strains (e.g., $> 70\%$), which result in fracture of the cheese mass to varying degrees. The behavior of the cheese when exposed to the different size-reduction methods constitutes a group of important functional attributes, including shreddability, sliceability, gratability, spreadability, hardness, and crumbliness (Guinee and Kilcawley, 2004). These attributes are determined largely by the rheological characteristics of the cheese, which define its deformation and/or flow when subjected to a stress and/or strain (O'Callaghan and Guinee, 2004).

Large-strain deformation testing, using uniaxial compression on a texture analyzer, may be used easily to apply strains in the range encountered during size-reduction operations. The cheese may be subjected to 1 or 2 compression cycles (Texture Profile Analysis; TPA). Large-strain compression is most commonly used to assess the rheological properties of cheese. Several rheological quantities, which may be related to the functional attributes of the cheese, can be obtained from the resultant stress strain/curve (O'Callaghan and Guinee, 2004). These include fracture stress (σ_f), fracture strain (ε_f), firmness (σ_{max}), cohesiveness, gumminess, chewiness, and adhesiveness. Other tests (large deformation shear, wire cutting, and bending) may also be used to assess the large-strain deformation properties (O'Callaghan and Guinee, 2004).

11.8.1. Effect of Fat Content on Fracture Properties

Altering the fat content has marked effects on the fracture-related properties of different cheese varieties, including Cheddar (Emmons *et al.*, 1980; Bryant *et al.*, 1995; Mackey and Desai, 1995; Fenelon and Guinee, 2000; Gwartney *et al.*, 2002), Mozzarella (Tunick *et al.*, 1991, 1993a, b, 1995; Tunick and Shieh, 1995) and Cottage cheese (Rosenberg *et al.*, 1995). Reducing the level of fat in Cheddar cheese results in increases in elasticity, σ_f, ε_f, σ_{max} (Figure 11.9), cohesiveness, springiness, chewiness, and gumminess and a decrease in adhesiveness; consequently, the texture of reduced-fat Cheddar tends to be much less acceptable to the consumer than that of full-fat Cheddar, which is much softer and less rubbery and tough. The adverse effects of fat reduction are expected because of the concomitant increase in the concentration of intact casein and its contribution to cheese elasticity (see Guinee, 2003; Guinee and Kilcawley, 2004). Moreover, liquid fat acts as a lubricant on fracture surfaces of the casein matrix, and reduction of the fat level is thereby expected to increase the stress required to fracture the matrix (Marshall, 1990; Prentice *et al.*, 1993). Thus, while Chen *et al.* (1979) found that the hardness of different cheese varieties generally tended to increase as the level of fat was reduced, the relationship between fat and hardness was not significant.

Similar trends have been noted for the effect of fat content on the rheological properties of Mozzarella cheese (Tunick *et al.*, 1993a, b; Tunick and Shieh, 1995). Reducing the fat content (e.g., 21–25%, w/w, to $\sim 9-11\%$, w/w) of low- and high-moisture Mozzarella cheeses (47.7–51.8%, w/w, and 52.2–57.4%, w/w, respectively) resulted in significant increases in hardness and springiness, with the magnitude of the effect being most pronounced for hardness (Figure 11.10). There was a significant effect of the interaction between scald temperature and fat content on hardness, with the effect of fat reduction on hardness being more pronounced as the scald temperature was raised from 32.4°C to 45.9°C.

11.8.2. Effect of Solid-to-Liquid Fat Ratio on Fracture Properties

Increasing the liquid-to-solid fat ratio, by raising the temperature of the cheese in the range 0–40°C, results in large decreases in the σ_f and σ_{max} of different cheese types including Gouda (Culioli and Sherman, 1976), Cheshire and Leicester (Dickinson and Goulding, 1980), and 4 week-old Brie (Molander *et al.*, 1990). However, in these studies, the change in ε_f on increasing temperature depended on cheese type: it did not change with

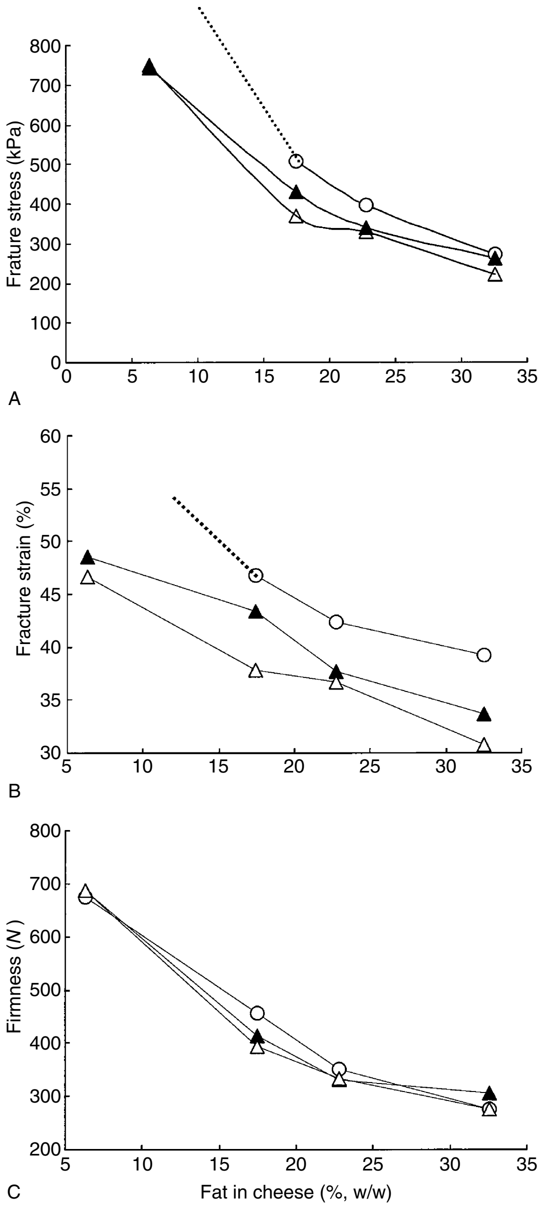

Figure 11.9. Effect of fat content on fracture stress (A), fracture strain (B) and firmness (C) of Cheddar cheeses aged for 120 (○), 180 (▲) or 225 (△) days. Broken line indicates that the sample did not fracture on compression at the early ripening times (drawn from data of Fenelon and Guinee, 1999; Guinee *et al.*, 2000a).

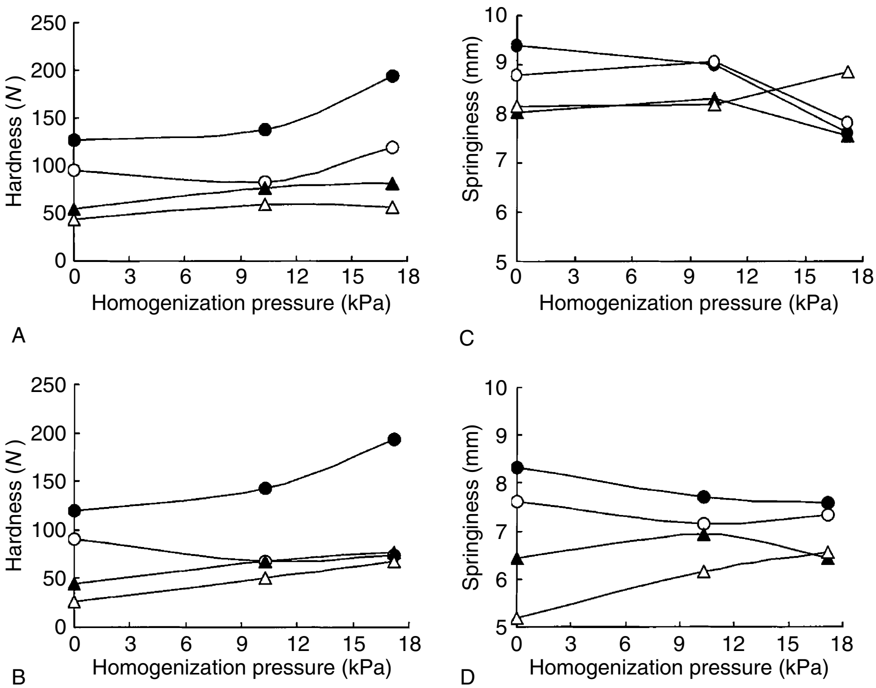

Figure 11.10. Effect of homogenization pressure on the hardness (A, B) and springiness (C, D) of 1 week (a, c) and 6 week (b, d) -old Mozzarella cheeses with different levels of fat-in-dry matter (low-fat, LF, ~22%, w/w; high-fat, HF, ~49%, w/w) and manufactured using a low (LT, 32°C) or high (HT, 45.9°C) curd scalding temperature: LFHT (●), LFLT (○), HFHT (▲), or HFLT (△) (drawn from data of Tunick *et al.*, 1993b).

temperature (0–40°C) for Leicester, increased slightly for 4 week-old Brie (5–20°C), increased by a factor of 2 for Cheshire over temperature range (0–40°C), and decreased slightly for Gouda (10–20°C).

The changes in σ_f and σ_{max} are expected as milk fat, which is predominantly solid at 0°C, is almost entirely liquid at 40°C (Wright *et al.*, 2002). Hence, at a low temperature (e.g., 4°C), the fat globules encased within the *para*-casein network are essentially solid and augment the elastic contribution of the casein matrix and increase the stress required to achieve a given deformation or fracture; deformation of the casein matrix would also require deformation of the fat globules enmeshed within its pores. However, the contribution of fat to cheese elasticity decreases as the ratio of solid-to-liquid fat decreases with increasing temperature, and is very low at 40°C. At the higher temperatures, the fat behaves more as a fluid and the fat globules confer viscosity rather than elasticity or rigidity on the cheese mass. Hence, on the application of a stress at 40°C, the fat globules flow to an extent dependent, *inter alia,* on the liquid-to-solid fat ratio and the strength of the casein matrix, which determines the degree of deformation of the matrix itself and its occluded fat globules.

This effect of temperature is readily apparent from the sharp decrease in elastic shear modulus, G', and increase in phase angle, δ, as measured using low-strain (e.g., 0.006) oscillatory rheometry, when cheese is heated from 20 to 40°C (see Section 11.9). The effect of temperature on the rheological properties is also observed by comparing the effect of temperature on the G' of cheeses differing in fat content. The magnitude of the G' for half-fat Cheddar is lower than that of full-fat Cheddar at 4°C when the fat is mainly solid but similar at 40°C when fat is mainly liquid, even though the dry matter content of the latter is higher than that of the former (Figure 11.11). Other studies on Cheddar cheese have also shown that G' at 40°C decreased as the fat content was increased in the range 1.3–34%, w/w (Ustanol *et al.*, 1995; Guinee *et al.*, 2000c). In contrast to the foregoing, Ma *et al.* (1997) reported that G' for full-fat Cheddar at 20°C was higher

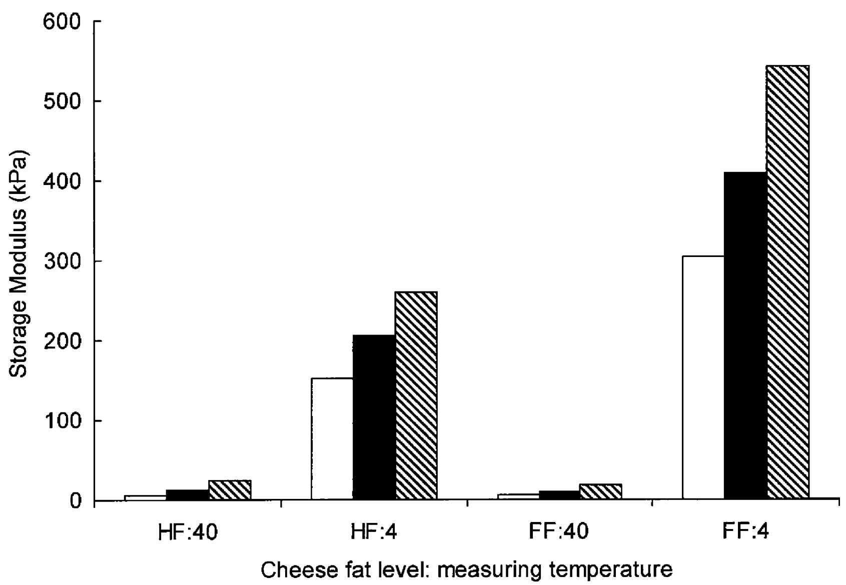

Figure 11.11. Effect of fat level (half-fat, HF, 17%, w/w; full-fat, FF, 32%, w/w) and assay temperature (4 or 40°C) on the elastic shear modulus of Cheddar cheeses measured using low-amplitude strain oscillation at a frequency of ~0.1(□), 1 (■) or 10 (▒), Hz.

than that of half-fat Cheddar. From a sensory viewpoint, it may be assumed that cheese rapidly approaches body temperature, (i.e., $\sim 37°C$), on ingestion. At this temperature, the cheese fat is almost fully liquid (Wright *et al.*, 2002) and therefore confers fluidity and lubrication to the cheese, and mouth coating, on mastication.

11.8.3. Effect of Homogenization of Milk or Cream, and Degree of Fat Emulsification on Fracture Properties

Homogenization of cheese milk or cream reduces the hardness and fracture stress of reduced-fat Cheddar (Emmons *et al.*, 1980; Jana and Upadhyay, 1993; Metzger and Mistry, 1994) and other varieties (Jana and Upadhyay, 1991, 1992). Because of these effects, homogenization improves the textural quality of reduced-fat Cheddar cheese (Metzger and Mistry, 1994). The positive effects of homogenization coincide with higher levels of MNFS and moisture, which has been found to reduce E, σ_f and/or σ_{max} and increase ε_f and adhesiveness in different cheese types, including Dutch-type Meshanger (de Jong, 1978a), Gouda (Luyten, 1988; Visser, 1991), and experimental Cheddar-type cheeses (Watkinson *et al.*, 2002).

Jana and Upadhyay (1991, 1993) reported that homogenization of milk for full-fat Mozzarella ($\sim$22% w/w fat) cheese, at a combined first and second stage pressure of 250 or 500 kPa, significantly reduced hardness and springiness, and increased cohesiveness; simultaneously, there were non-significant decreases in gumminess and chewiness. The magnitude of the effect, which increased with homogenization pressure, coincided with a decrease in protein content and increases in the levels of moisture and MNFS. In contrast, studies on reduced-fat Mozzarella (8–13%, w/w, fat) showed that homogenization of cheese milk or cream [first and second stage pressures of 13.8 and 3.45 MPa (Rudan *et al.*, 1998b) or 17.3 and 3.4 MPa, (Poduval and Mistry, 1999)] did not significantly affect the hardness or springiness of reduced-fat ($\sim$8% w/w) Mozzarella cheese at 30 d. An opposite trend to the above was reported by Tunick *et al.* (1995); two-stage homogenization of milk, at a combined first and second stage pressure of 10.3 or 17.2 MPa, resulted in a general increase in the hardness (at 23°C) of low-fat or high-fat Mozzarella after storage for 1–6 wk (Figure 11.10). There was a significant effect of the interaction between homogenization pressure and scald temperature used in cheese manufacture, with the increase in hardness being more pronounced in the cheese scalded at the higher temperature (Figure 11.10a, b). The magnitude of the effect also tended to be more pronounced at the lower fat content. Likewise, there was significant interaction between fat content and homogenization pressure on springiness (Figure 11.10 c, d) (Tunick *et al.*, 1995); the increase in springiness as the fat content was reduced tended to diminish with increasing homogenization pressure.

Discrepancies between the above studies *vis-à-vis* the effects of homogenization on the textural and rheological characteristics of cheese may be due to differences in homogenization conditions, assay conditions, age of cheese, and fat content.

11.9. Effect of Fat on the Functional Properties of Heated Cheese

As an ingredient, cheese is used extensively in cooking applications (e.g., grilled cheese sandwiches, pizza pie, cheeseburgers, pasta dishes, and sauces) in which it reaches a temperature of ~80–100°C. A key aspect of the cooking performance of cheese is its heat-induced functionality, which is a composite of different attributes. Depending on the application, one or more functional attributes may be required. The various functional properties of heated cheese and their interpretation have been reviewed by Kindstedt *et al.* (2004) and Guinee and Kilcawley (2004). Some of the main functional attributes include meltability (softening), stretchability, flowability (or spreadability), apparent viscosity, succulence, sheen, and/or tendency to brown. Flowability, a measure of the ability of cheese to spread during heating, and stretchability, a measure of the ability of the heated cheese to form strings and/or sheets when extended, involve strain displacement as a result of stresses on the *para*-casein matrix. Stress may occur spontaneously during quiescent heating (e.g., baking), as a result of collapse, flow and coalescence of melted fat globules/pools, which when solid (e.g., at 4°C) contribute to rigidity and physical support of the matrix. Conversely, an external stress may be applied to the hot molten cheese mass after cooking, (e.g., during consumption or instrumentally during testing; shear during viscometric testing, compression during squeeze flow evaluation, or extension during stretchability testing).

11.9.1. Effect of Fat Level on Cooking Properties

The effects of fat reduction on the heat-induced functionality of cheeses, especially Mozzarella and Cheddar, has been investigated extensively and reviewed in the last decade (Tunick *et al.*, 1993a, b; Merrill *et al.*, 1994; Fife *et al.*, 1996; McMahon *et al.*, 1996; Rudan and Barbano, 1998; Poudaval and Mistry, 1999; Rudan *et al.*, 1999; Guinee *et al.*, 2000a; Metzger *et al.*, 2001a, b; Sheehan and Guinee, 2004; Kindstedt *et al.*, 2004; Guinee and Kilcawley, 2004). The concentration on Cheddar and Mozzarella reflects their economic importance (together, they account for ~30% of total cheese produced globally) and their extensive use as ingredient in cooked foods.

Reducing the fat content impairs the functionality of Cheddar and Mozzarella, as reflected by decreases in flowability and stretchability and an increase in the apparent viscosity of the melted cheese (Figures 11.12, 13, 14). The flowability of 1 and 6 wk-old Mozzarella decreased by 9–25% as the fat content was reduced from ~25–9% (w/w) (Tunick *et al.*, 1993b, 1995; Tunick and Shieh, 1995), while the flowability of Cheddar cheeses ripened for $\leq$180 d decreased by 70 to 95% on reducing the fat level from 32 to 6% (w/w) (Guinee *et al.*, 2000a).

Olson and Bogenrief (1995) noted that the difference in flowability between full-fat and reduced-fat Cheddar cheese (with 50 or 75% of fat content in full-fat cheese) decreased with ripening time and had disappeared after 200 d storage at 7°C, when the cheese was melted for 5 min. In contrast, Guinee *et al.* (2000 a, c) reported that the differences in flowability between Cheddar cheeses with a fat content ranging from 6 to 32%, w/w, persisted throughout an 180 day ripening period; moreover, the negative relationship between flow and fat level was essentially linear at all ripening times investigated (Figure 11.12). The discrepancy between the foregoing studies may be due to differences in the measurement technique, which affected the propensity of the cheese to dehydrate during cooking. Olson and Bogenrief (1995) used a cylinder of cheese in a Pyrex glass tube in a water bath at 94°C,

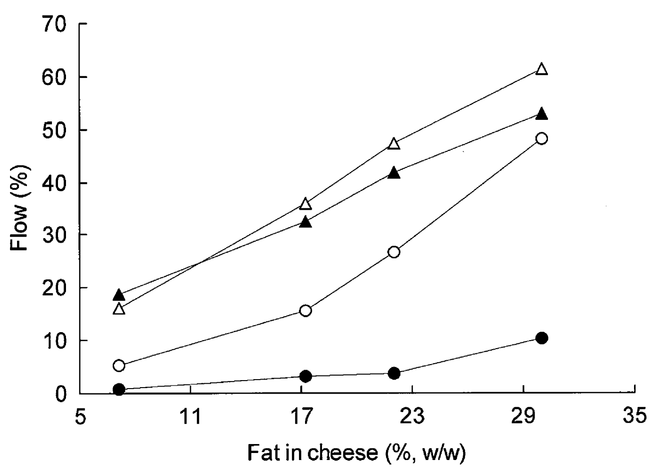

Figure 11.12. Effect of fat content on the flowability of Cheddar cheeses aged for 1 (●), 30 (○), 90 (▲), or 180 (△) days, on baking at 280°C for 4 min (redrawn from data of Guinee *et al.*, 2000a).

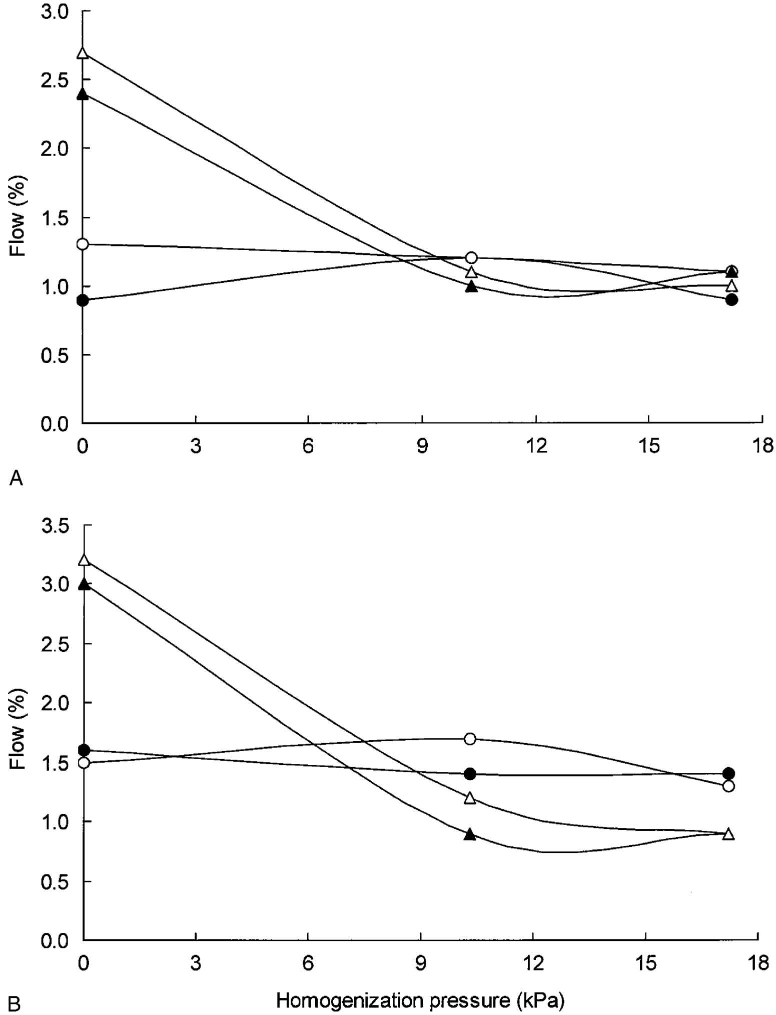

Figure 11.13. Effect of homogenization pressure on the flowability of 1 week (A) and 6 week (B) -old Mozzarella cheeses with different levels of fat-in-dry matter (low fat, LF, ~22%, w/w; high fat, HF, ~49%, w/w) and manufactured using a low (LT, 32°C) or high (HT, 45.9°C) curd scalding temperature: LFHT (●), LFLT (○), HFHT (▲), or HFLT (△) (redrawn from data from Tunick *et al.*, 1993b).

while Guinee *et al.* (2000a,c) used a disc of cheese placed on a stainless steel surface in a convection oven at 280°C for 4 min.

The adverse effects of fat reduction on flowability may be attributed to a number of factors, including the increased level of intact casein, and

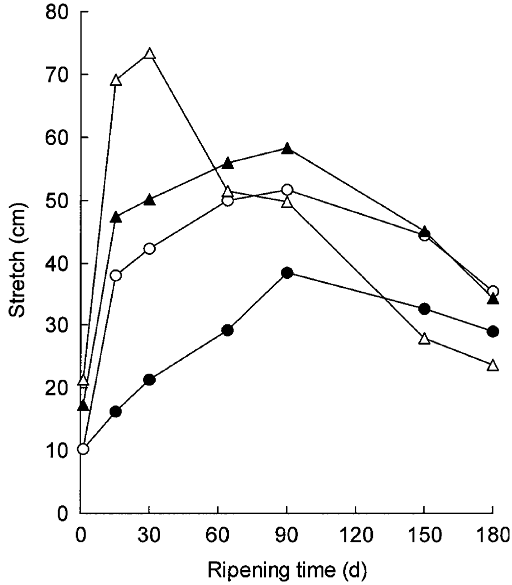

Figure 11.14. Age-related changes in the stretchability of Cheddar cheeses of different fat content on baking at 280°C for 4 min: low-fat (7.2%, w/w; ●), half-fat (17.2%, w/w; ○), reduced-fat (21.9%, w/w; ▲) and full-fat (30.5%, w/w; △) (redrawn from data of Guinee *et al.*, 2000a).

reduced levels of proteolysis, moisture-to-protein ratio and heat-induced fat coalescence. The net impact of these changes, which are discussed below, is a reduction in the level of displacement of adjoining layers of the casein matrix for a given level of stress.

Large reductions in fat content are paralleled by increases in the volume fraction of the casein matrix and level of intact casein in the unheated cheese (Figures 11.1, 2). The increase in intact casein is positively correlated with the apparent viscosity, and negatively with flowability, of the heated cheese (Guinee *et al.*, 2000a). The concomitant reduction in the number of fat globules is conducive to a higher degree of aggregation and fusion of casein strands during gel formation (McMahon *et al.*, 1993). Fat in the gel exists as globules occluded within the casein network, which physically impede casein aggregation. The higher degree of casein aggregation is unfavorable to heat-induced flow due to:

- A probable decrease in the degree of heat-induced slippage of contiguous casein layers
- A reduction in the content of MNFS (Table 11.1, Figure 11.1)
- A lower degree of casein hydration in the resultant cheese

The low degree of casein hydration is less favorable to moisture retention during baking and results in dehydration, crusting and poor flow (Kindstedt, 1995; Kindstedt and Guo, 1997). Moisture also acts as a lubricant between the protein layers and between protein and fat layers during melting and thereby facilitates heat-induced slippage of different parts of the matrix (McMahon *et al.*, 1993; Prentice *et al.*, 1993).

The level of primary proteolysis in cheese decreases as the level of fat is lowered (Section 11.6). However, several studies have shown a positive relationship between the level of proteolysis and flowability in cheeses with a fixed fat content, [e.g., Mozzarella (Yun *et al.*, 1993a, b; Madsen and Qvist, 1998), Cheddar (Arnott *et al.*, 1957; Bogenrief and Olson, 1995) and model acid-curd cheeses (Lazaridis *et al.*, 1981)]. The positive effect of proteolysis on flowability may be due to a number of concomitant changes, including the increased water-binding capacity (Kindstedt, 1995), and an increase in the number of discontinuities or 'breaks' in the *para*-casein matrix at the micro-structural level (de Jong, 1978a). The latter factors are expected to promote a decrease in casein aggregation, an occurrence that should enhance heat-induced displacement of adjoining layers of the casein matrix.

A reduction in fat level results in a decrease in the moisture-to-protein ratio, as reflected by the lower content of MNFS (Figure 11.1; Table 11.1). However, the flowability of rennet-curd cheeses is positively correlated with the content of MNFS (Rüegg *et al.*, 1991; McMahon *et al.*, 1993), an effect which may, in part, be due to the concomitant increase in casein hydration and the lubrication effect of moisture.

The degree of fat globule clumping and coalescence in both the unheated and heated (to 90°C) Cheddar cheese decrease as the fat level is reduced (Section 11.3). Coalescence of fat results in an increase in the continuity of the fat phase and in the free oil (FO) on heating the cheese. FO forms a layer on the surface of melting cheese, which limits the evaporation of moisture during cooking and the occurrence of associated defects such as skin formation, scorching, and impaired flow and stretch (Rudan and Barbano, 1998). Moreover, FO lubricates the displacement of adjoining layers of the casein matrix and thereby contributes positively to flow and stretch. An increase in FO may also alter the polarity of the solvent system (water and oil) in contact with the *para*-casein matrix and thereby increase the degree of solvation of the *para*-casein *per se*. A study by Hokes *et al.* (1982) on model analogue cheese systems showed that the addition of water to a dispersion of calcium caseinate in heated vegetable oil (or other polar solvents such as dioxane) resulted in gelation of the calcium caseinate. The latter effect was attributed to an increase in the polarity of the solvent system. Considering this, it is conceivable that free oil formed on heating cheese may be conducive to a structural rearrangement (e.g., exposure of hydrophobic groups), and increase in level of solvation of the *para*-casein molecules. An increase in casein solvation is, in turn, expected to favor an increase in the heat-induced fluidity and flowability of the cheese. Hence, it is noteworthy that:

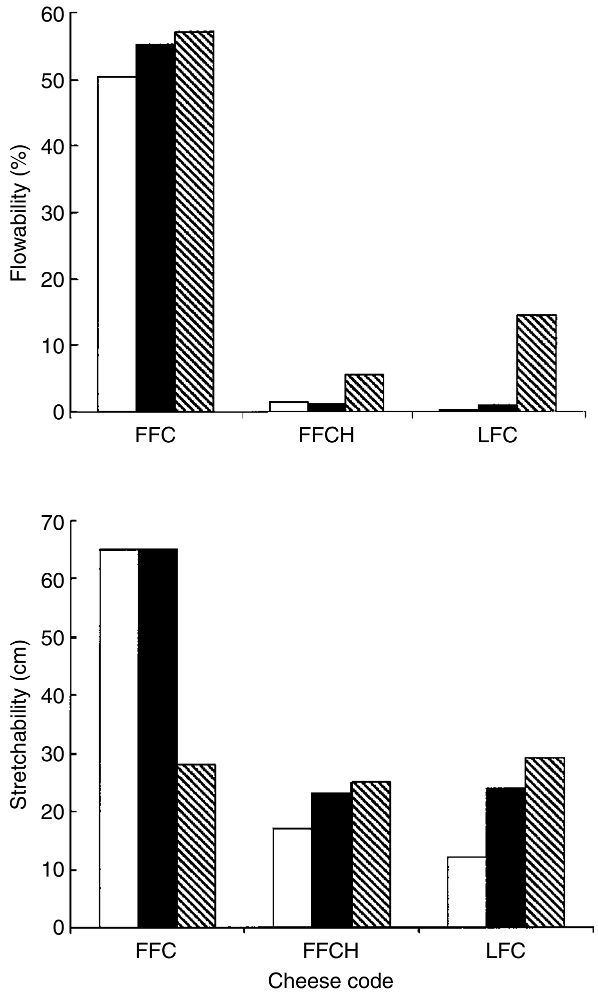

Figure 11.15. Effect of homogenization pressure on the flowability and stretchability of full-fat Cheddar-type cheese prepared from non-homogenized control milk (FFC) or control milk homogenized at respective first and second stages pressures of 25 and 5 Pa (FFCH), and of low fat Cheddar-type cheese from non-homogenized skimmed milk (LFC), after storage at 7°C for 5 (□), 70 (■) or 156 (▨) days (redrawn from data of Guinee *et al.*, 2000c).

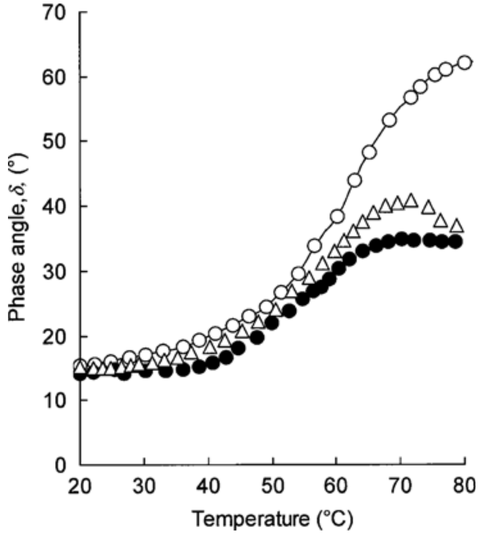

Figure 11.16. Phase angle, δ, as a function of temperature for 5 day-old Cheddar type cheese of different fat content, from non-homogenized (△, ○) or homogenized (●) milks. The cheeses were low-fat (1.3%, w/w; △); full-fat (30.0%, w/w; ○), and full-fat homogenized (30.6%, w/w; ●) (redrawn from data of Guinee *et al.*, 2000c).

- The flowability of melted Cheddar and Mozzarella increases with fat content (Figures 11.12, 13) and
- The increase in phase angle, and thus fluidity, on heating low-fat Cheddar cheese or full-fat Cheddar cheese made from homogenized milk are much lower than that on heating full-fat Cheddar cheese made from non-homogenized milk (Figure 11.16).

11.9.2. Effect of Milk Homogenization and Degree of Fat Emulsification

Increasing the degree of fat emulsification (DE) in cheese by homogenization of the cheese milk (e.g., at first and second stage pressures of 25 and 5 MPa) reduces the flowability, stretchability and fluidity of heated full-fat Cheddar (Figure 11.15; Guinee *et al.*, 2000a, c). The effect of homogenization, which for Cheddar is similar to reducing its fat content from 30 to 1.3%, w/w (Figure 11.15), would be highly undesirable in applications such as pizza but highly desirable where a high degree of flow resistance is required, (e.g., in frying). Similarly, increasing the homogenization pressure of milk, in the range 0.4–6.7 MPa, leads to a progressive deterioration in the flowability and stretchability of Halloumi cheese prepared from fresh milk or from reconstituted low-heat skim milk powder (RSMP) and anhydrous milk fat (AMF) (Lelievre *et al.*, 1990). However, the adverse effects of homogenization at the high pressure (6.7 MPa) on the melt characteristics of Halloumi were reversed by the addition of lecithin to the RSMP/AMF during the preparation of the recombined milk. Homogenization of cheese milk, or the cream used to standardize cheese milk, has also been found to impair flowability and reduce the level of free oil released from Mozzarella cheeses with a fat content of ~19–25% (w/w) on baking, with the effect generally becoming more pronounced as the homogenization pressure is increased (Jana and Upadhyay, 1992; Tunick *et al.*, 1993b, Rowney *et al.*, 2003). In contrast, homogenization of milk or cream at similar pressures does not adversely affect the flowability or apparent viscosity (AV) of reduced-fat Mozzarella ($\leq\sim$11%, w/w, fat), but leads to a significant reduction in the content of free oil (FO) released on baking (Rudan *et al.*, 1998b; Poduval and Mistry, 1999). The similarity in flowability and AV for low-fat cheeses made from homogenized, or unhomogenized, milks despite the differences in FO was probably due to the very low level of FO in all cheeses; FO as a percentage total fat in cheese was ~0.5, 0.5 and 3.9 for the homogenized milk cheese, homogenized cream cheese and the control at 40 d. Using the same analytical methodology, the FO values for commercial low-moisture part-skim Mozzarella ranged from ~10 to 40% of total fat in cheese, depending on the FDM and age (Kindstedt and Rippe, 1990; Kindstedt, 1993). Thus, it is noteworthy that Tunick *et al.* (1993b) reported that the interaction between fat content and homogenization pressure had a significant effect on the flowability of 1 or 6 week-old Mozzarella (Figure 11.13). Homogenization of milk had little effect on the melt properties of low-fat Mozzarella (~10%, w/w, fat) but markedly impaired those of Mozzarella with a higher fat level (~25%, w/w).

Increasing the degree of emulsification of fat in pasteurized processed, and analogue, cheese products (by selective use of emulsifying salts and extending the duration of processing) also leads to a marked reduction in flowability (Rayan *et al.*, 1980) and loss of fluidity, as reflected by a decrease in the loss tangent ($\tan\delta$) at 80°C (Neville, 1998).

The adverse effects of increasing the degree of emulsification on the functional properties of heated cheese probably ensue from the combined effects of:

- The interaction of *para*-casein-covered emulsified fat particles (which may be considered as pseudo-protein particles) with the casein matrix of the cheese, and
- A lower degree of heat-induced fat coalescence on melting the cheese.

On homogenization of milk, the native fat globule membrane is largely displaced and replaced by an adsorbed layer comprised of casein micelles, sub-micelles and whey proteins (Walstra and Jenness, 1984; Keenan, 1988). The newly formed recombined fat globule membrane interacts with the "free" micelles and thereby enables the emulsified fat particles to become an integral part of the matrix formed during cheese making, rather than being occluded within the matrix as inert particles (van Vliet and Dentener-Kikkert, 1982; Walstra and van Vliet, 1986). The incorporation of the emulsified fat particles into the casein matrix increases the effective protein concentration and the overall level of protein-protein interactions. Consequently, it is expected that functional properties relying on displacement of contiguous layers of the casein matrix (e.g., flow or stretch) would be impaired by the homogenization of cheese milk. Moreover, the recombined fat globule membrane stabilizes the newly formed fat globules to heat-induced coalescence (Guinee *et al*., 2000c), as reflected by the general reduction in free oil when the cheese milk, or cream used for standardization of the cheese milk, is homogenized at combined first and second stage pressures of 0.5–20 MPa (Lelievre *et al*., 1990; Jana and Upadhyay, 1991, 1993; Metzger and Mistry, 1995; Poduval and Mistry, 1999; Rowney *et al*., 2003). The consequent reduction in FO predisposes the cheese to dehydration during heating (Rudan and Barbano, 1998) and reduces the lubricating effect of oil on the surfaces of adjoining layers of the *para*-casein matrix during displacement. Thus, the adverse effects of homogenization on flowability and stretchability may be reduced by (Lelievere *et al*., 1990):

- Lowering the homogenization pressure that has the effect of reducing the surface area of the fat phase and the number of newly formed fat globules;
- Preventing the casein micelles from adsorbing at the fat-water interface by using a surface film of lecithin that has the effect of making the newly formed globules more susceptible to heat-induced coalescence.

11.9.3. Effect of Milk Fat Fraction on Cooking Properties

Rowney *et al*. (2003) compared the effect of anhydrous milk fat (AMF, melting point 29.5°C) and fat fractions of different melting points, obtained from the AMF and dispersed into the cheese milk at a low homogenization pressure (2.6 MPa at 50°C), on the functionality of Mozzarella cheese. The fractions and their melting points were: olein, (27°C) and stearin (43.2°C). Increasing the melting point led to a significant increase in the level of free oil in the cheese and a reduction in the viscosity of the heated (60°C) 3 d-old cheese. The results suggest the higher fluidity of the low meting point fraction is conducive to the movement of the non-globular fat (formed during cheese manufacture) throughout the cheese mass during storage and its coalescence (see Section 11.3).

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