

1 The Quality of Milk for Cheese Manufacture

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1.1 Introduction

World production of milk in 2008 is estimated at $\sim 576 \times 10^6$ tonnes (ZMP, 2008), with India/Pakistan, the Americas and Europe being the major producing regions. The proportions of total milk produced by cow, water buffalo, goat, ewe, camel and other are ~ 84.0 , 12.1, 2.0, 1.3, 0.2 and 0.2, respectively (International Dairy Federation – IDF, 2008). Cows' milk is the major milk used for cheese manufacture; however, significant quantities of cheese are also made from goat, sheep and water buffalo milks in some European Union (EU) countries, such as France, Italy and Spain.

Based on an estimated yield of 1 kg cheese 10 kg^{-1} milk, the percentage of total milk used for cheese is $\sim 25\%$, but varies widely from ~ 70 – 90% in some European countries (Italy, France, Denmark and Germany) to $\sim 0.5\%$ in China. While cheese-like products are produced in most parts of the world, the principal cheese-producing regions are Europe, North America and Oceania. Cheese production has increased consistently over the last two decades at an annual average rate of $\sim 1.5\%$. As discussed in Chapter 8, this may be attributed to a number of factors including increases in global population and per capita income, globalisation of eating trends/habits, changing lifestyles, growth in use of cheese as an ingredient in the food service (in pizza-type dishes, cheese burgers and salad dishes) and industrial sectors (cordon bleu entrees, co-extruded products with cheese and gratins).

The increase in consumption has been paralleled by a greater emphasis on improved quality and consistency with respect to the levels of particular nutrients (fat, protein, calcium $-\text{Ca}^{2+}$ and sodium $-\text{Na}^+$), physical properties (texture and cooking attributes), sensory characteristics and processability (size reduction attributes, such as shredability; ability to yield processed cheeses or other cheese products when subjected to secondary processing). Consequently, this has necessitated an increase in the quality and consistency of all inputs (milk composition/quality, enzyme activity/purity, starter cultures characteristics, for example, acid productivity, phage resistance, autolytic properties and flavour-imparting characteristics) and standardisation of the manufacturing process (cf. Chapter 8). In an overall context, milk quality for cheese manufacture may be defined as its suitability for conversion into cheese and deliver cheese of the desired quality and yield. The current chapter examines milk quality for cheese manufacture and the factors affecting it, together with broad-based strategies for improving quality and consistency.

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1.2 Overview of milk composition

Milk consists of protein (caseins and whey proteins), lipid, lactose, minerals (soluble and insoluble), minor components (enzymes, free amino acids, peptides) and water (Table 1.1).

The casein fraction coexists with the insoluble minerals as a calcium phosphate–casein complex. The water and its soluble constituents (lactose, native whey proteins, some minerals, citric acid and minor components) are referred to as serum. During cheese manufacture, the milk is subjected to a partial dehydration, involving controlled expulsion of serum and concentration of fat, caseins (and in some cases denatured, aggregated whey proteins) and some of the minerals. The methods engaged to affect the dehydration include limited destabilisation and aggregation of the calcium phosphate casein in the form of a gel network which

Table 1.1 Compositional and gelation characteristics of cows' milks.

Characteristic	Mean	Range
Gross composition		
Dry matter (g 100 g ⁻¹)	12.04	11.52–12.44
Fat (g 100 g ⁻¹)	3.55	3.24–3.90
Lactose (g 100 g ⁻¹)	4.42	4.21–4.56
Total protein (g 100 g ⁻¹)	3.25	2.99–3.71
True protein (g 100 g ⁻¹)	3.06	2.77–3.47
Casein (g 100 g ⁻¹)	2.51	2.29–2.93
Whey protein (g 100 g ⁻¹)	0.54	0.48–0.64
Non-protein nitrogen (N) (g 100 g ⁻¹ N)	5.33	4.79–6.16
Urea (mg 100 g ⁻¹)	27.60	22.00–37.50
Ash (g 100 g ⁻¹)	0.74	0.71–0.77
Calcium (mg 100 mL ⁻¹)	118	108–137
Iron (mg 100 mL ⁻¹)	976	460–1490
Magnesium (mg 100 mL ⁻¹)	107	96–117
Chloride (mg 100 mL ⁻¹)	100	95–116
Vitamins/vitamin components		
β-Carotene (μg g ⁻¹ fat)	3.18	0.48–8.37
Thiamine (μg mL ⁻¹)	0.18	0.09–0.35
Riboflavin (μg mL ⁻¹)	0.88	0.19–1.85
Vitamin A (μg g ⁻¹ fat)	9.41	2.18–27.85
Vitamin E (μg g ⁻¹ fat)	25.56	6.84–42.15
Iodine (I) (μg mL ⁻¹)	0.28	0.20–0.51
Cobalt (Co) (μg mL ⁻¹)	0.96	0.44–1.70
Gelation properties^a		
RCT (min)	6.15	4.50–7.44
A ₃₀ (mm)	46.80	43.00–51.38
1/k ₂₀ (mm ⁻¹)	0.23	0.3–0.19
Other components		
Total free fatty acids (mg kg ⁻¹ fat)	3769	2629–5108

Source: Compiled from O'Brien *et al.* (1999b–d), Mehra *et al.* (1999) and Hickey *et al.* (2006b) for manufacturing milks.

^aBased on the analysis using the Formagraph (Type 1170, Foss Electric, Denmark) on milks at pH 6.55 and rennet-treated at a level corresponding to ~0.18 mL L⁻¹ (Chymax Plus, Pfizer Inc., Milwaukee, WI); RCT is an index of rennet coagulation (gelation) time, A₃₀ of the curd firmness after 30 min, and 1/k₂₀ of gel firming rate.

encloses the fat and serum via specific enzymatic hydrolysis of the casein, acidification (by fermentation of milk lactose to lactic acid by added bacterial cultures), elevated temperature and various mechanical operations as discussed in Chapter 8. Amongst others, the degrees of casein aggregation and dehydration are critical parameters controlling the properties and quality of the final cheese.

Although manufacturing procedures for most cheese types are very defined (at least in large modern cheesemaking facilities) in terms of technology applied and the type and levels of operations imposed on the milk (cf. Chapter 8), variations in cheese quality do occur. Seasonal variation in the composition and quality of milk are considered to be crucial factors contributing to the inconsistency in quality. Consequently, an overview of milk composition in terms of its relevance to cheese manufacture is presented below. The main focus of this chapter is on cows' milk, which accounts for an estimated 95% of total milk used in cheese manufacture; the characteristics of other milks are discussed elsewhere (Anifantakis, 1986; Juárez, 1986; Remeuf & Lenoir, 1986; Muir *et al.*, 1993a,b; Garcia-Ruiz *et al.*, 2000; Bramanti *et al.*, 2003; Huppertz *et al.*, 2006; Kuchtik *et al.*, 2008; Caravaca *et al.*, 2009).

1.2.1 Casein

The nitrogenous fraction of cows' milk typically consists of casein, whey protein and non-protein nitrogen (urea, proteose-peptones, peptides) at levels of ~ 78 , 18 and 4 g 100 g⁻¹, respectively, of total nitrogen (Table 1.1).

Casein, which is typically present at a level of 2.5 g 100 g⁻¹ in cows' milk, is the main structural protein of both rennet- and acid-induced milk gels (Table 1.1). The casein is heterogeneous, comprising four main types: α_{s1} , α_{s2} , β and κ , which represent ~ 38 , 10, 35 and 15 g 100 g⁻¹ of the total casein, respectively (Fox & McSweeney, 1998; Fox, 2003; Swaisgood, 2003). Model studies in dilute dispersions indicate that the individual caseins vary in the content and distribution of phosphate (Table 1.2); the respective number of (serine) phosphate residues *per* mole of casein are ~ 8 , 10–13, 5 and 1 for α_{s1} -, α_{s2} -, β - and κ -caseins, respectively. The serine phosphates bind calcium and calcium phosphate, and consequently, different caseins have different calcium-binding properties. Generally, α_{s1} -, α_{s2} - and β -caseins bind calcium strongly and precipitate at relatively low calcium concentrations (~ 0.005 – 0.1 M CaCl₂ solutions), inclusive of the calcium level in milk (30 mM); in contrast, κ -casein is not sensitive to these calcium concentrations and can, in fact, stabilise up to 10 times its mass of the calcium-sensitive caseins.

Casein in milk exists in the form of spherical-shaped colloid particles (~ 40 – 300 nm diameter), known as casein micelles (Fox & Brodkorb, 2008; McMahan & Oommen, 2008). Different models have been proposed for the structure of the casein micelle on the basis of the location of individual caseins (in response to their calcium sensitivity) and the calcium phosphate. These include:

- sub-micelle model (Schmidt, 1982), in which sub-micelles are 'cemented' together by colloidal calcium phosphate (CCP) and κ -casein-rich sub-micelles are mainly concentrated at the surface of the micelle; the hydrophilic C-terminal region of the κ -casein

Table 1.2 Characteristics of cows' milk proteins of relevance to cheese manufacture.

Protein	Level in skimmed milk (g 100 g ⁻¹ protein)	Variants	Amino acids mole ⁻¹	Glu residues mole ⁻¹	Asp residues mole ⁻¹	-SH groups mole ⁻¹	Disulphide bonds (S-S) mole ⁻¹	Phosphate residues mole ⁻¹	Glycosylated residues	Sensitivity to Ca ²⁺ at temperatures >18°C	Approximate isoelectric pH in milk	Approximate isoelectric pH in milk	Sensitivity to chymosin hydrolysis during milk gelation
Caseins													
α _{s1} -casein	38	α _{s0}	199	7	0	0	0	9	No	High at >4 mM	—	—	Low
		α _{s1}		24	0	0	0	8			4.96		
α _{s2} -casein	10	α _{s2}	207	4	2	0	0	10	No	High at >4 mM	5.4	4.6	Low
		α _{s3}		25	2	0	0	11			5.3		
		α _{s4}			2	0	0	12			5.3		
		α _{s6}			2	0	0	13			5.2		
β-casein	35		209	11	4	0	0	5	No	High	5.3		Low
κ-casein	15		169	12	4	2	0	1	Yes, with galactose, GalNAc, sialic acid	Very low	5.5		High
γ-casein	3	γ ₁	183	11	4	0	0	1	No	—	—	—	Low
		γ ₂	103	4	2	0	0	0			—	—	
		γ ₃	1001	4	2	0	0	0			—	—	
Whey proteins													
β-lactoglobulin (β-Lg)	55	—	162	—	—	1	2	0	No	—	5.4	5.13	Very low
α-lactalbumin (α-La)	21	—	123	—	—	0	4	0	No	—	—	4.4	Very low
Serum albumin	7	—	34	—	—	1	17	0	No	—	5.1	4.8	Very low
Others													
Immunoglobulins	16	—	—	—	—	—	—	—	—	—	—	—	—
Lactoferrin													

Source: Compiled from Mulvihill & Donovan (1987), Fox & McSweeney (1998), Fox (2003) and Swaisgood (2003).

- orient into the serum as a highly hydrated 'hairy layer' that is in a state of constant flux and confers stability to the micelle by steric repulsion;
- dual bonding model (Horne, 1998), in which the interior of the micelle is composed of α_s - and β -caseins which form a lattice through interactions between hydrophobic regions (hydrophobic-induced) and between hydrophilic regions containing phosphoserine clusters (that attach to CCP clusters), while κ -casein molecules located at the surface interact hydrophobically with the other caseins (α_s - or β -) and orient their highly hydrophilic regions (hairs) into the serum;
 - tangled, cross-linked web model (Holt & Horne, 1996), comprising a 'tangled' mass of rheomorphic casein chains cross-linked by calcium phosphate nanoclusters, similar in casein composition throughout but with the chains becoming more diffuse at the micelle periphery (on moving outwards from the dense centre); and
 - interlocked lattice model (McMahon & Oomen, 2008), featuring a system of interlocking sites composed of anchoring calcium phosphate nanoclusters (several hundred per micelle), which bind the phosphoserine domains of α_s - and β -caseins; the hydrophobic ends of these caseins orientate away from the calcium phosphate nanocluster and interact hydrophobically with other α - and β -caseins, while κ -casein is predominantly surface located because of its lack of phosphoserine domains (to bind to the calcium phosphate nanoclusters) and its highly charged C-terminal regions (which prevents strong electrostatic interactions).

In all of the above models, the arrangement of casein within the micelle is such that the interior is mainly occupied by the calcium-sensitive caseins (α_s - and β -) and κ -casein is principally located at the surface, with its hydrophilic C-terminal region (caseinomacropptide) oriented outwards toward the serum phase in the form of protruding negatively charged hairs, which create an electrokinetic potential of ~ -20 mV and confer stability to the micelle by electrostatic repulsion, Brownian movement and a consequent steric repulsion (de Kruif & Holt, 2003; Horne & Banks, 2004). The κ -casein C-terminal projecting from the micelle surface has been considered as an extended polyelectrolyte brush (de Kruif, 1999), a region containing 14 carboxylic acid groups and immersed in a milk serum with a high ionic strength (~ 0.08 M) due to the presence of various ions (e.g. potassium, sodium, chloride, phosphate, citrate). Consequently, electrostatic interactions (between the C-terminal regions) at physiological conditions are very short and highly screened (by the high ionic strength). This is conducive to a high degree of 'solvency' and extension of the κ -casein C-terminal hairs and to the stability of the micelle as a whole. Moreover, the C-terminal region of the κ -casein is glycosylated to varying degrees (Table 1.2; Saito & Itoh, 1992; Mollé & Leonil, 1995; Fox & McSweeney, 1998; Mollé *et al.*, 2006), containing galactose, *N*-acetylgalactosamine (GalNAc) and/or *N*-acetylneuraminic (sialic) acid (NANA) (Dziuba & Minkiewicz, 1996). These may further enhance the ability of κ -casein to increase micelle stability by steric impedance and electrostatic repulsion via their contribution to increase in water binding (to carbohydrate moieties) and to negatively charged carboxylic groups (on the NANA molecule). O'Connell & Fox (2000) found that the level of glycosylation of κ -casein and protein surface hydrophobicity increased as a function of micelle size.

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While a predominant surface location of κ -casein confers stability to the casein micelle in native milk, it renders it susceptible to aggregation/flocculation by processes which reduce the solvency of (and collapse/flatten) the κ -casein hairs or remove them, and thereby enable contact between the more hydrophobic micelle cores, for example cleavage of the κ -casein by acid proteinases, reducing the negative charge by acidification, reducing ionic strength by microfiltration/diafiltration at native pH. However, the interactions between the micelle cores are modified by many factors, including pH, composition of the serum phase, ionic strength, protein concentration and conditions to which milk is subjected (heat, acidification, ultrafiltration/diafiltration homogenisation, shearing).

The casein micelles on a dry weight basis consist of ~ 7 g 100 g⁻¹ ash (mainly calcium and phosphorous), 92 g 100 g⁻¹ casein and 1 g 100 g⁻¹ minor compounds including magnesium and other salts. They are present in milk at 10^{14} – 10^{16} mL⁻¹, are highly hydrated (~ 3.7 g H₂O g⁻¹ protein), are spherical and have a diameter of ~ 80 nm (100–500 nm), a surface area of $\sim 8 \times 10^{-10}$ cm² and a density of ~ 1.063 g cm⁻³ (Fox & McSweeney, 1998).

1.2.2 Whey protein

Whey protein in cows' milk is typically ~ 0.6 – 0.7 g 100 g⁻¹ and consists of four main types – β -lactoglobulin (β -Lg), α -lactalbumin (α -La), immunoglobulin(s) (Ig) and bovine serum albumin (BSA) at levels of ~ 54 , 21, 14 and 6 g 100 g⁻¹ of total (Table 1.2). The properties of the individual whey proteins have been extensively reviewed (Table 1.2; Mulvihill & Donovan, 1987; Brew, 2003; Fox, 2003; Hurley, 2003; Sawyer, 2003). In milk, they exist as soluble globular proteins and are characterised by a relatively high level of intramolecular disulphide bonding, and β -Lg and BSA each contain one cysteine residue per mole. On heat-induced denaturation, the whey proteins can interact via thiol–disulphide bonds with other whey proteins and with κ -casein. The latter results in the formation of κ -casein/ β -Lg aggregates either at the surface of the casein micelle or in the serum phase or both (cf. Chapter 8). The size and location (serum/micelle surface) of these aggregates are affected by severity of heat treatment of milk, pH at heating, ionic strength, calcium level and casein-to-whey protein ratio. The degree of interaction and size/location of aggregates have a profound effect on the structure and physical properties of rennet- and acid-induced milk gels, and hence on cheeses (see Chapter 8). For example, a high level of casein–whey protein interaction, induced by high heat treatment of the milk (e.g. 95°C for ≥ 1 –2 min, $\sim \geq 40\%$ denaturation of total whey protein; Guinee *et al.*, 1995), is highly favoured in the manufacture of yoghurt and smooth-textured cheeses with a high moisture-to-protein ratio, such as cream cheese and ultrafiltration-produced Quark. In these products it increases protein recovery and moisture binding (reduce syneresis), contributes smoothness and enhances yield (Guinee *et al.*, 1993). In contrast, high heat treatment of milk is unsuitable for acid-curd cheeses with a granular structure (Cottage cheese) or for Quark manufactured using a mechanical separator, as it impedes whey expulsion during separation and makes it difficult to achieve the desired dry matter and texture characteristics. High heat treatment of milk is generally undesirable for rennet-curd cheeses as denatured protein at levels of $\geq 25\%$ of total (at heat treatments of 82°C for 26 s, or greater) impedes the ability of the milk to gel on rennet addition, causes

marked deterioration in melt properties of the cheese (Rynne *et al.*, 2004) and reduces the recovery of fat from milk to cheese (see Chapter 8). However, a higher-than-normal heat treatment that gives a moderate degree of whey protein denaturation may be desirable as a means of modulating the texture of reduced fat cheese, e.g. reduce firmness (Guinee, 2003; Rynne *et al.*, 2004).

1.2.3 Minerals

Cows' milk contains ~ 0.75 g 100 g $^{-1}$ ash, which comprises K^+ , Ca^{2+} , Cl^- , P^{5+} , Na^+ and Mg^{2+} at concentrations (mg 100 g $^{-1}$) of ~ 140 , 120, 105, 95, 58 and 12, respectively (Table 1.2; White & Davies, 1958a; Chapman & Burnett, 1972; Keogh *et al.*, 1982; Grandison *et al.*, 1984; O'Brien *et al.*, 1999c). These minerals are partitioned to varying degrees between the serum (soluble) and the casein (colloidal or insoluble) in native milk (pH ~ 6.6 – 6.7) at room temperature. Serum concentrations as a percentage of the total concentration for each of the minerals are ~ 100 , 100, 100, 66, 43 and 34 for Na^+ , K^+ , Cl^- , Mg^{2+} , P^{2+} and Ca^{2+} , respectively. The partition concentrations of Ca^{2+} and P^{2+} between the colloidal and soluble states in native milk is controlled mainly by the degree of ionisation of the casein (micelle), which in milk may be considered as a very large dominant anion that regulates the degree of binding of the counterion calcium, to an extent affected by the concentration of calcium *per se* and those of citric acid and phosphate. A major difference between the calcium salts of citrate (tricalcium citrate – $Ca_3(C_6H_5O_7)_2$) and phosphate (tricalcium phosphate – $Ca_3(PO_4)_2$) is their solubility, with the solubility product of the latter being very low (2.07×10^{-33} mol L^{-1} at $25^\circ C$) compared to the former (3.23×10^{-3} mol L^{-1} at $25^\circ C$).

Cows' milk typically contains ~ 120 mg 100 mL $^{-1}$ calcium (~ 30 mM), which exists as colloidal inorganic calcium (~ 12.5 mM), caseinate calcium (8.5 mM), soluble unionised calcium (6.5 mM) and serum ionic calcium (2.5 mM). Calcium attached to the casein micelle, referred to as micellar calcium phosphate, is composed of the colloidal inorganic Ca^{2+} (more frequently denoted CCP) and caseinate Ca^{2+} . The former occurs as a calcium phosphate complex attached indirectly to the organic serine phosphate groups, while the latter is attached directly to casein via the dissociated ϵ -carboxyl groups of acidic amino acids including aspartic ($pK_a \sim 3.9$) and glutamic ($pK_a \sim 4.1$) acids. Owing to the high molarity of glutamic and aspartic acids (~ 25 and 7 mM) in milk (with a casein content of 2.5 g 100 g $^{-1}$), it can be inferred that only ~ 26 g 100 g $^{-1}$ of the available ϵ -carboxyl groups are titrated with calcium and that these groups could potentially bind with added calcium to increase the susceptibility of the casein to aggregation, especially on rennet treatment. The sensitivity of the individual caseins to calcium precipitation as found from model studies in dilute solutions varies and tends to increase with the number of moles of both phosphate and glutamic acid per mole of casein. Hence, the concentration of Ca^{2+} at which the individual caseins precipitate is lowest for α_{s2} -casein (< 2 mM), intermediate for α_{s1} -casein (3–8 mM) and β -casein (8–15 mM), and highest for κ -casein, which remains soluble at all of these concentrations and can prevent the precipitation of the other caseins (Aoki *et al.*, 1985).

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In the context of the milk salt system, the milk may be viewed as a ‘soup’ consisting of a large colloidal anion (calcium phosphate casein) dispersed in a serum containing various soluble salt and ionic species (calcium citrate, sodium phosphate, potassium and ionic calcium). The insoluble (colloidal salts associated with the casein) and soluble (serum) salts exist in equilibrium. While the soluble citrate and phosphate compete with the casein for calcium ions (resulting in the formation of calcium citrate and insoluble calcium phosphate), the polyvalent casein is the main player controlling the equilibrium concentrations of salts. However, slight changes in pH and concentrations of serum salts (e.g. as a consequence of natural variation or fortification) can affect the equilibrium balance, and consequently the charge and reactivity of the casein.

1.2.4 Milk lipids

Cows’ milk typically contains ~ 3.7 g 100 g⁻¹ lipid, but the level varies significantly (from ~ 3.0 to 5.0 g 100 g⁻¹) with breed, diet, health, stage of lactation and animal husbandry. Triacylglycerols, denoted as milk fat, represent ~ 96 – 99 g 100 g⁻¹ lipid. The remaining (1 – 2 g 100 g⁻¹) consists of phospholipids (0.8 g 100 g⁻¹), diacylglycerols, sterols (0.3 g 100 g⁻¹) and trace quantities of carotenoids, fat-soluble vitamins and traces of free fatty acids (FFA) (Jensen, 2002; Huppertz *et al.*, 2009). The fat in milk exists in the form of dispersed globules (~ 2 – 6 μm average volume weighted diameter) (Wiking *et al.*, 2004), surrounded by a lipoprotein membrane (milk fat globule membrane, MFGM) (Keenan & Maher, 2006). The MFGM stabilises the enclosed fat against coalescence and fusion (and hence, phase separation) and access from lipases, such as the lipoprotein lipase (LPL) naturally present in native milk, or from lipases of contaminating microorganisms, such as *Pseudomonas* spp. (Ward *et al.*, 2006). Inadvertent damage of the membrane, as, for example, by manhandling of the milk (e.g. excessive shearing, turbulence, cavitation; see Section 1.5.4), is highly undesirable in cheese manufacture. It leads to free fat in the cheese milk, lower recovery of milk fat to cheese, lipolysis of the fat by lipases that survive pasteurisation treatment, high levels of FFA and undesirable flavours (e.g. bitter, soapiness, metallic), especially in some cheese types (e.g. Emmental, Gouda, Cheddar). In the latter cheeses, only low to moderate levels of FFA are required for satisfactory flavour (Cousin & Marth, 1977; Woo, 1983; Gripon, 1993; Brand *et al.*, 2000; Collins *et al.*, 2004; Ouattara *et al.*, 2004; Deeth & FitzGerald, 2006; see also Chapter 8). Nevertheless, there are a number of applications in cheese manufacture where the cheese milk is homogenised, resulting in physical breakage of the MFGM and its replacement by a newly formed membrane composed of casein and whey proteins, and smaller fat globules (Huppertz & Kelly, 2006). The reformed fat globule, owing to its smaller size (~ 1.0 μm), is stable to flocculation and creaming, but does not isolate the enclosed fat from lipolytic enzymes. These properties are exploited in the manufacture of cheeses (see Chapter 8):

- high-fat acid-curd cheeses, such as Cream cheese, where the smaller fat globules prevent flocculation and creaming during the relatively long incubation/gelation period and where the reformed fat globule membrane enables the fat globule to behave as a fat-filled protein

Table 1.3 Free fatty acid profile of milk fat triacylglycerols.

Fatty acid/ common name	Number of carbon atoms	Number of double bond	Typical level in milk fat	
			mol mol ⁻¹ fat	g 100 g ⁻¹ fat ^a
Saturated				
Butyric	4	0	10.1	3.9 (2–5)
Caproic	6	0	4.9	2.5 (1–5)
Capryllic	8	0	2.4	1.5 (1–3)
Capric	10	0	4.3	3.2 (2–4)
Lauric	12	0	4.1	3.6 (2–5)
Myristic	14	0	11.1	11.1 (8–14)
Palmitic	16	0	24.9	27.9 (22–35)
Stearic	18	0	9.8	12.2 (9–14)
Unsaturated				
Myristoleic	14	1	0.8	0.8 (0.5–1.1)
Palmitoleic	16	1	1.4	1.5 (1–3)
Oleic	18	2	17.1	21.1 (20–30)
Linoleic	18	2	2.0	2.5 (1–3)
Linolenic	18	3	0.8	1.0 (0.5–2)

Source: Compiled from Jensen (2002) and MacGibbon & Taylor (2006).

^aValues in parentheses indicate the range of values reported in the literature.

particle, become an integral part of the gel network during acid gelation and contribute to the desired texture characteristics (Guinee & Hickey, 2009; cf. Chapter 8); and

- rennet-curd cheeses where a high level of lipolysis is desirable (e.g. blue-type cheeses), where added lipases or lipases from secondary starter cultures can access the fat more easily, bring about selective hydrolysis of the triacylglycerols and release the FFA that lead to the desired flavour.

The principal fatty acids in milk fat on a total weight basis are C_{16:0} (palmitic), C_{18:1} (oleic) and C_{14:0} (myristic) in decreasing order (Table 1.3). While the shorter chain fatty acids (C_{4:0} to C_{12:0}) are present in lower quantities on a weight basis, they are primarily responsible for the piquant flavour of hard Italian cheeses, such as Parmesan and Romano, or the sharp goaty/sheep-like flavours of soft goat milk cheeses. These fatty acids are hydrolysed from the milk fat triacylglycerols by lipase enzymes, which gain access owing to damage of the MFGM during cheese manufacture and maturation. The principal sources of these lipases are added exogenous enzymes (added rennet paste, pregastric esterase), secondary flora (*Brevibacterium linens*, *Penicillium roqueforti*, *Geotrichium candidum*; see also Chapter 6), starter culture lactic acid bacteria and culture adjuncts (*Lactococcus* spp., *Lactobacillus helveticus*) (Collins *et al.*, 2004; Hickey *et al.*, 2006b; Santillo *et al.*, 2007; Hashemi *et al.*, 2009; Jooyandeh *et al.*, 2009).

1.3 Principles of cheese manufacture

Cheese is a concentrated protein gel, which occludes fat and moisture. Its manufacture essentially involves gelation of cheese milk, dehydration of the gel to form a curd and

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treatment of the curd (e.g. dry stirring, cheddaring, texturisation, salting, moulding, pressing). The moulded curd may be consumed fresh (shortly after manufacture, for example within 1 week) or matured for periods of ~ 2 weeks to 2 years to form a ripened cheese. The gelation of milk may be induced by:

- selective hydrolysis of the κ -casein at the phenylalanine₁₀₅–methionine₁₀₆ peptide bond by the addition of acid proteinases, referred to generically as rennets (chymosin, pepsin);
- acidification (using starter cultures or food-grade acids and/or acidogens), at a temperature of 20–40°C, to a pH value close to the isoelectric pH of casein, i.e. ~ 4.6 ; and/or
- a combination of acid and heat, for example heating milk at pH ~ 5.6 to $\sim 90^\circ\text{C}$.

1.3.1 Rennet-induced gelation

On treatment of milk with chymosin (rennet), the κ -casein is hydrolysed, with the primary cleavage point being the peptide bond phenylalanine₁₀₅–methionine₁₀₆, and the liberation of the highly charged, hydrophilic methionine₁₀₆–valine₁₆₉ caseinomacropeptide into the milk serum (whey). This results in an effective ‘shaving’ of the hairy layer from the micelle surface, a marked reduction in the negative surface charge to ~ -10 mV, and an increase in the attractive forces between, or ‘stickiness’ of, the *para*-casein micelle surfaces. Consequently, the latter begin to aggregate when sufficient κ -casein is hydrolysed (~ 80 – 90 g 100 g⁻¹ of total; Green *et al.*, 1978; Dalgleish, 1979), resulting in the formation of clusters/aggregates of *para*-casein micelles that fuse gradually and eventually ‘knit’ into a restricted, periodic repeating, solid-like viscoelastic gel network (Fig. 1.1). The enzymatic stage of rennet coagulation and the aggregation of enzymatically altered sensitised *para*-casein micelles overlap. While the exact contribution of calcium to rennet coagulation is unclear, it is likely that the casein calcium (which in effect may be considered as pre-bound ionic calcium) is the principal agent inducing cross-linking and aggregation of the *para*-casein micelles into a gel. The serum ionic calcium in milk is in equilibrium with the casein calcium. Hence, apart from reflecting the level of casein-bound calcium, serum ionic calcium probably plays little, or no, direct role in rennet-induced casein aggregation and gelation of milk. Similarly, the progressive increase in gel firmness of rennet-treated milks on the addition of calcium chloride (ionic calcium) while retaining a constant pH (Fig. 1.2) probably reflects the consequent increases in the levels of casein calcium and CCP rather than an increase in the serum ionic calcium *per se*. Hence, it is noteworthy that on concentration of milk by evaporation, the calcium ion activity slightly decreases from ~ 1.0 to 0.75 mM L⁻¹ while the levels of micellar calcium increase (Nieuwenhuijse *et al.*, 1988). Rennet-induced gelation of milk is hindered by a variety of factors, which either:

- restrict access of the rennet to its substrate (κ -casein), for example complexation of denatured whey protein with κ -casein at the micelle surface, as a result of high heat treatment of the cheese milk (Fig. 1.1; Guinee, 2003);
- act as obstacles to the aggregation and fusion of rennet-treated casein micelles, for example κ -casein/ β -Lg appendages at micelle surface, or serum κ -casein/ β -Lg particles (Guyomarc’h, 2006);

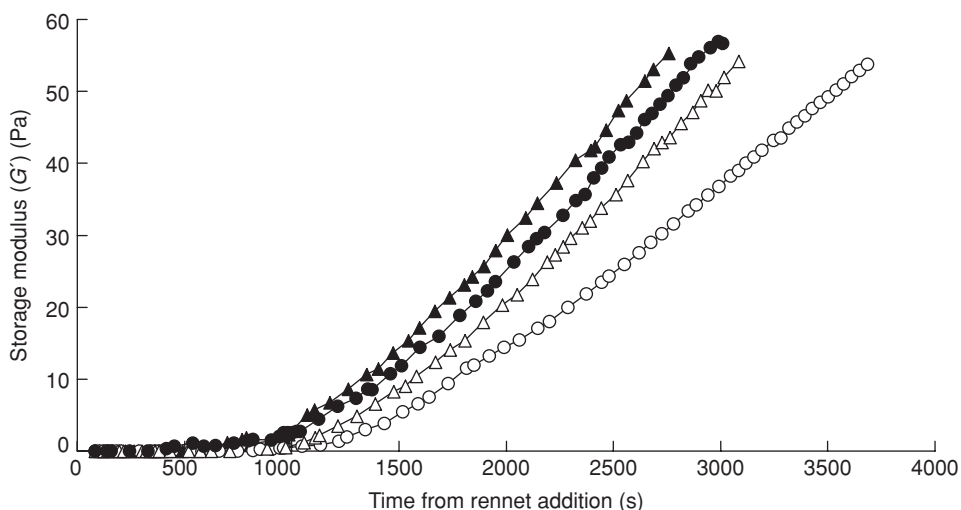


Fig. 1.1 Effect of pasteurisation temperature on changes in storage modulus G' during the rennet gelation of milk. Note: Milks were heated to various temperatures (in $^{\circ}\text{C}$) for 26 s prior to rennet addition: 72 (\bullet), 74.6 (\blacktriangle), 75.9 (\circ) or 78.5 (\triangle); the milks were cooled to 31°C , adjusted to pH 6.55 if necessary with lactic acid solution ($5\text{ g } 100\text{ g}^{-1}$), treated with chymosin (Chymax Plus, Pfizer Inc., Milwaukee, WI) at a rate of 0.18 mL of undiluted rennet per litre of milk; all milks had similar contents of protein ($3.3\text{ g } 100\text{ g}^{-1}$) and fat ($3.4\text{ g } 100\text{ g}^{-1}$); G' was measured dynamically using low-amplitude strain oscillation rheometry (controlled stress rheometer).

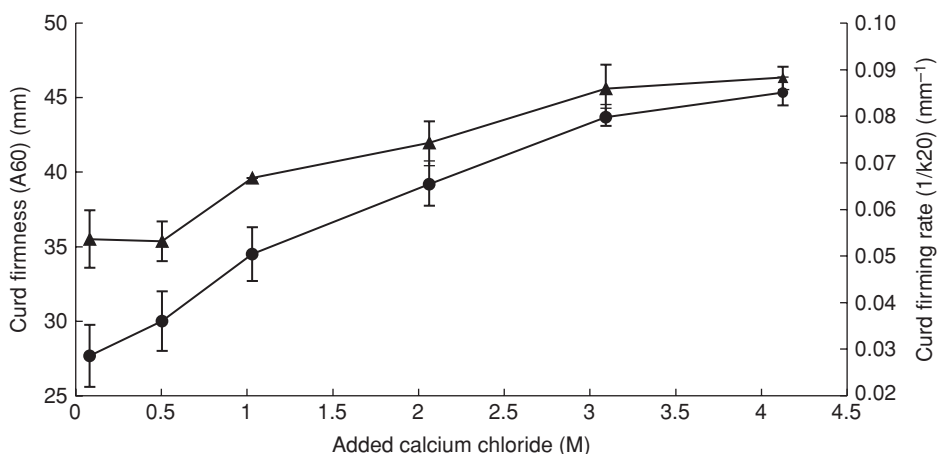


Fig. 1.2 Changes in curd firmness at 60 min (A60; \bullet) and curd firming rate ($1/k_{20}$; \blacktriangle) of skimmed milk as a function of the level of added calcium chloride. Note: All milk samples ($\sim 3.45\text{ g protein } 100\text{ g}^{-1}$ of milk) were adjusted to pH 6.55 prior to measuring the rennet gelation properties at 31°C on the Formagraph (Type 1170, Foss Electric, Denmark); the following parameters were measured k_{20} , a measure of time from the onset of gelation to a output signal width of 20 mm, and A60, the width of the output signal at 60 after rennet addition.

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- reduce the ‘stickiness’ of rennet-altered casein micelles, for example increased ionic strength (e.g. by the addition of NaCl to the cheese milk as in Domiati cheese) (Awad, 2007; Huppertz, 2007), negative charge (high pH); and/or
- reduce the degree of bonding between touching micelles, for example reducing the level of calcium by the addition of ethylenediaminetetraacetic acid (EDTA) or other chelants (Shalabi & Fox, 1982; Mohammad & Fox, 1983; Choi *et al.*, 2007), ion exchange (Mei-Jen-Lin *et al.*, 2006) and/or dialysis (Wahba *et al.*, 1975), or by a naturally low level of Ca^{2+} as in late lactation milks or milks from cows with subclinical mastitis (White & Davies, 1958a).

Following gel formation, the resultant milk gel is subjected to a number of operations that promote the release of whey, an approximate tenfold concentration of the casein, fat and micellar calcium phosphate components, and a transformation to a curd with much higher dry matter content than the original milk gel (45 g 100 g⁻¹ for Cheddar curd at whey drainage). These operations include cutting the gel into pieces (referred to as curd particles, ~0.5–1.5-cm cubes), stirring and heating the particles in expressed whey, reducing the pH of the aqueous phase inside the curd particle by fermentation of lactose to lactic acid (by the lactic bacteria in the starter culture added to the milk prior to rennet addition), and physical draining of the whey from the curd particles by pumping the curd particle–whey mixture onto perforated screens (cf. Chapter 8). Following whey drainage, the curd particles knit together into a cohesive mass of curd, which is treated to enhance further whey expulsion and concentration to the desired dry matter content of the cheese variety being manufactured; these treatments differ according to variety but typically include further lactose fermentation and pH reduction, cutting the curd mass into pieces (slabs), moulding the pieces to the desired shape and weight of finished cheese, salt addition and pressing. During the dehydration process of the gel, protein concentration and aggregation continues via various types of intra- and intermolecular interactions (Lucey *et al.*, 2003), including calcium bridging (between glutamate/aspartate residues, calcium–CCP bridges between phosphoserine residues), hydrophobic interactions between lipophilic domains and electrostatic interactions (other than calcium bridging). The strength of these interactions is modulated by ionic strength, pH, calcium and temperature, and hydrolysis of proteins to peptides, which alters the hydrophile/lipophile balance of the proteinaceous fraction.

Following manufacture, rennet-curd cheeses are usually matured or ripened by holding under specific conditions of temperature and humidity for periods which range from ~2 to 4 weeks for soft cheeses (for Camembert-type cheeses) to ~2 years for some hard cheeses (for Parmesan-style cheeses). During this period, a host of physico-chemical changes take place which transform the ‘rubbery/chewy’-textured fresh cheese curd to the finished cheese with the desired variety quality characteristics, for example a soft, smooth, short and adhesive texture with a mushroom-like flavour and creamy mouth-feel for Camembert, or a long, elastic sliceable texture and mild, sweet flavour for Leerdammer cheese. These physico-chemical changes include:

- glycolysis, conversion of residual lactose to lactic acid by the starter culture and of lactic acid to other compounds, such as acetic acid and propionic acid by secondary starter

cultures such as *Propionobacteria freudenreichii* subsp. *shermanii* in Emmental-style cheese;

- proteolysis, hydrolysis of caseins to peptides and free amino acids by proteinases and peptidases present in the cheese (residual rennet; plasmin, and proteinases and peptidases from the cells of starter culture and non-starter lactic acid bacteria); and
- lipolysis, involving the hydrolysis of triacylglycerols to FFA, di- and monoacylglycerols by lipases and esterases from various sources, including native milk LPL, added pregastric esterases and/or secondary cultures.

The physico-chemical and biochemical changes that occur during ripening are discussed in Chapter 8 and several comprehensive reviews are available (Collins *et al.*, 2004; McSweeney & Fox, 2004; Upadhyay *et al.*, 2004; Kilcawley, 2009).

Of particular interest in relation to milk composition and cheese quality is the impact of the proportion of intact α_{s1} -casein content in milk on casein aggregation, strength of the rennet-induced milk gel and texture of the final cheese. The sequence of residues 14–24 is a strongly hydrophobic domain and confers intact α_{s1} -casein with strong self-association and aggregation tendencies in the cheese environment (Creamer *et al.*, 1982); interestingly, this domain also has 3 mol of glutamate, which are expected to contribute to intra- and intermolecular calcium bridges. It has been suggested that self-association of α_{s1} -casein in cheese, via these hydrophobic ‘patches’, leads to extensive cross-linking of *para*-casein molecules and thus contributes to the overall continuity and integrity of the casein matrix in the cheese curd (de Jong, 1976, 1978; Creamer *et al.*, 1982; Lawrence *et al.*, 1987). The early hydrolysis of α_{s1} -casein at the phenylalanine₂₃–phenylalanine₂₄ peptide bond, by residual rennet retained in the cheese curd following manufacture (~10% of that added), results in a marked weakening of the *para*-casein matrix and reductions in fracture stress and firmness of the cheese during maturation (de Jong, 1976, 1977; Creamer & Olson, 1982; Malin *et al.*, 1993; Tunick *et al.*, 1996; Fenelon & Guinee, 2000). This hydrolysis is a key step in mediating the conversion from a fresh rubbery curd to a mature cheese with the desired textural and cooking properties (meltability) (cf. Chapters 7–10).

1.3.2 Acid-induced gelation

The caseins in milk are insoluble at their isoelectric points (pH ~4.6) at temperatures $> \sim 8^{\circ}\text{C}$ (Mulvihill, 1992). This property is exploited in the formation of acid-curd cheeses, such as Cottage cheese, Quark and Cream cheese, the manufacture of which involves slow quiescent acidification of the cheese milk to pH ~4.6–4.8 by starter culture, acidogens (e.g. glucono- δ -lactone) at temperatures of 20–30°C (Guinee *et al.*, 1993; Lucey & Singh, 1997; Fox *et al.*, 2000; Farkye, 2004; Lucey, 2004; Schulz-Collins & Zenge, 2004). Acidification results in a number of physico-chemical changes promoting hydration/dispersion or dehydration/aggregation effects on the casein micelle, with the ratio of these effects changing as the pH declines during the acidification (fermentation) process. Reducing the pH from 6.6 to ~5.2–5.4 results in a decrease in the negative charge of the micelles due to titration of negative charges with H^{+} ions. Nevertheless, this is generally not accompanied by the onset

of gelation because of:

- solubilisation of micelle ‘cementing’ agent CCP (fully soluble at pH \sim 5.2 at 20°C);
- diffusion of all caseins from the micelle to the serum (owing to a decrease in the degree of electrostatic interaction between phosphoserine residues of α_s - and β -caseins and the CCP nanoclusters);
- increases in ionic strength of the serum phase; and
- hydration of the casein micelles.

However, further reduction in pH in the range \sim 5.2–4.6 results in aggregation of casein and gel formation, as forces promoting dispersion of casein micelles are overtaken by the sharp reductions in the negative charge and hydration of the casein, the collapse in steric effect associated with the κ -casein C-terminal ‘hairs’ and the increase in hydrophobic interactions. The onset of gelation typically occurs at pH \sim 5.1 and further reduction in pH toward 4.6 coincides with the eventual formation of a continuous gel structure with sufficient rigidity to enable separation of whey from the curd by physical means (e.g. breakage, stirring, and whey drainage, or centrifugation). The increase in gel rigidity coincides with a sigmoidal increase in the elastic shear modulus of the gelling cheese milk, as the pH continues to decrease towards 4.6 during incubation and the fermentation of lactose to lactic acid (Fig. 1.3).

High heat treatment of the cheese milk (e.g. 95°C for \geq 1 min) leads to an increase in the pH at the onset of gelation (from \sim 4.7 to 5.3) and the rigidity of the resultant gel (cf. Chapter 8; Vasbinder *et al.*, 2003; Anema *et al.*, 2004). These changes coincide with increases in the level of whey protein denaturation and its covalent interaction with κ -casein,

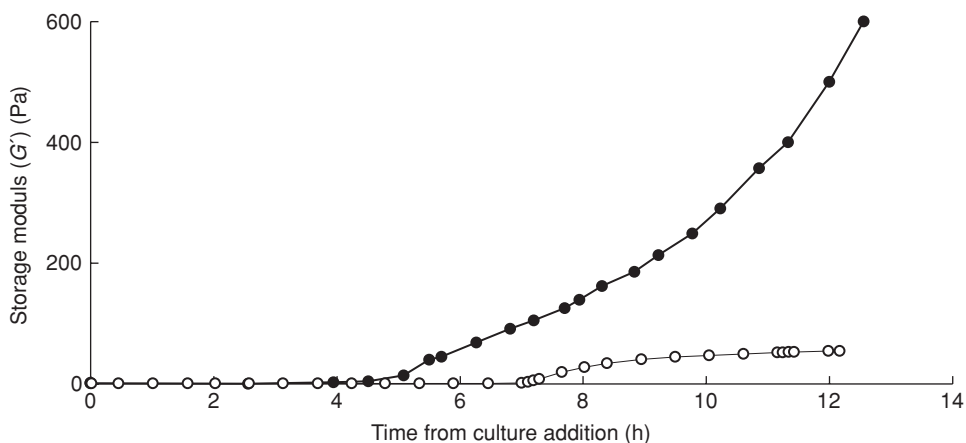


Fig. 1.3 Effect of milk treatment (heated to 90°C for 30 min – ●, or unheated – ○) on changes in modulus G' during acid gelation of milk. Note: The milks (1.5 g fat 100 g⁻¹, 3.2 g protein 100 g⁻¹) were cooled to 40°C and a thermophilic starter culture (*Streptococcus thermophilus*) was added at a rate of 2.5 g 100 g⁻¹; G' was measured dynamically using low-amplitude strain oscillation rheometry (controlled stress rheometer).

via thiol–disulphide interchange. This interaction occurs both at the surface of the micelle, resulting in the formation of filamentous appendages projecting from the micelle surface, as well as in the serum phase when κ -casein dissociates from the micelle into the serum and interacts with β -Lg to form soluble complexes that sediment as the pH is reduced during fermentation and/or rennet treatment. The different types of interactions are influenced by pH and the level of whey proteins (Donato & Guyomarc'h, 2009; cf. Chapter 8). *In situ* denatured whey protein increases the concentration of gel-forming protein, the spatial uniformity of the gel matrix and the number of stress-bearing strands in the matrix. Denatured whey proteins, whether in the form of filamentous appendages (κ -casein/ β -Lg) that occur at the surface of micelle and 'flatten' on pH reduction or that occur as serum-soluble κ -casein/ β -Lg particles that sediment on pH reduction (and/or rennet treatment), act as obstructions that physically obstruct/prevent a high level of interaction of the native casein micelles and, thereby, lead a more continuous gel structure with higher rigidity. The increase in micelle size resulting from complexation with denatured whey protein (Anema & Li, 2003) is conducive to an earlier touching of the casein micelles during the acidification/gelation process and the onset of gelation at a higher pH. The changes in gel structure associated with high heat treatment of milk lead to significant increases in the stiffness (G') and visual smoothness of the resultant acid gel (Fig. 1.3), a principle which has long been exploited in the manufacture of yoghurt.

In the manufacture of acid-curd cheeses, the milk gel is cut or broken, and whey removal is achieved by various means including centrifugation, ultrafiltration and/or straining the broken gel in muslin cheese bags. In some varieties (e.g. cream cheese), whey separation is further enhanced by heating the broken gel to temperatures of $\sim 80^\circ\text{C}$ prior to centrifugation or to $\sim 50^\circ\text{C}$ prior to ultrafiltration or straining. Treatments of the curd differ with cheese variety. In the manufacture of Quark, the temperature of the concentrated curd ($\sim 18 \text{ g } 100 \text{ g}^{-1}$ dry matter) is cooled rapidly to $< 8^\circ\text{C}$ by passing through suitable heat exchangers so as to limit hydrophobic interactions between the proteins and to, thereby, minimise the likelihood of defects associated with excessive protein interaction in the final cheese, for example sandy, chalky, or grainy mouth-feel, and/or wheying-off. In contrast, the manufacture of Cream cheese involves high heat treatment of the curd ($\sim 80^\circ\text{C}$), the addition of NaCl ($\sim 0.5 \text{ g } 100 \text{ g}^{-1}$) and hydrocolloids (blends of xanthan/guar gums: $\sim 0.3 \text{ g } 100 \text{ g}^{-1}$), mixing, homogenisation and cooling. The added hydrocolloids, hydrated and dispersed at high temperature, increase the viscosity of the hot molten cheese curd and reduce the growth of protein aggregates and the occurrence of chalky/grainy texture.

The degree to which the attributes (stiffness, structure) of the gel prior to whey separation and concentration influence the characteristics of the final acid-curd cheese is influenced by the type and extent of operations (whey separation method, heat treatment, type/level of hydrocolloid) following gel formation; this subject is beyond the scope of this chapter and the reader is referred to earlier reviews (Guinee *et al.*, 1993; Lucey *et al.*, 2003; Farkye, 2004; Schulz-Collins & Zenge, 2004; Guinee & Hickey, 2009).

1.4 Quality definition of milk

In an overall context, the quality of milk for cheese may be defined as its characteristics that fulfil the requirements of its users – direct (the cheese manufacturer) and indirect (the cheese

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user, consumer) (Peri, 2006). The quality requirements may be defined as:

- safety, which denotes the absence of associated risk (e.g. pathogenic microorganisms, 'toxic' residues) in milk from consuming the cheese from which it is made;
- compositional/nutritional, which indicate the conformity to minimum levels of particular components (fat, protein, casein, calcium) that make it suitable for cheese manufacture, for example enable the milk to form a gel suitable for cutting within a certain time after addition of rennet; to give desired manufacturing efficiency (percentage recovery of fat and casein; product yield), composition (levels of protein, calcium, moisture) and sensory characteristics;
- microbiological, ensuring that total bacterial count does not exceed a maximum value so as to reduce the risk of the milk quality (level of intact casein, absence of rancidity associated with hydrolysis of milk fat) being compromised in terms of its cheesemaking capacity (rennet coagulability, altered levels of pH at different stages of manufacture), cheese yield efficiency (recoveries of fat and casein, cheese yield) and cheese quality (flavour and physical properties);
- sensory and functional, implying its possession of the desired hedonic (absence of taints) and physico-chemical characteristics (coagulability by rennet under defined conditions), enabling it to be satisfactorily made into cheese with the desired hedonic (taste, smell), usage (techno-functional) and nutritional characteristics; and
- ethical, in terms of its naturalness (non-adulterated) and its compliance to production standards including those pertaining to animal breeding, animal welfare and agricultural/husbandry systems.

Requirements of the first four aspects either can be quantified directly by tests (microbiological, chemical, physical) undertaken by the cheese manufacturer or regulatory agencies, or can generally be perceived by both the manufactures and users of the milk, as they may impact on cheesemaking capacity of the milk, yield efficiency or product quality. Generally, ethical requirements (apart from adulteration) cannot be tested and/or perceived directly by the users; for example, analysis of milk or consumption of the resultant cheese cannot verify that the milk was produced in compliance with organic farming methods. Compliance to ethical requirements is generally considered to be fulfilled by the milk producer and, moreover, is ensured by specifications set by government agencies (EU, 1992, 2004) and organisations such as dairy cooperatives and organic milk supplier organisations.

In the current chapter, milk quality for cheese manufacture will be discussed under the following criteria, each of which involves different types of sub-criteria or characteristics.

1.4.1 *Safety/public health (pathogens including Mycobacterium tuberculosis, Brucella spp., toxic residues, and contaminants)*

Directive 92/46 (EU, 1992) specifies that raw milk must come from healthy animals and should not endanger human health by way of infectious diseases or foreign substances that are communicable to human beings through the milk. A recent study has attributed 9% of foodborne disease cases to milk consumption (Adak *et al.*, 2005).

Pathogenic bacteria

The presence of potentially pathogenic bacteria in milk is well documented (Rea *et al.*, 1992; Jayarao & Henning, 2001). The pathogens reported as the most common agents implicated in milkborne disease include *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* (Gillespie *et al.*, 2003), but others found in milk could also have public health implications, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes* (Jayarao & Henning, 2001). Reed and Grivetti (2000) reported that surveys on Californian dairies revealed the presence of a variety of bacteria that could make people ill, and raw milk consumption has often been associated with foodborne epidemics due to pathogens, such as *Campylobacter* spp., *Listeria* spp. and *Salmonella dublin*. These microorganisms may enter the mammary gland and thus the milk, from the external environment through the teat orifice during the milking process or during the interval between milkings. Contamination of the external surface of the teat with faecal and other environmental organisms is scarcely avoidable, but is minimised by compliance to the highest standards of hygiene at milking. However, if initial contamination levels are low and subsequent milk storage conditions (hygiene and temperature) are correct, then further bacterial growth will be minimised.

Mycobacterium bovis

This organism has a broad host range and is the principal agent of tuberculosis in wild and domestic animals. This organism can also infect humans causing zoonotic tuberculosis. The transmission of tuberculosis to humans in the United Kingdom following consumption of unpasteurised milk was reported by de la Rua-Domenech (2006). *Brucella* spp. are pathogens, which are highly infectious and capable of causing disease in both animals and humans. The pathogenic strain *Brucella abortus* is more associated with cows, whereas *Brucella melitensis* is more commonly found in sheep and goats. Transmission to humans can be (amongst other routes) via milk and milk products (Gupta *et al.*, 2006).

Regulation 853/2004 (EU, 2004) (Annex III, Section IX) states that raw milk must come from animals that do not show symptoms of infectious diseases communicable to humans through milk. In particular, as regards tuberculosis and brucellosis, this regulation states that raw milk must come from cows (or buffalos) belonging to a herd which, within the meaning of Directive 64/432 (EU, 1964), is free or officially free of tuberculosis and brucellosis, and if not, the milk may only be used with the authorisation of the competent authority. In addition to compliance with directivities on milk quality, perhaps the most effective means of ensuring the safety of milk from a public health perspective may be to implement ongoing training of dairy farmers and their employees in the areas of cow management, milk handling and storage procedures, fundamentals of toxin and disease transmission, and pathogen effects on human health. In addition, pasteurisation of milk represents possibly the most significant and successful contribution to milk safety (Holsinger *et al.*, 1997).

Toxic residues/contaminants

These compounds in the animal's body may be shed into milk and thus pose a threat to human health. Chemical residues are remnants of purposeful additions to the food chain (see Section 1.5.5), whereas contaminants represent any biological or chemical agent and

any other foreign substances (e.g. dioxins, pesticides) that could gain entry to the milk and, as a result, compromise food safety or suitability for use. The most common chemical residues found in milk are antibiotics, following administration to treat mastitis. Regulation 853/2004 (EU, 2004) (Annex III, Section IX) states that raw milk must come from animals to which no unauthorised substances have been administered and that where authorised products or substances have been administered, the withdrawal periods for those products have been observed. The most effective means of controlling toxic residues/contaminants is by legislation, voluntary codes of practice, monitoring and surveillance of animal feeds, and prudent use of all animal inputs (Buncic, 2006).

1.4.2 *Composition (protein, casein, fat, total solids, lactose, and mineral)*

Regulation 2597/97 (EU, 1997) outlines marketing standards to guarantee compositional quality of non-standardised whole milk, and include minimum fat and protein concentrations ($\text{g } 100 \text{ g}^{-1}$) of 3.5 and 2.9 (based on a fat content of $3.5 \text{ g } 100 \text{ g}^{-1}$), respectively. The specific combination of milk characteristics required for cheese depends on the type of cheese being manufactured. For example, sheep's milk is more suited than cows' milk for the production of piquant-flavoured cheeses, such as Pecorino Romano owing to the higher concentration of short-chain fatty acids ($\text{C}_{4:0}$, $\text{C}_{6:0}$, $\text{C}_{8:0}$, $\text{C}_{10:0}$ and $\text{C}_{12:0}$) in its milk fat, which contributes to this flavour profile (Nelson *et al.*, 1977; Lindsay, 1983; Woo & Lindsay, 1984; Medina & Nunez, 2004). The low carotenoid content of sheep's and goat's milk relative to cows' milk is also more suited to the manufacture of white-coloured cheese varieties, such as Manchego and Roquefort cheeses (Anifantakis, 1986; Fox *et al.*, 2000). However, cows' milk can vary dramatically in carotenoid content from ~ 4 to $13 \mu\text{g g}^{-1}$ fat, depending on breed, feed type and stage of lactation (Noziere *et al.*, 2006; Calderon *et al.*, 2007). In contrast, sheep's milk because of the above characteristics is unlikely to be suitable for the manufacture of Cheddar cheese, in which the rich straw-yellow colour, relatively low level of lipolysis (Hickey *et al.*, 2006a,b, 2007) and non-rancid flavour are key quality criteria. Owing to its low ratio of α_{s1} - to α_{s2} -casein, goat milk gels much more slowly than cow milk on rennet addition and forms markedly weaker gels and curds, and is consequently much more suited to the manufacture of soft cheese (Storry *et al.*, 1983; Juárez & Ramos, 1986; Medina & Nunez, 2004), but much less so to the large-scale manufacture of hard cheeses, such as Emmental, Gouda, Mozzarella and Cheddar. Apart from the altered proportions of individual caseins, other factors such as the (generally) lower contents of calcium and total casein may also contribute to the relatively poor rennet coagulation characteristics of goat milk.

Optimising manufacturing procedures for milks of varying compositions

Rennet-curd cheese is a product created through controlled enzymatic destabilisation and aggregation of colloidal calcium phosphate casein micelles in the form of a calcium phosphate *para*-casein gel, enclosing fat and moisture. The gel is subjected to various operations (e.g. breaking/cutting, pH reduction, temperature elevation) to induce expulsion of whey and transition from a low-solids gel to a high-solids cheese curd. During this dehydration, involving breakage and shrinking of the gel, the gel/matrix structure continually rearranges, resulting

in further aggregation and fusion of the *para*-casein. The compositional characteristics of good quality milk for the manufacture of all cheeses are those that enhance this controlled aggregation under optimised cheesemaking conditions to give an acceptable manufacturing time, cheese with the desired composition, high yield and excellent quality.

However, a given set of milk compositional characteristics may not fulfil all three requirements simultaneously unless the manufacturing procedure is optimised. For example, the potential of milk with a higher than normal intact casein content to deliver a high yield of cheese with the desired composition and quality may not be realised if the standard operating procedure (SOP) was developed using milks with lower casein content. A critical step in the SOP for any cheese recipe is the firmness of the gel at cut, which can affect the cheese moisture, pH, salt in moisture, yield and quality (cf. Chapter 8). Yet in most modern cheesemaking operations, rennet is added to the milk on a volume basis (rather than on basis of casein load: volume \times concentration) and the gel is cut at a fixed time after rennet addition (rather than on the basis of firmness). While such a process may appear to be standardised (fixed rennet dosage per volume of milk, fixed set-to-cut time), it automatically promotes variable curd firmness at cutting when the properties of the cheese milk (e.g. casein number, casein content, pH, calcium content) presented to the SOP change seasonally. Such SOPs are frequently established by the investigations of production support personnel, working over relatively short-time periods on milks with composition parameters falling within a narrow range. However, seasonal variations in milk composition can be relatively large; for example, in Ireland, protein can vary from ~ 3.1 to $3.8 \text{ g } 100 \text{ g}^{-1}$ in milk from pasture-fed, spring-calved herds (Mehra *et al.*, 1999; O'Brien *et al.*, 1999d; Guinee *et al.*, 2006). Significant seasonal changes in milk composition are also common elsewhere, including the United Kingdom (Grandison 1986; Banks & Tamime, 1987), France (Martin & Coulon, 1995), New Zealand (Auldism *et al.*, 1998; Nicholas *et al.*, 2002), Australia (Auldism *et al.*, 1996; Broome *et al.*, 1998a; Walker *et al.*, 2004) and Canada (Kroeker *et al.*, 1985). Hence, there is a need to standardise basic parameters, such as protein-to-fat ratio, casein content (ideally), ratios of starter culture and rennet to casein load, starter culture activity, firmness at cut and the pH at different stages of manufacture (e.g. at set) to achieve the optimum performance from good quality milk. Using such an approach to develop SOPs should minimise seasonal variations in cheese composition, manufacturing efficiency, biochemical changes during maturation and quality (cf. Chapter 8). The use of the most-up-to-date technology (including milk casein standardisation), process modelling, in combination with on-line monitoring (in-vat curd firmness sensors), is seen as an approach for further optimisation of process control and improvement in cheese quality.

Effects of variations in different compositional parameters

The effects of many compositional parameters of milk on cheese manufacture (rennet coagulation characteristics), cheese yield and/or cheese quality have been investigated (Okigbo *et al.*, 1985a-c; Guinee *et al.*, 1994, 1997, 2006; Broome *et al.*, 1998a,b; Auldism *et al.*, 2004; Mei-Jen-Lin *et al.*, 2006; Wedholm *et al.*, 2006; Jōudu *et al.*, 2008) and are summarised in Table 1.4. Generally, numerically higher values of the following variables are positively correlated with enhanced rennet coagulation properties (more rapid curd firming rate, higher curd firmness and shorter set-to-cut time in manufacture) and cheese yield: casein number; contents of total casein, individual (α_{s1} -, β - and κ -) caseins, β -Lg, calcium;

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Table 1.4 Characteristics of milk ex-farm important for cheese manufacture.

Characteristic	Suggested values	Comment
Visual/sensory characteristics		
Appearance		Should be typical of milk (cream-white colour, homogeneous, no free fat or froth)
Smell		Free from atypical smells and taints
Biochemical/physical characteristics		
Colour (instrumental measurement)	ND ^a	Ideally should have instrumental measured (colour coordinates L^* , a^* , b^* values; for further detail, see Chapter 8)
pH	≤ 6.7 to ≥ 6.5	
Protein content (g 100 g ⁻¹)	3.3	
Casein (g 100 g ⁻¹)	≥ 2.55	
Casein number	≥ 77	
Non-protein nitrogen (N) (g 100 g ⁻¹ total N)	<6	
Serum casein (g 100 g ⁻¹ total casein)	<4	Serum casein as percentage of total casein should ideally be very low
κ -casein (g 100 g ⁻¹ total casein)	>15	
γ -casein (g 100 g ⁻¹ total casein)	<3	
Fat content (g 100 g ⁻¹)	>3.6	Should remain relatively consistent to avoid large changes in liquid-to-solid fat ratio and rheology of fat phase in cheese
Free fatty acid (mg kg ⁻¹)	<3500	Should be low to avoid rancid off-flavours
Lactose content (g 100 g ⁻¹)	>4.3	
Somatic cell count (cells mL ⁻¹)	$\leq 100 \times 10^3$	
Total bacterial count (colony forming units (cfu) mL ⁻¹)	$\leq 30 \times 10^3$	
Plasmin (AMC units mL ⁻¹) ^b	<0.18	
Plasminogen (AMC units mL ⁻¹) ^b	<0.18	
Residues		
Antibiotics	Not detectable	
Iodine ($\mu\text{g kg}^{-1}$)	<250	
Trichloromethane ($\mu\text{g kg}^{-1}$)	<2	
Processability characteristics		
Gel firmness		
Rheometer (G' , Pa)	50 Pa at 31°C in 60 min ^a	
Formagraph (A60, mm)	>45 mm	
Syneresis	ND	Gel should undergo syneresis readily on cutting (could be measured empirically, for example, by centrifugation under defined condition, or $\mu\text{g kg}^{-1}$)

^aND, not defined.

^bAminomethyl cumarin.

and ratios of κ -casein to total casein and to individual (α_{s2} - and β -) caseins. For a given rennet-to-casein ratio, the positive effect of the increases in the above milk characteristics on rennet coagulation and/or cheese yield are consistent with a higher concentration of gel-forming casein and/or enhanced aggregation via calcium bridges, calcium phosphate bridges and hydrophobic interactions. The positive effect of a high κ -casein-to-total casein ratio is expected because of:

- the presence of three hydrophobic domains and a high level of aspartic acid (4 mol) on the N-terminal (AA₁₋₂₀) region of the *para*- κ -casein;
- the reduction in casein micelle size that generally accompanies an increase in the ratio of κ -casein to total casein (Dalglish *et al.*, 1989; Umeda & Aoki, 2002);
- the relatively high hydrophobicity of the *para*- κ -casein, which would enhance the aggregation of the rennet-altered micelles.

However, it is noteworthy that while an increase in the proportion of κ -casein to total casein has been found to enhance the rennet gelation characteristics of milk, it has been found to give a non-significant decrease in the yield of laboratory-scale cheeses (Wedholm *et al.*, 2006); this contrasts with the results of studies on the influence of genetic variants of κ -casein and β -Lg, which show that the B-alleles of these proteins have, in addition to other factors (casein micelle size), higher levels of κ -casein as a percentage of total casein. While a higher κ -casein, as a percentage of total casein, may coincide with a higher loss of caseinomacropptide, care must be taken when interpreting results on cheese yield as affected by any parameter, owing to the confounding effects of indirect variables (e.g. variation in firmness of gel at cut, moisture content of curd).

The genetic variant of κ -casein has a major influence on cheesemaking properties of milk, with κ -casein BB variant giving superior rennet coagulation characteristics, fat recovery from milk to cheese and cheese yield capacity compared to milk having the κ -casein AB, which in turn is superior to milk with the corresponding AA or AE genotypes (van den Berg *et al.*, 1992; Walsh *et al.*, 1995, 1998a; Ng-Kwai-Hang & Grosclaude, 2003; Wedholm *et al.*, 2006). Reported increases in moisture-adjusted cheese yield with the κ -casein BB variant, compared to κ -casein AA variant range from ~3 to 8%, depending on milk composition and cheese type. Generally, the κ -casein AB variant has been found to exhibit rennet coagulation and cheese-yielding characteristics that are intermediate between those of κ -casein AA and BB. The superior rennet coagulation and cheese-yielding characteristics of the κ -casein BB variant compared to the AA variant appear to be related to its higher casein content, higher level of κ -casein as a percentage of total casein, smaller micelles and lower negative charge. These properties are conducive to a higher degree of casein aggregation and a more compact arrangement of the *para*-casein micelles, which in turn favours more numerous intermicellar bonds during gel formation. Indeed, it has been shown using model rennet coagulation studies that for a given casein concentration, the curd firming rate of rennet-treated micelle suspensions was inversely proportional to the cube of the micelle diameter (Horne *et al.*, 1996).

In milk there is an inverse relationship between the concentrations of lactose and chloride, which is the basis of the test for Koestler number, to distinguish between normal and

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abnormal (e.g. mastitic) milks (Ferreiro *et al.*, 1980; Horvath *et al.*, 1980; Fox & McSweeney, 1998)

$$\text{Koestler number} = \frac{100 \times \text{Chloride (g 100 g}^{-1}\text{)}}{\text{Lactose (g 100 g}^{-1}\text{)}}$$

where a value of <2 is normal and >2.8 – 3.0 is abnormal. Mastitis increases the concentrations of Na^+ , K^+ , Cl^- ions but decreases the concentration of lactose in the milk, as a response to maintain osmotic pressure within the mammary gland system. While a high level of Cl^- (or Na^+ , K^+) *per se* probably has little direct negative impact on *para*-casein aggregation and curd formation, apart from giving a slight increase in ionic strength, its occurrence is indicative of high somatic cell count (SCC) (250 to $>400 \times 10^3$ and $>1000 \times 10^3$ cells mL^{-1} for subclinical and clinical mastitis, respectively). Elevated SCC results in a marked increase in γ -caseins, proteose-peptones and the ratio of soluble to micellar casein (Anderson & Andrews, 1977; Ali *et al.*, 1980a,b; Schaar 1985a; Saeman *et al.*, 1988). These changes ensue from hydrolysis of β - and α_{s2} -caseins by the elevated activity of plasmin (and probably other proteinases) in the milk; κ -casein is hydrolysed more slowly by plasmin than β - and α_{s2} -caseins. The ensuing decrease in the intact casein level reduces the degree of casein aggregation as reflected by a marked deterioration in rennet gelation properties, syneretic properties and cheese yield (Donnelly *et al.*, 1984; Okigbo, *et al.*, 1985a; Mitchell *et al.*, 1986; Politis & Ng-Kwai-Hang, 1988a–c; Barbano *et al.*, 1991; Barbano, 1994; Auldust *et al.*, 1996; Klei *et al.*, 1998). An increase in SCC from 1×10^5 to $>5 \times 10^5$ cells mL^{-1} typically results in a reduction of ~ 3 – 7% in the moisture-adjusted (to $37 \text{ g } 100 \text{ g}^{-1}$) yield of Cheddar cheese. However, it is noteworthy that the decrease is also relatively large ($\sim 0.4 \text{ kg Cheddar cheese } 100 \text{ kg}^{-1} \text{ milk}$) on increasing the SCC from 1×10^5 to 2×10^5 cell mL^{-1} , a range that would be considered relatively low for good quality bulk milk. Losses of fat and protein during Cheddar cheese manufacture increased, more or less linearly, by ~ 0.7 and $2.5 \text{ g } 100 \text{ g}^{-1}$, respectively, with SCC in the range 1×10^5 to 1×10^6 cells mL^{-1} (Politis & Ng-Kwai-Hang, 1988a,c).

1.4.3 Microbiology (total bacterial count)

Microbial contamination of milk can occur pre-milking as a consequence of animal infection or during, or post-milking as a consequence of direct contact with bacteria in the environment or milk handling equipment and/or, for example, milking machine, on-farm storage, transport. Directive 92/46 (EU, 1992), which became effective from 1 January 1994, contained animal health requirements for raw milk, hygiene requirements for registered holdings and hygiene requirements for milking, collection and transport of milk to collection centres. A package of new hygiene regulations was adopted in April 2004 by the European Parliament and the Council (Regulation 853/2004) (EU, 2004). These became applicable from January 2006, and in the case of milk and milk products, these replace Directive 92/46 (EU, 1992). The new regulations are binding in EU Member States without the necessity of national legislation to be enacted to implement their provisions. However, instead of all of the hygiene requirements being incorporated in a single piece of legislation, the requirements for the dairy sector are

contained in three main regulations. One specific Regulation 853/2004 (EU, 2004) lays down specific hygiene rules for food of animal origin, with Annex III (Section IX) containing specific requirements for raw milk and dairy products. Specifically, with regard to plate count standards, milk-processing operators must ensure that raw milk meets the following criteria:

- plate count at 30°C $\leq 100 \times 10^3$ colony forming units (cfu) mL⁻¹ for cows' milk, corresponding to the rolling geometric average over a 2-month period, with at least two samples per month;
- plate count at 30°C $\leq 150 \times 10^4$ cfu mL⁻¹ for milk from other species, corresponding to the rolling geometric average over a 2-month period, with at least two samples per month;
- plate count at 30°C $\leq 50 \times 10^4$ cfu mL⁻¹ for raw milk from species other than cows when to be used for manufacture of products using processes that do not involve pasteurisation, corresponding to the rolling geometric average over a 2-month period, with at least two samples per month.

1.4.4 *Sensory (appearance, colour, smell, and taste)*

Sensory analysis may be used to test the characteristics of milk and may be considered as part of the overall quality control of the product. The sensory attributes of appearance and aroma are important factors in determining the quality of milk. Factors that influence the sensory evaluation of cows' milk include cow health and feed and the absorption of foreign flavours after milking (Ishler & Roberts, 1991). Flavour defects that are chemically induced (rancidity – specific chemical flavour) cannot be removed or improved, and may become more pronounced on storage (Mounchili *et al.*, 2005). For example, off-flavours in milk may arise as a consequence of improper milking practices (inadequate removal of teat disinfectant prior to milking) and milk handling procedures (excessive agitation leading to free fat) and reduce consumer acceptability. Hence, milk with good sensory characteristics may be maintained by: (a) control of cow diet, (b) optimisation of milking practices, (c) milk handling/pumping procedures, (d) storage conditions and (e) minimisation of storage time prior to processing.

1.4.5 *Authenticity (non-adulteration with residues or other milks/milk fractions)*

Authenticity of milk may be detected by specific tests, often using advanced instrumentation and methods in specialised laboratories; for example, detection of cows' milk fat in ovine milk using differential scanning calorimetry or free fatty profile using gas chromatography. The current EU reference method for the detection of cows' milk in goat or sheep milks is based on the separation of the γ -casein peptides after digestion of the sample by plasmin (EU, 1996). Further examples of fraudulent addition of ingredients include water, whey proteins or non-dairy proteins (of plant or animal origin).

1.5 Factors affecting the quality of milk for cheese manufacture

The quality of milk for cheese manufacture is affected by five key parameters, namely composition, microbiology, SCC, enzymatic activity and levels of residues/contaminants.

1.5.1 Milk composition

Several studies have shown seasonal variations in composition of milk (Chapman & Burnett, 1972; Phelan *et al.*, 1982; Auld *et al.*, 1998; O'Brien *et al.*, 1999c,d). The gross composition of cheese milk, especially the concentrations of protein, casein and fat, has a major influence on several aspects of cheese manufacture, including rennet coagulability, gel strength, curd syneresis, cheese composition, yield and quality (Chapman, 1974; Grandison *et al.*, 1984; Fox & Guinee, 2000; Guinee *et al.*, 2006b; cf. Chapter 8).

Ceteris paribus, Cheddar cheese yield increases by $\sim 0.25\text{--}0.30$ kg 100 kg⁻¹ milk for every 0.1 g 100 g⁻¹ increase in milk protein in the range 3.0–4.5 g 100 g⁻¹ while retaining the protein-to-fat ratio constant at 0.96 (Guinee *et al.*, 1994, 1996, 2006), and by ~ 0.11 kg 100 kg⁻¹ milk for every 0.1 g 100 g⁻¹ increase in milk fat in the range 3.4–4.7 g 100 g⁻¹ while retaining the protein level constant at 3.7 g 100 g⁻¹ (Guinee *et al.*, 2007a). The importance of casein and fat to yield is reflected by the following general equation for the prediction of cheese yield:

$$Y = aF + bC$$

where Y is the yield (kg cheese 100 kg⁻¹ milk), F and C are the concentrations (g 100 g⁻¹) of milk fat (F) and casein (C) in the milk, a and b are coefficients, the magnitude of which depend on the contributions of fat and casein to yield. The values of a and b have been found to range from ~ 1.47 to 1.6 and from 1.44 to 1.9, respectively, for Cheddar cheese (Emmons, 1991). The relatively high contribution of casein is expected as it forms the continuous *para*-casein matrix, which, acting like a sponge, occludes the fat and moisture (serum) phases. Occluded moisture contributes directly to cheese yield and indirectly due to the presence of dissolved solids such as lactate and soluble salts. While fat on its own has little water-holding capacity, its presence in the *para*-casein matrix affects the degree of matrix contraction and hence moisture content and cheese yield. The occluded fat globules physically limit contraction, and hence aggregation, of the surrounding *para*-casein network and, therefore, reduce the extent of syneresis. Hence, as the fat content of the curd is increased, it becomes more difficult to expel moisture; consequently, the moisture-to-casein ratio generally increases unless the cheesemaking process is modified to enhance casein aggregation, for example, by reducing the firmness of gel at cut, reducing curd particle size, cooking more slowly and/or increasing the scald temperature (Gilles & Lawrence, 1985; Fenelon & Guinee, 1999). However, if the content of moisture-in-non-fat substances is maintained constant (e.g. by process modifications), fat contributes less than its own weight to cheese yield (~ 0.9 kg kg⁻¹), because $\sim 8\text{--}10$ g 100 g⁻¹ of the milk fat is normally lost in the cheese whey.

The levels of fat, protein and moisture in cheese are interdependent, with the levels of protein and fat decreasing pro rata as the moisture content increases (Fenelon & Guinee,

1999; Guinee *et al.*, 2006b). Reducing the protein-to-fat ratio of the cheese milk (by increasing fat content while retaining the protein level constant) leads to lower moisture and protein and higher levels of fat and fat-in-dry matter. The effects of increasing the protein content, for a given protein-to-fat ratio, on cheese composition can vary with the SOP applied during manufacture. In the absence of process intervention, it increases the moisture content of the cheese, where rennet is added to the milk on a casein load basis (kilogrammes of casein *per* unit volume) and cutting of the rennet-induced milk gel is performed on the basis of time (cf. Chapter 8), as is typical in large modern cheese factories with production capacities of 10–15 tonnes h⁻¹. The higher moisture coincides with an attenuated ability of the calcium phosphate *para*-casein curd matrix to rearrange and contract during cutting and the early stages of stirring/cooking of the curd particles in the whey, as a consequence of the higher gel firmness/stiffness at cutting. Conversely, when the gel is cut at a defined firmness, increasing the protein content of the cheese milk leads to a reduction in moisture content (Bush *et al.*, 1983; Guinee *et al.*, 1994, 1996; Broome, 1998a) of ~0.29 g 100 g⁻¹ *per* 0.1 g 100 g⁻¹ increase in milk protein (Guinee *et al.*, 2006). The latter effect probably resides in the concomitant increases in the ratio of protein to serum calcium and the collision frequency of curd particles in the cheese vat during stirring, because of a concomitant increase in the volume fraction of the curd in the cheese vat (cf. Chapter 8).

Variation in fat content of the raw milk is generally of little practical significance, as milk for cheese manufacture is easily standardised to a protein-to-fat ratio within a defined range (~0.85–0.90 for Cheddar cheese) as part of a SOP (cf. Chapter 8) by the appropriate removal of fat *via* mechanical separation (skimming) (for low-moisture, partly skimmed Mozzarella) or the addition of cream (for cream cheese). Similarly, the protein content, or more specifically the casein content, of raw milk has little effect when the protein content of the cheese milk is standardised to a defined level, e.g., by the low concentration factor (1–1.5×) ultrafiltration of the raw milk or by the addition of milk protein supplements. However, protein standardisation is not a universal practice, and consequently variation in milk protein levels can have significant effects on cheese composition, yield and quality (Banks & Tamime, 1987; Kefford *et al.*, 1995; Auldish *et al.*, 1996; Guinee *et al.*, 2007a). In such a situation, the following should assist in minimising variation in moisture content, and hence other compositional parameters:

- optimising firmness of gel at cutting, by using *ex-post* information on the relationship between gel firmness at cutting and moisture content for the particular cheese recipe; and
- standardising the levels of starter culture and rennet per unit weight of casein, and pH at different stages of manufacture (set, whey drainage, salting).

The calcium content of milk changes with stage of lactation and season. The mean concentration in milk from individual cows showed a marked decrease (from 150 to 155 mg 100 g⁻¹) during the first 16 days of lactation and an increase (from 115 to 170 mg 100 g⁻¹) after 300 days in lactation (DIL) (White & Davies 1958a); however, between the extremes of the lactation period, the calcium concentration typically fluctuates between 105 and 130 mg 100 g⁻¹ and shows little or no trend with stage of lactation. A similar trend for seasonal changes in total calcium occurs in manufacturing milks and milks from spring- and autumn-calved herds (White & Davies, 1958a; O'Brien *et al.*, 1999a). Likewise, the concentrations

of ionic and soluble unionised calcium vary between ~ 10 and $14 \text{ mg } 100 \text{ g}^{-1}$ (2.5–3.5 mM) and between ~ 20 and $34 \text{ mg } 100 \text{ g}^{-1}$ (4–8 mM), respectively, with stage of lactation and season (White & Davies, 1958b). Other factors have also been found to affect the concentrations of different forms of calcium, with the transition from stall to pasture grazing during Spring resulting in decreases in the concentrations of both total and ionic calcium, citrate and Mg^{2+} (Grimley *et al.*, 2008) and increases in Na^+ and casein. While little information is available on the direct effects of natural (seasonal) variation in calcium content of milk on its cheesemaking properties, available results suggest that calcium concentration is a factor in the pool of compositional-related parameters (e.g. level of intact casein, citrate, pH, casein micelle size, ionic strength) that interactively affect rennet gelation of milk and cheesemaking efficiency yield (Chapman & Burnett, 1972; Grandison *et al.*, 1984). Keogh *et al.* (1982) found that the content of colloidal calcium in spring-calved herd milks and bulked-herd manufacturing milks remained relatively constant between March and September and increased slightly ($65\text{--}70 \text{ mg } 100 \text{ g}^{-1}$) in October/November before returning to baseline values of $\sim 65 \text{ mg } 100 \text{ g}^{-1}$ (Keogh *et al.*, 1982).

The casein content of the same milks increased progressively from July ($\sim 2.4 \text{ g } 100 \text{ g}^{-1}$) to October/November ($3 \text{ g } 100 \text{ g}^{-1}$), and thereafter decreased (Phelan *et al.*, 1982). Further analyses of these data (Keogh *et al.*, 1982; Phelan *et al.*, 1982) indicate that as the proportional increase in casein from mid to late lactation is higher than that of calcium, the ratios of colloidal calcium and ionic calcium decrease from ~ 26 and $4.8 \text{ mg } \text{g}^{-1}$ casein in mid lactation (July) to ~ 23 and $3.9 \text{ mg } \text{g}^{-1}$ casein in late lactation (November). Similarly, the data of White and Davies (1958a) indicated a reduction in the ratios of ionic calcium and soluble unionised calcium to casein between mid and late lactation, but an increase in the ratio of colloidal calcium to casein. The reductions in the former ratios, amongst other factors (such as increase in milk pH and casein hydrolysis by plasmin and/or SCC proteinases), are likely to contribute to the deterioration in rennet coagulability and impaired curd syneretic properties frequently observed in manufacturing milks (cf. Sections 1.2 and 1.3), especially those from spring-calved herds, in late lactation (O’Keeffe 1984; O’Keeffe *et al.*, 1982). Increases in the levels of ionic ($10\text{--}14 \text{ mg } 100 \text{ g}^{-1}$) and soluble unionised ($19\text{--}28 \text{ mg } 100 \text{ g}^{-1}$) calcium in seasonal milk have coincided with reductions in rennet gelation time (White & Davies, 1958b). This trend is consistent with the results of experimental studies reporting an improvement in rennet gelation properties on the addition of CaCl_2 at the levels of $0\text{--}2 \text{ mM}$ to mid-lactation (Fig. 1.2) and late-lactation milks (Lucey & Fox, 1992). An investigation on the commercial manufacture of Swiss-type cheese showed that the addition of CaCl_2 (0.1 g L^{-1}) gave insignificant increases in the mean recoveries of milk fat (85.3 vs 84.7%) and non-fat milk solids (33.85 vs 33.75) and a significant increase in the mean cheese yield ($0.038 \text{ kg } 100 \text{ kg}^{-1}$) (Wolfschoon-Pombo, 1997). The proportion of large curd particles (i.e. $5.5\text{--}7.5 \text{ mm}$) increased while the proportion of small particles ($<3.5 \text{ mm}$) decreased on the addition of CaCl_2 . These trends suggest that the positive effects of CaCl_2 on recoveries and cheese yield probably ensue from the enhanced degree of casein aggregation, which reduces the susceptibility of the curd to fracturing during cutting and the initial phase of stirring (cf. Chapter 8).

Apart from variations in the levels of gross constituents, seasonal variation can also occur in the ‘quality’ of the protein in terms of its ability to form a gel with satisfactory curd firming and syneretic (wheying off) properties and to produce cheese curd of satisfactory

moisture content. Late-lactation milk generally gives poor rennet coagulability (low curd firmness), impaired curd syneresis, high moisture Cheddar cheese and lower recovery of milk fat to cheese (O’Keeffe, 1984; Banks & Tamime, 1987; Auldism *et al.*, 1996). These defects coincide with low levels ($<4.3 \text{ g } 100 \text{ g}^{-1}$) of milk lactose, low casein number (<72 , casein as a percentage of true protein) and increased levels of serum casein ($>40 \text{ g } 100 \text{ g}^{-1}$ total protein), which was non-sedimentable at $30\,000 \times \text{g}$ (O’Keeffe, 1984). In this context it is noteworthy that low lactose levels in milk generally coincide with high SCC and levels of plasmin activity (Somers *et al.*, 2003), and may be indicative of udder infection and increased excretion of blood constituents into the milk. Similarly, Lucey *et al.* (1992) found that late-lactation milk from spring-calved herds (258–280 DIL in October) had impaired rennet coagulability, and resulted in Mozzarella cheeses, which had a higher moisture level, were softer and had a lower apparent viscosity when melted compared to the corresponding cheeses from mid-lactation milk from an autumn-calved herd. In contrast, Kefford *et al.* (1995) reported no differences between the compositions of Cheddar cheeses made from early- or late-lactation milk; they also observed a higher recovery of milk fat to cheese with late-lactation milk compared to mid-lactation milk. Discrepancies between the above studies may be due to differences in diet, SCC and the definition of late-lactation milk, which for Irish studies (O’Keeffe, 1984; Lucey *et al.*, 1992) typically refer to milk from cows >250 DIL compared to ~ 200 – 220 DIL in Australia (Kefford *et al.*, 1995; Auldism *et al.*, 1996) and New Zealand (Auldism *et al.*, 1998; Nicholas *et al.*, 2002). O’Keeffe (1984) found that the extent of these cheesemaking defects in late-lactation spring-calved herds in Ireland was accentuated when both the plane of nutrition of the cow and the milk yield at drying off were low (e.g. high stocking density on pasture in October and November without dietary supplementation, and $<6 \text{ L}$ of milk $\text{cow}^{-1} \text{ day}^{-1}$). Hence, Guinee *et al.* (2007a) reported satisfactory composition and functionality of low moisture Mozzarella cheese made from late-lactation milk (266–284 DIL) from spring-calved cows maintained on a high plane of nutrition and with a high milk yield ($>6 \text{ L}$ of milk $\text{cow}^{-1} \text{ day}^{-1}$).

Effect of cow nutrition on milk composition

One alternative to influence the manufacturing potential of milk is through the nutrition of the cow, but the response may vary depending on the stage of lactation.

- *Early lactation:* In pasture-fed systems, calving date is targeted to commence with the start of the grass herbage-growing season. The objective of the system is to allow grazed grass herbage to make up as large a contribution as possible to the total diet of the cow. Recommendations over a number of years have been to allow cows out to pasture from mid to late February when the soil conditions allow (firm under foot) and herbage mass is sufficient, i.e. from a milk production viewpoint (Dillon *et al.*, 1995). However, supplementing spring-calved cows on grass silage and concentrates with grazed grass in late February to late April (by allowing cows on pasture for 2–4 hours day^{-1}) was also found to significantly improve the gelation properties of milk (Dillon *et al.*, 2002), an effect concomitant with numerical increases in protein concentration (3.06–3.17 $\text{g } 100 \text{ g}^{-1}$).

- *Mid lactation:* Increasing herbage allowance from 16 to 24 kg grass dry matter in mid lactation resulted in significant increases in both the yields and concentrations of total protein (3.2–3.4 g 100 g⁻¹), casein (2.43–2.61 g 100 g⁻¹) and lactose (4.60–4.65 g 100 g⁻¹). However, the concentrations of calcium and phosphorous, the rennet gelation properties or the alcohol stability of the milk was not affected (O'Brien *et al.*, 1997). In a complimentary study, Guinee *et al.* (1998) showed that increasing herbage allowance increased the moisture-adjusted yield of low-moisture Mozzarella cheese, but did not significantly affect the gross composition, rheological characteristics or cooking properties. An increase in milk casein of 0.1 g 100 g⁻¹ raised the yield of moisture-adjusted cheese by more than 0.5 kg 100 kg⁻¹ of milk. A similar trend was observed by Kefford *et al.* (1995) for Cheddar cheese. A further study (O'Brien *et al.*, 1999a) found that increasing stocking density above a standard limit (defined as post-grazing grass height of 60 mm) resulted in significant reductions in milk fat and protein yields, the concentrations of total protein (3.22 vs 3.40 g 100 g⁻¹), casein (2.48 vs 2.58 g 100 g⁻¹) and whey proteins and a deterioration in rennet coagulability. Imposing concentrate supplementation on the standard system increased the levels of total protein (3.40 vs 3.49 g 100 g⁻¹), casein (2.58 vs 2.65 g 100 g⁻¹) and whey protein but generally did not affect processing characteristics; alcohol stability was measured at alcohol levels of 76–79 g 100 mL⁻¹. It can be inferred from the Irish studies (O'Brien *et al.*, 1997a; Dillon *et al.*, 2002) that adequate herbage is a necessary requirement for quality milk and, if not available, concentrate supplementation of the grass diet is recommended in order to increase the energy supply to cows. However, in the presence of adequate grass, concentrate supplementation increases the concentrations of milk constituents but has little effect on milk processability characteristics (rennet coagulation, alcohol stability).
- *Late lactation:* O'Brien *et al.* (2006) and Guinee *et al.* (2007a) found that good management of spring-calved cows close to the end of lactation (261–307 DIL) gave good milk composition (lactose \geq 4.3 g 100 g⁻¹, protein 3.6 g 100 g⁻¹, casein 2.8 g 100 g⁻¹), rennet gelation (Formagraph Type 1170, Foss Electric, Denmark; curd firmness, A60 = 42.1 mm at 60 min) (Auldust *et al.*, 2001) and Mozzarella cheesemaking properties. These practices included maintenance of milk yield at >6 kg cow day⁻¹ and supplementation of pasture and/or silage with concentrates.

Effect of stage of lactation on milk composition

Milk from cows in late lactation has been found to have lower casein as a percentage of true protein and a higher level of FFA than milk from cows in early lactation (Sapru *et al.*, 1997). In the same study, cheese manufactured from late-lactation milk had higher moisture content, a trend also reported by Broome *et al.* (1998a). Stage of lactation also affected cheese pH and degradation of α_{s1} -casein in cheese during ageing. Late-lactation milk has also been found to give lower recoveries of fat and protein from milk to cheese (Auldust *et al.*, 1996; Sapru *et al.*, 1997). Furthermore, Auldust *et al.* (1996) found adverse effects of a high SCC milk on the yield and quality of Cheddar cheese in late lactation, and concluded that the effect of stage of lactation was magnified by an elevated bulk milk SCC and that many of the problems encountered when processing late season milk could be overcome by controlling mastitis at this time.

The generally detrimental impact of late lactation on cheesemaking quality of milk may be reduced by maintaining a high plane of nutrition in combination with application of a strict cow drying-off policy, i.e. ceasing to milk individual cows when milk yields decrease below 8–9 kg day⁻¹ or drying-off herds at average yields of 10–11 kg day⁻¹ (Guinee *et al.*, 2007a; O'Brien, 2008). This practice would eliminate extremely late-lactation milk from the product manufacturing process, and assist in retaining the characteristics (gel formation and gel syneresis) required for satisfactory manufacture of Mozzarella cheese into late lactation (276 DIL).

Effect of genetic variants of milk proteins on composition

All the major proteins in milk (α_s -, β -, κ -caseins, β -Lg, α -La) exhibit genetic polymorphism (Ng-Kwai-Hang & Grosclaude, 2003). The genetic variants, which have been investigated most thoroughly for their effects on the rennet coagulation and cheesemaking characteristics of milk, are those of κ -casein and β -Lg. Compared to the AA variants, the BB genotypes of both κ -casein and β -Lg are generally associated with a higher concentration of casein and superior rennet coagulation properties, as reflected by higher curd firming rates and gel firmness after a given renneting time (Schaar, 1985b; Green & Grandison, 1993; Walsh *et al.*, 1998a,b). The BB variants of κ -casein and β -Lg have also been associated with superior cheesemaking properties, as reflected by the higher recovery of fat, a lower level of curd fines in cheese whey, and higher actual and moisture-adjusted cheese yields for a range of varieties, including Cheddar, Svecia, Parmigiano-Reggiano, Edam and Gouda, low-moisture Mozzarella and Camembert (Aleandri *et al.*, 1990; van den Berg *et al.*, 1992; Walsh *et al.*, 1998a,b; Ng-Kwai-Hang & Grosclaude, 2003). Reported increases in moisture-adjusted cheese yield with the κ -casein BB range from ~3 to 8%, depending on milk composition and cheese type. The superior milk gelation and cheese-yielding capacity of κ -casein BB milks compared to AA milks are probably associated with the higher levels of casein, ratio of κ -casein to other casein and of casein to whey protein, smaller casein micelle size, lower negative charge, and possible alteration in the interactivity of the caseins (due to the amino acid substitutions). It is also noteworthy that the κ -casein B allele induced higher levels of non-glycosylated κ -casein than the corresponding A, C or E alleles (Lodes *et al.*, 1996) and that a lower level of glycosylation of κ -casein is associated with smaller, more hydrophobic micelles (O'Connell & Fox, 2000). The latter factors are expected to favour firmer rennet gels because of a more rapid hydrolysis of κ -casein by chymosin (Dziuba & Minkiewicz, 1996) and a more close pack arrangement (and aggregation between) of *para*-casein micelles forming the basic building blocks (*para*-casein aggregates) of the gel matrix. The generally higher casein content of milk containing the κ -casein BB compared to the AA variant also contributes to superior rennet coagulation and cheese-yielding properties. Generally, the κ -casein AB variant has been found to exhibit rennet coagulation and cheese-yielding characteristics which are intermediate between those of κ -casein AA and BB.

The different genotypes of β -Lg have also been found to be important in cheese manufacture, even though it does not have a direct role *per se* in the formation of rennet-curd cheeses. Milk containing the β -Lg BB produced firmer curds than that containing the AA or AB variants (Marziali & Ng-Kwai-Hang, 1986). Similarly, Schaar *et al.* (1985) reported a higher cheese yield (9.25 vs 8.94 kg 100 kg⁻¹) and dry matter content (53.1 vs 50.8 g

100 g⁻¹) from milk with β -Lg BB than from milk with β -Lg AA. Thus, there appears to be potential for the cheese industry to include selection of milk protein genotypes in breeding programmes. Auldrist *et al.* (2002) examined composition and rennet gelation characteristics of milk from conventional dairy breeds (Holstein-Friesian) and dual-purpose breeds (Montbelliard and Normandy) under Irish pasture grazing conditions. Higher frequency of the κ -casein B variant for both the latter breeds was associated with higher concentrations of protein and casein (3.49 vs 3.20 g 100 g⁻¹ and 2.77 vs 2.50 g 100 g⁻¹, respectively), smaller casein micelle size (151 vs 158 nm diameter) and improved rennet gelation characteristics (48.0 vs 35.0 mm curd firmness, A30).

Effect of season on milk composition

The influence of season on milk quality normally relates to changes in climate, lactation and natural feed. In general, environmental factors affect the protein content of milk in the same way as fat content, but less markedly. Protein content of bovine milk tends to be higher in winter than in summer.

Effect of parity (lactation number) on composition

It is unlikely that age of the cow is an important factor influencing cheese-yielding capacity of bulk milk since herds usually include cows of varying ages. A Canadian study (Ng-Kwai-Hang *et al.*, 1987) showed that the concentrations of total protein and serum protein increased slightly from 2 to 3 years of age, whereas casein content remained unchanged. After 3 years, the casein level decreased and serum protein remained the same. Maintaining a young herd is generally considered important in optimising milk composition. Sheldrake *et al.* (1983) reported that there was little change in SCC with parity if the cow remained free of infection between lactations. However, Schutz *et al.* (1990) reported that SCC increased as parity increased. Fuerst-Waltl *et al.* (2004) found that the relationship between cow age and SCC was inconsistent, while Valde *et al.* (2004), Carlén *et al.* (2004) and Walsh *et al.* (2007) all observed an increase in SCC with parity. While parity may affect milk quality directly from milk component concentration or indirectly through milk SCC, it is likely that parity has a relatively minor influence compared to cow nutrition or the presence of contagious/environment mastitis pathogens.

Effect of milking frequency on milk composition

Once-a-day (OAD) milking may be used as a labour-saving technique on farms. Stelwagen and Lacy-Hulbert (1996) suggested that OAD milking may initiate changes in the permeability of the mammary gland through impairment of tight junctions between the alveolar cells, leading to changes in milk composition through increased influx of serum proteins and ions and increased efflux of lactose and potassium. A study by Kelly *et al.* (1998) reported reduced lactose, elevated plasmin concentrations and increased SCC levels with OAD milking. O'Brien *et al.* (2005) reported that the fat and protein contents of OAD milk (4.41 and 3.65 g 100 g⁻¹) were significantly higher than those of twice-a-day (TAD) milk (4.09 and 3.38 g 100 g⁻¹). However, the total yield of milk solids (fat + protein yield) was reduced

due to the significant decrease in milk volume associated with OAD milking. Milk SCC was not significantly affected by milking frequency; only cows with $SCC < 250 \times 10^3$ cells mL^{-1} had been selected for trial. Gel strength for the OAD milk was significantly higher than that of the TAD milk (105 vs 85 Pa), an effect attributed to the higher casein content of the OAD milk. Overall, OAD milking increased concentrations of protein, casein and fat, improved the gelation properties of milk and did not affect the SCC or level of plasmin activity in milk, provided that cows had a good nutritional status and an acceptable udder health history.

1.5.2 *Microbial activity of milk*

Microbial contamination of milk is important from two perspectives: (a) public health, as discussed above (see Section 1.4), and (b) dairy product manufacture.

Hygienic milk production on-farm

The hygienic production of milk is critically important to dairy product manufacture from two perspectives: efficiency of manufacture and quality of product; for example, poor hygiene leads to high counts of somatic cells and bacteria, which enhance undesirable protein hydrolysis and loss of yield (see Sections 1.4.2, 1.5.1 and 1.5.3). In accordance with legislation and driven by demands for efficient production of high-quality products, processors are now increasing their demands on the quality of raw milk produced on-farm (Vissers, 2007; Vissers & Driehuis, 2009). Consequently, they are increasingly developing and implementing schemes to accommodate differential payment for milk according to its quality. Legal requirements for safety, quality and hygienic production conditions are currently enforced under European Directive 92/46 (EU, 1992) and Regulation 853/2004 (EU, 2004) (Annex III, Section IX). This legislation specifies that raw milk must come from healthy animals and that equipment and conditions under which it is produced must fulfil certain minimum requirements. The milk itself must also satisfy specified hygienic standards in terms of bacterial numbers present, for example the plate count at 30°C for raw cows' milk is $\leq 100 \times 10^3$ cells mL^{-1} , corresponding to the rolling geometric average over a 2-month period, with at least two samples per month.

Milk is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources: from within the udder, the exterior of the udder (cow environment) and the surface of milk handling and storage equipment. The health and hygiene of the cow, the environment in which the cow is housed and milked, the procedures used in cleaning and sanitising the milking and storage equipment, and the temperature and length of time of storage are all key factors in influencing the level of microbial contamination of raw milk.

Microbial contamination from within the mammary gland

Microbial contamination from within the udder of healthy animals is not considered to contribute significantly to the total numbers of bacteria in milk. However, a cow with mastitis has

the potential to shed large numbers of bacteria into the milk supply, for example *Staphylococcus aureus*, which is commonly responsible for mastitis in cows. If bacteria penetrate the teat canal and proliferate and induce an inflammation process in the mammary gland, mastitis develops. A number of factors predisposing the cow to mastitis include an impaired teat canal defence mechanism, an unhygienic cow environment, poorly maintained or malfunctioning milking machine facility and transfer of bacteria from affected cows to unaffected cows. The influence of mastitis on the total bacterial count of bulk milk depends on the strain of the infecting bacteria, the stage of infection and the percentage of the herd infected. Infected cows have the potential to shed in excess of 1×10^7 cfu mL⁻¹.

Microbial contamination from outside of the mammary gland

Contamination outside the udder can originate from two main sources, namely the environment of the cow and milk contact surfaces. Potential for microbial contamination of milk during the on-farm production process is present in the general environment. Microbes may be transferred to milk through the medium of feed, faeces, bedding material and soil, and, if not removed prior to milking, are washed into the milk during milking. The influence of dirty cows on total bacterial count in milk depends on the extent of soiling of the teat surface and the teat cleaning procedures used immediately before milking. Milking heavily soiled cows could potentially result in bulk milk counts exceeding 1×10^4 cfu mL⁻¹. For example, contamination of milk by unclean teats can potentially contaminate the milk with heat-resistant bacterial spores, which are problematic for the dairy industry, especially in the manufacture of milk powders where these organisms survive pasteurisation and grow during evaporation. The presence of *Bacillus cereus* is a limiting factor for the potential shelf life of pasteurised dairy products (te Giffel *et al.*, 1997), and may be a potential food poisoning agent. *B. cereus* is commonly found in soil and may be frequently found in milk during the grazing season when the risk of teat contamination with soil is greatest (Slaghuis *et al.*, 1997; Christiansson *et al.*, 1999). Also, spore-forming bacteria of the clostridium species (*Clostridium tyrobutyricum*) can cause problems with late gas-blowing development in some types of cheese (cf. Chapter 8). The main source of clostridia in milk is feeding of poor-quality silage (Stadhouders & Spoelstra, 1990). Spores can then be found in faeces of the animals consuming the silage and are transferred to the milk via the teat (Stadhouders & Jorgensen, 1990; Herlin & Christiansson, 1993).

A further source of microorganisms in milk and frequently the principal cause of consistently high bacterial counts is the build-up of contaminated deposits within the milking machine. Milk residue left on equipment contact surfaces supports the growth of a variety of bacteria (*Micrococcus*, *Streptococcus* and *Bacillus* spp.) (Bramley & McKinnon, 1990). Except in very cold and dry weather, bacteria can multiply on these surfaces during the interval between milkings. This risk can only be corrected by an appropriate machine washing routine. This is particularly relevant for thermophilic bacteria, which may be removed with hot water. Insufficient cleaning may result in persistent growth of thermophilic bacteria on surfaces (Vissers, 2007; Vissers & Driehuis, 2009). During the next milking, adhered microorganisms may be released into the milk. Hence, thorough cleaning of all surfaces

in contact with milk, including the bulk tank, is essential in order to minimise bacterial contamination and growth.

Milk storage conditions

Refrigerated storage of milk is conducive to the growth of psychrotrophic bacteria. These bacteria typically come from the cows' environment, such as dirt and manure. The extent to which the bacterial count increases in milk during storage depends on both the temperature and duration of storage as well as the numbers and types of bacteria present in the milk. The total bacterial count of milk at the end of a refrigerated storage period on-farm is also influenced by the initial count of that milk. When milk is stored at 4°C, one and two doublings of bacterial growth occur after 2 and 3 days of storage, respectively (O'Brien, 2008). For example, in situations of non-hygienic milk production, the initial bacterial count when milk enters the bulk tank may be high (20×10^3 cfu mL⁻¹) and lead to a very large bacterial count (40×10^3 – 120×10^3 cfu mL⁻¹) after 2–3 days at refrigerated temperature (O'Brien, 2008). Hence, refrigerated cooling is not a substitute for unhygienic milk practices. Efficient cooling of milk to 4°C immediately after production in conjunction with good milking hygiene makes it possible to maintain good quality milk for up to 2–3 days on the farm, provided that the milk container/tank is well insulated.

Psychrotrophic bacteria are often associated with poorly cleaned refrigerated farm bulk tanks (Thomas *et al.*, 1966; MacKenzie, 1973; Murphy & Boor, 2000). The longer the period of refrigerated storage of raw milk prior to processing, the greater is the chance that psychrotrophic bacteria increase in number. While milk produced under ideal conditions may have an initial psychrotrophic bacterial population of <10% of the total bacteria, psychrotrophic bacteria become the dominant microflora after 2–3 days at ~4°C (Gehringer, 1980).

The temperature of the large volumes of milk in road tankers (used to transfer the milk from the farm to factory) is unlikely to rise significantly during transport. Milk collected daily from the farm and having a mean initial psychrotrophic bacteria count of 1×10^4 cfu mL⁻¹ (on arrival at the factory) showed an increase to over 1×10^6 cfu mL⁻¹ on storage for 3 days at 5°C at the factory (Cousins & Bramley, 1984). Bacterial contamination of milk is likely to occur during collection and transport as a result of contact with transport tankers, hoses, pumps, metres and automatic samplers. Although the extent of contamination is difficult to assess, milk collection/transport is likely to augment the bacterial content of milk being transferred to bulk storage at processing plants. Heat treatment of milk (thermisation, pasteurisation) at the dairy may destroy the psychrotrophic bacteria (cf. Chapter 8), but not necessarily the products of their metabolism (FFA) or their enzymes that can adversely affect rennet coagulation properties of the milk, cheese yield and quality (cf. Chapter 8). Psychrotrophic bacteria commonly produce extracellular enzymes capable of hydrolysing proteins and fats of milk and milk products. Thus, they can increase the likelihood of off-flavours and odours and cause changes in body, texture and colour. Weatherup and Mullen (1993) indicated that storage of milk at 3°C for periods of 3 or more days resulted in a significant reduction in cheese yield, with a considerable loss in revenue to the cheesemaker. The latter also found that cheese manufactured from stored milk gave a significant reduction in quality, with the results being more pronounced after 5 days of storage.

1.5.3 Somatic cell count

The influence of somatic cells and mastitis on the composition of milk and its suitability for cheese manufacture has been studied extensively. Somatic cells are of three main types, namely lymphocytes (L), phagocytes and mammary gland epithelial cells (E) (Burvenich *et al.*, 1995). Lymphocytes function in humoral and cell-mediated immunity, while phagocytes, of which there are two types – polymorphonuclear leucocytes (PMN) and macrophages (M ϕ), ingest and kill pathogenic microorganisms, which invade the mammary gland. Somatic cells are present at low levels ($<100 \times 10^3$ cells mL $^{-1}$) in normal milk from healthy animals during mid lactation, with M ϕ , L, PMN and E cells typically at a ratio of $\sim 2.1:1.0:0.4:0.2$, respectively. It is generally agreed that somatic cells are released from the blood to combat udder infection, and thereby prevent or reduce inflammation (mastitis). Factors that contribute to increases in SCC of bulk manufacturing milk include subclinical mastitis, advance in stage of lactation, lactation number, stress and poor nutrition. During clinical mastitis, there is a rapid increase in SCC primarily due to PMN. Depending on the type and extent of bacterial infection, milk from infected quarters of the udder may have an SCC of 200×10^3 to 5000×10^3 cells mL $^{-1}$. However, the milk from animals suffering from clinical mastitis is excluded from the commercial milk supply. Such milk frequently forms clots within the udder, formed from a mixture of somatic cells and precipitated milk proteins; in severe mastitis, these clots block the drainage ductules and ducts in the mammary gland, thereby preventing milk drainage. The initial stage of mastitic infection is subclinical, with inflammation so slight that it is not detectable by visual examination. Hence, the milk from cows suffering from subclinical mastitis becomes part of bulk herd milk and bulk manufacturing milk, unless individual cows are tested routinely at farm level for subclinical mastitis (by monitoring SCC), which is not routinely conducted. While bulking dilutes such as milk, subclinical mastitis may contribute to an increased SCC of manufacturing milk, and thereby impact negatively on the suitability of milk for cheese manufacture.

Increasing SCC in milk is associated with marked changes in both the concentrations of milk constituents, the state (degree of hydrolysis) of the milk components and the cheese-making properties (Kosikowski & Mistry, 1988; Klei *et al.*, 1998; Cooney *et al.*, 2000; Kalit *et al.*, 2002; Franceschi *et al.*, 2003; Jaeggi *et al.*, 2003; Albenzio *et al.*, 2004; Mazal *et al.*, 2007). An increase in SCC in the range 100×10^3 to 1000×10^3 cells mL $^{-1}$ has generally been found to:

- reduce lactose, fat and casein contents in milk, casein as a percentage of true protein, gel firmness, recoveries of protein from milk to cheese, and cheese yield; and
- increase milk pH, levels of chloride, whey protein, and non-protein nitrogen in milk, curd fines in cheese whey, cheese moisture, rates of primary and secondary proteolysis during maturation (as monitored by urea polyacrylamide gel electrophoresis, levels of water-soluble and trichloroacetic acid-soluble nitrogen).

Increasing SCC in the range 1×10^5 to 6×10^5 cells mL $^{-1}$ resulted in an increase in rennet coagulation time and reductions in curd-firming rate (reciprocal of k_{20} , as measured using Formagraph Type 1170) and curd firmness (Politis & Ng-Kwai-Hang, 1988b). Fat and protein losses during Cheddar cheese manufacture increased, more or less linearly, by ~ 0.7

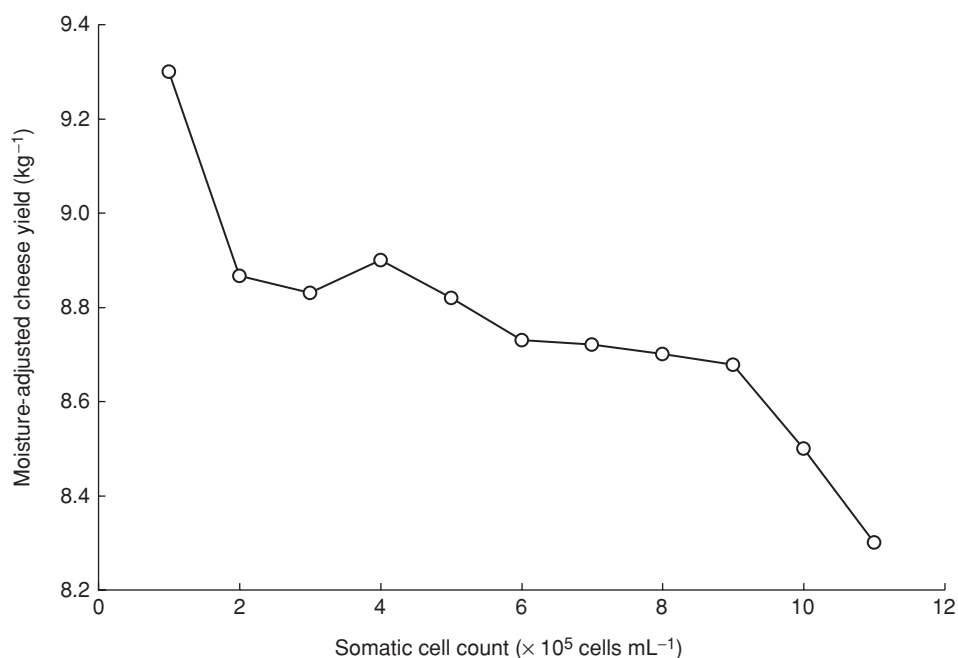


Fig. 1.4 Influence of somatic cell count on the moisture-adjusted (to 37 g kg^{-1}) Cheddar cheese yield from milks from individual cows. (Redrawn from Politis & Ng-Kwai-Hang, 1988a.)

and 2.5%, respectively, with SCC in the range 10^5 – 10^6 cells mL^{-1} (Politis & Ng-Kwai-Hang, 1988a,c). The increase in SCC from 1×10^5 to 6×10^5 cells mL^{-1} resulted in an approximate 6% reduction in moisture-adjusted (to 37.0 g 100 g^{-1}) Cheddar cheese yield (Fig. 1.4). It is noteworthy that there was also a relatively large decrease in yield (i.e. $\sim 0.4 \text{ kg } 100 \text{ kg}^{-1}$ milk) on increasing the SCC from 1×10^5 to 2×10^5 cells mL^{-1} , a range which would be considered relatively low for bulk milk of good quality. Hence, Barbano *et al.* (1991) concluded that any increase in SCC to values greater than 100×10^3 cells mL^{-1} for bulk milk herd will have a negative impact on cheese yield efficiency when milk from all the contributing herds had similar SCC. Auld *et al.* (1998) found that an increase in SCC from $>3 \times 10^5$ to $>5 \times 10^5$ cells mL^{-1} in late lactation (220 DIL) resulted in a 9.3% decrease in moisture-adjusted (to 35.5 g 100 g^{-1}) yield of Cheddar cheese and decreases in the recovery of fat (from 90.1 to 86.6 g 100 g^{-1} fat) and protein (from 78.3 to 74.4 g 100 g^{-1} protein). Significant decreases have also been reported in the yield of uncreamed Cottage cheese, with a 4.3% reduction in the percentage yield efficiency on increasing the mean SCC from 83×10^3 to 872×10^3 cells mL^{-1} (Klei *et al.*, 1998).

The negative impact of SCC on yield and recoveries are due in large part to the increase in proteolysis of α_s - and β -caseins to products (γ -caseins, proteose-peptones and other peptides) that are soluble in the serum and are not recovered in the cheese. Such proteolysis ensues from the elevated proteolytic activity of plasmin (and probably other proteinases), plasminogen, plasminogen activator in the milk that parallels increasing SCC (Mijacevic *et al.*, 1993; Rogers & Mitchell, 1994; Gilmore *et al.*, 1995; Kennedy & Kelly, 1997). Moreover, the lower effective concentration of gel-forming protein results in a slower curd-firming

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rate, and hence a lower degree of casein–casein interaction in the gel following cutting (at a given firmness) and during the early stage of stirring. A gel with the latter characteristics exhibits:

- a greater susceptibility to shattering during cutting and the early stages of stirring, resulting in higher losses of curd fines and milk fat; and
- an impaired syneretic capacity, with a consequent increase in moisture level.

A high SCC may also inhibit the activity of some strains of lactococci during cheese manufacture, an effect expected to further impair curd firming rate and reduce firmness at cutting. In commercial practice, the gel is generally not cut on the basis of firmness, but rather on the basis of a preset renneting time, which gives curd firmness within the acceptable range for normal milk. In large modern factories, the conditions are not conducive to testing curd firmness of cheese vats from separate milk silos because of the large scale of operation (frequently $>1 \times 10^6$ L day⁻¹) and the use of pre-programmed vats with limited operator access. In such operations, the effects of increases in SCC may be accentuated, as the slower-than-normal curd firming rate is conducive to lower-than-optimum firmness at cutting.

In conclusion, high SCC is detrimental to cheese yield and cheesemaking profitability. It is estimated that the monetary loss resulting from a 2% reduction in cheese yield on increasing the SCC from 1×10^5 to 5×10^5 cells mL⁻¹ would be $\sim\text{€}4000$ day⁻¹ for a Cheddar cheese plant processing 1×10^6 L of milk day⁻¹ (at a fresh curd value of $\sim\text{€}2.0$ kg⁻¹). Consequently, a concerted effort is being undertaken to reduce SCC through the use of good on-farm practices, for example reducing the percentage of animals in herds with subclinical mastitis, meeting regulations and the introduction of payment incentives for lower SCC. The EU has set the legislative limit of $\leq 400 \times 10^3$ SCC mL⁻¹ as the value above which milk cannot be sold by producers or used for further processing (EU, 2004). The permitted limit count varies internationally, for example $\leq 400 \times 10^3$ cells mL⁻¹ in New Zealand and $\leq 750 \times 10^3$ cells mL⁻¹ in the United States. However, it is noteworthy that Hamann (2003) suggests that milk constituents ‘abandon their physiological ranges’ at SCC $>100 \times 10^3$ cells mL⁻¹.

1.5.4 *Enzymatic activity of milk*

Milk enzymes are proteins that have biological functions and originate from a number of sources, for example milk itself, bacterial contamination and somatic cells present in milk. In the context of cheese manufacture, proteinase and lipase enzymes can have significant effects on cheesemaking properties, yield and quality.

Proteolytic activity

Native milk contains proteinases from a number of sources, the indigenous milk trypsin-like proteinase, plasmin proteinase (EC 3.4.21.7), lysosomal proteinases of somatic cells and bacterial proteinases of bacteria (especially psychrotrophic bacteria, such as *Pseudomonas*

spp. or *Bacillus* spp.). These proteinase systems hydrolyse caseins, are complex in their regulation and vary in activity according to factors such as stage of lactation and mastitis status (Kelly *et al.*, 2006). Excessive proteolytic activity is undesirable as it hydrolyses caseins to water-soluble peptides that are lost in whey and not recovered during the manufacture of products such as casein or cheese. Moreover, hydrolysis alters the chemistry and interactivity of the remaining (recovered) protein and thus the techno-functionality of the resultant products, such as the ability of the resultant cheese to shred or grate, or the ability of casein to hydrate, form gels or impart structure/texture to products in which it is used as an ingredient (e.g. gluten substitute in bakery products, imitation cheese and processed cheese products).

Plasmin proteinase

The native proteinase system of milk comprises plasmin as the active enzyme, its zymogen (plasminogen) and enzyme activators/inhibitors (Verdi & Barbano, 1991; Bastian & Brown, 1996; Nielsen, 2002). While plasminogen, plasminogen activator and plasmin are all very heat stable (Lu & Nielsen, 1993; Bastian & Brown, 1996), the plasmin inhibitor is heat labile (Richardson, 1983). Plasmin and plasminogen in milk fully survive pasteurisation temperature at pH 6.8 (Dulley, 1972; Driessen and van der Waals, 1978; Richardson, 1983; Metwalli *et al.*, 1998). Plasmin is associated with the casein micelles and readily hydrolyses α_{s1} -, α_{s2} - and β -caseins, resulting in an increase in γ -caseins (Ali *et al.* 1980a,b; Le-Bars & Gripon, 1989; McSweeney *et al.*, 1993). κ -Casein can also undergo some degree of hydrolysis by plasmin but reports differ on the extent of hydrolysis, which may be due to environmental conditions or the concentrations of enzymes and substrates used (Grufferty & Fox, 1988).

Discrepancies exist between various studies in relation to the effects of plasmin on the cheesemaking properties of milk (Pearse *et al.*, 1986; Bastian *et al.*, 1991; Farkye and Fox, 1992; Mara *et al.*, 1998), which may be related to many factors such as method of assessment (based on indigenous plasmin or added plasmin), plasmin activity, variation in the storage of milk with added plasmin, degree of casein hydrolysis at rennet addition, the presence of varying degrees of bacterial proteinases, assay pH and manufacturing process (pH of curd at whey drainage). However, high levels of plasmin activity and corresponding proteolysis (>40–50% of total α_{s1} - and β -caseins), as affected by the addition of plasmin to milk, have generally been found to give longer rennet gelation times and markedly lower gel firmness (Grufferty & Fox, 1988; Mara *et al.*, 1998; Srinivasan & Lucey, 2002). The impaired rennet gelation characteristics coincide with a more porous open structured gel and less connectivity between the particles and clusters making up the gel matrix (Srinivasan & Lucey, 2002). Despite its adverse effects on rennet gelation, addition of plasmin to milk (1.2 Sigma units L^{-1} milk) and incubating for up to 48 h at 4°C prior to rennet addition had little effect on the composition, rheological or cooking properties of low-moisture, partly skimmed Mozzarella cheese (Somers *et al.*, 2002). This suggests that the adverse effects of high plasmin activity on gel structure, which may be considered as equivalent to a reduction in gel-forming protein, are by and large overcome by ongoing contraction and shrinkage of the gel matrix during the dehydration stages (cutting, stirring, whey removal) of manufacture. Farkye & Fox (1992) and Farkye & Landkammer (1992) added plasmin to milk for Cheddar cheese manufacture, resulting in levels in the experimental Cheddar that were 1.5–6

times that in the control cheese. Plasmin addition resulted in greater hydrolysis of β -casein and higher levels of γ -caseins and water-soluble N, but did not effect cheese composition. The organoleptic quality of the plasmin-enriched cheeses was judged superior to that of the controls and ripening was considerably accelerated; a plasmin level 3–4 times the indigenous value appeared to be optimal. O'Farrell *et al.* (2002) reported that the addition of plasmin (0.125 or 0.25 mg L⁻¹) to milk increased the rates of primary proteolysis, as measured by levels of pH 4.6-soluble N and urea-polyacrylamide gel electrophoresis, in the cheese. A similar effect was obtained on addition of 10–20% mastitic milk (with an SCC of $>1 \times 10^6$ cells mL⁻¹) to control milk, reflecting a high content of plasmin or plasminogen activators in mastitic milk. However, Kelly & O'Donnell (1998) reported that plasmin addition (6 mg L⁻¹) to milk (and incubation at 37°C for 6 h) for Quark manufacture resulted in higher moisture content, a lower level of protein and a reduced moisture-adjusted cheese yield.

The plasmin activity of milk is markedly affected by stage of lactation. Nicholas *et al.* (2002) found increases in plasminogen activity associated with advancing lactation. This was in agreement with the studies of Politis *et al.* (1989) and Bastian *et al.* (1991). Plasmin activity has also been shown to increase with advancing lactation (Donnelly & Barry, 1983; Gilmore *et al.*, 1995), but this is not consistent in all studies (Richardson, 1983), which may be due to variation in cows. Management practices such as nutritional status and milking frequency (Lacy-Hulbert *et al.*, 1999), udder health (Auldish & Hubble, 1998) and onset of involution (Politis *et al.*, 1989) may also contribute to inter-study discrepancy. Stelwagen *et al.* (1994) suggested that a likely mechanism for the increase in plasmin activity in late-lactation milk was by *para*-cellular leakage from the blood system, assisted by disruption of tight junctions between mammary epithelial cells. Those authors suggested a positive correlation between loosening of the mammary tight junctions and plasmin and plasminogen-derived activity in milk. This phenomenon is normally associated with reductions in milk yield and lactose level (Kelly *et al.* 1998). Nicholas *et al.* (2002) concluded that increased proteinase activity occurs in milk with advanced lactation because more of both plasmin and plasminogen enter milk rather than solely because of increased plasminogen activation. However, maintenance of cow nutritional level together with milk yield at the approach of lactation end can assist in significantly restricting proteolytic activity due to plasmin (O'Brien *et al.*, 2006).

Lysosomal proteinases of somatic cells

The lysosomes of somatic cells in milk are a significant source of proteinases, for example cathepsin D (Larsen *et al.*, 1996). Lysosomes of somatic cells also contain a number of serine proteinases (cathepsin B), which are also involved in the hydrolysis of proteins. The level of cathepsin D in milk is correlated significantly with SCC (O'Driscoll *et al.*, 1999), and the elevated activity derived from cathepsin D is due to an increased level of procathepsin D rather than mature cathepsin D (Larsen *et al.*, 2006). SCC of milk is an indicator of the intensity of the cellular immune defence in cows. When mastitis infection occurs, cellular damage at the site of this infection initiates chemical signals that attract white blood cells to the area of infection. Some of the white blood cells are transferred to milk and therefore the SCC of milk increases during mastitis. Many studies have shown different patterns of proteolytic activity between milk samples of low and high SCC (Le Roux *et al.*, 1995;

Larsen *et al.*, 2004). In consideration of a number of studies, Kelly *et al.* (2006) indicated that there is a consensus that proteolysis in low SCC milk is dominated by plasmin with a minor contribution by cathepsin D, while in milk of increasing SCC the relative significance of plasmin decreases and the activity of other enzymes (e.g. cathepsin D, procathepsin D) increases.

Leitner *et al.* (2006) examined the effects of four different pathogens frequently associated with the occurrence of subclinical mastitis (*S. aureus*, *Staphylococcus chromogenes*, *Escherichia coli* and *Staphylococcus dysgalactiae*) on quality of cheese milk. Infection with these pathogens increased SCC and increased proteolysis of casein. Regardless of pathogen type, the plasmin activity in milk from the infected glands increased twofold compared with that in milk from uninfected quarters. These changes coincided with increased rennet clotting time and lower curd firmness for the milk from infected glands, indicating that cheese milk quality was negatively affected by infection. These authors concluded that indices of casein proteolysis proved to be a much better prediction of cheese milk quality than SCC alone.

Milk produced from cows with mastitis or high SCC has different cheesemaking properties to that produced by cows free of mastitis (Barbano, 1994). An SCC standard of 400×10^3 cells mL^{-1} for bulk milk is adopted in European milk quality schemes, with many milk purchasers now applying bonus payments for milk with $\leq 200 \times 10^3$ cells mL^{-1} , and this has reduced the effects of mastitis and high SCC on product quality. However, Barbano *et al.* (1991) reported that milk SCC begins to affect product quality as the SCC increases above 100×10^3 cells mL^{-1} .

Increased SCC in milk coincides with an increase in the proteolytic activity (Politis & Ng-Kwai-Hang, 1988c; Mijacevic *et al.*, 1993; Rogers & Mitchell, 1994), which, as discussed earlier (see Sections 1.4.2 and 1.5.3), impacts negatively on cheese manufacture, including giving higher moisture cheese, and lower component recoveries and cheese yield. The increase in moisture content is undesirable as it can easily place the product outside of specification. Moreover, elevated cheese moisture often causes a reduction in curd firmness and fracture stress, an increase in stickiness, a deterioration in shredability and an alteration of cooking properties (a melted cheese with a liquid, 'soupy' consistency, a loss of stretchability) (Guinee, 2003; Guinee & Kilcawley, 2004). The defects associated with high SCC has thus forced processors to target low SCC milk supplies – hence the current trend in penalty or bonus payments for low SCC milk.

Proteinases from psychrotrophic bacteria

Although the refrigerated storage of raw milk is used to prolong shelf life and reduce spoilage by mesophilic bacteria, it favours the growth of psychrotrophic microorganisms, which produce heat-resistant extracellular enzymes such as proteinases and lipases (Ali *et al.*, 1980a–c; Cromie, 1992; Shah, 1994; Guinot-Thomas *et al.*, 1995; van den Berg *et al.*, 1996; Haryani *et al.*, 2003). These proteinases hydrolyse the caseins in milk, to a degree dependent on temperature (2–7°C) and duration of cold storage (Celestino *et al.*, 1996; Haryani *et al.*, 2003). The caseins are particularly susceptible to hydrolysis at low temperatures because of the solubilisation of CCP, lower degree of hydrophobic-induced casein interactions, loosening of the micelle structure and the solubilisation and dissociation

of all caseins, especially β -casein, into the serum phase (cf. Chapter 8, Fox, 1970; Dalgleish & Law, 1988, 1989; Roupas, 2001).

Hydrolysis of casein by psychrotrophic proteinases is undesirable because of the associated defects in milk quality (e.g. off flavours) and processability (lower protein recoveries), especially at high total counts of psychrotrophic bacteria (Shah, 1994). Hydrolysis of caseins in cold-stored milk has been found to result in increased rennet coagulation, reduced curd firmness, higher losses of protein in cheese whey, lower cheese yield and/or higher cheese moisture (McCaskey & Babel, 1966; Ali *et al.*, 1980c; Hicks *et al.*, 1982). The extent of these effects generally increased with storage temperature in the range 1–10°C and time, though the rate of change appeared highest in the first 24–48 h. Kumaresan *et al.* (2007) found that storage of raw milk at 2°C supported significantly lower growth, proteolytic and lipolytic activities of psychrotrophic bacteria and had better sensory qualities when compared to milk stored at 4 and 7°C for a period of up to 14 days. They concluded that raw milk should be stored at 2°C before processing to protect the nutritional and sensory qualities of raw milk. Conversely, very extensive hydrolysis as affected by prolonged storage at 20°C led to very high bacterial counts ($>10^7$ cfu mL⁻¹), extensive casein hydrolysis, very short gelation times, spontaneous gelation and marked losses in cheese yield (Ali *et al.*, 1980c); it is probable that a concomitant reduction in pH and proteolytic-induced release of sialic acid from the casein macropeptide region of the κ -casein (Zalazar *et al.*, 1993) accelerated such defects.

Lipolytic activity

The hydrolysis of triacylglycerols by lipases into mono- and diacylglycerols and FFA is commonly referred to as lipolysis. Inadvertent lipolysis in milk and cheeses can give off-flavours (rancid, soapy, bitter) and flavour inconsistency. Hence, it is undesirable in all cheeses, even in those where the make procedure is designed to promote hydrolysis by the addition of exogenous lipases/esterases and/or lipolytic cultures (Blue cheese) (see Section 1.2.4).

Most lipolysis in milk is caused by the native LPL enzyme (EC 3.1.1.3.4), which is normally present in milk (Olivecrona *et al.*, 2003). LPL is essentially completely inactivated by conventional pasteurisation treatment (72°C for 15 s) (Martin *et al.*, 2005), and it therefore makes little contribution to lipolysis in milk or cheese, unless the milk fat globule in the raw milk is physically damaged, allowing access of the LPL to the milk fat triacylglycerols (Deeth & Fitz-Gerald, 1976). In addition to the indigenous lipolytic activity, milk may contain lipase/esterase activities from contaminating bacteria (Shah, 1994; Celestino *et al.*, 1996; Ouattara *et al.*, 2004).

Lipolysis in milk can be broadly classified into two types, depending on the causative/activating factor, namely induced lipolysis and spontaneous lipolysis.

Induced lipolysis

This is defined as lipolysis promoted by both mechanical damage and temperature alterations of the milk (Deeth, 2006). The degree to which lipolysis occurs depends generally on the extent of contact or association between the enzyme and the fat. Thus, little or no lipolysis

occurs normally in fresh milk, because the access of the enzyme to the milk fat is denied by the presence of the intact native MFGM (see Section 1.2.4). However, damage to the MFGM or its replacement by a reformed membrane of caseins and whey proteins (during homogenisation) increases the susceptibility of the milk fat triacylglycerols to the lipolytic and esterolytic activities present in the milk. Such damage may be accelerated by subjecting the milk to mechanical processes and/or temperature cycling (cooling/reheating). Physical actions that promote mechanically induced lipolysis include agitation and pumping (especially with air incorporation), homogenisation, and freezing and thawing of milk (Deeth, 2006).

The method of milk agitation can influence the degree of lipolysis. With low-speed agitation, the fat globules coalesce, while under high-speed agitation the fat globules are dispersed and form much smaller globules similar to the effect of homogenisation (Deeth & Fitz-Gerald, 1977). While the extent of the globule membrane damage may be similar in both cases, the extent of the lipolysis resulting from the high-speed agitation is much greater because the surface area of the lipase accessible fat is greater (Deeth, 2006). Once induced by agitation, lipolysis proceeds rapidly for a short time, followed by no further accumulation of FFA. Downey (1980a) attributed this to the accumulation of FFA at the fat globule interface, and failure of the enzyme to desorb from the interface. However, if vigorous agitation is repeated, accumulated FFA are swept from the interface and formation of a new enzyme substrate complex leads to resumption of lipolysis until the interface again becomes blocked. The incorporation of air during agitation/pumping of milk results in significantly more lipolysis than agitation/pumping of milk without air inclusion.

Homogenisation of milk breaks down the fat globules into a smaller, uniform size and can result in very strong activation of lipolysis. The newly reformed membrane of caseins and whey proteins (see Section 1.2.4) is more permeable to lipase, and consequently, the fat is more vulnerable (Deeth & Fitz-Gerald, 1976). Lipolysis proceeds very quickly after homogenisation, and rancidity may be evident within 5–10 min. Ideally, milk should be pasteurised prior to, or immediately after, homogenisation to minimise the lipolysis as a result of LPL or other lipases/esterases.

Freezing and thawing disrupt the native MFGM and allow access to the fat by the lipase (Willart & Sjoström, 1966). The amount of disruption is increased by repeated freezing and thawing. Freezing by slow cooling causes more damage to the globules than fast cooling.

Temperature-activated lipolysis is induced by temperature cycling, which can occur at several stages on the farm and during milk collection and assembly at the factory. Milk as it leaves the cow is at $\sim 37^{\circ}\text{C}$. Kitchen and Aston (1970) suggested that maximum activation of LPL occurred at 30°C and marked decreases were observed at temperatures $>37^{\circ}\text{C}$ and $<12^{\circ}\text{C}$. However, change in temperature can also promote the development of lipolysis, for example cooling to 5°C followed by re-warming to $25\text{--}37^{\circ}\text{C}$ and re-cooling (Kon & Saito, 1997). A maximum degree of lipolysis occurs when milk is warmed to $\sim 30^{\circ}\text{C}$, followed by cooling to $<10^{\circ}\text{C}$ (Deeth & Fitz-Gerald, 1976; Kon & Saito, 1997). Temperature activation appears to be related to the release of an LPL-inhibitory component from the MFGM and an increase in the association between lipase(s) present in the milk with fat globules on heating to 30°C ; the decrease in lipolysis on heating cooled milk to temperatures $>37^{\circ}\text{C}$ may be associated with an inhibitory effect of skimmed milk components associated with the fat globule.

Spontaneous lipolysis

This is defined as lipolysis that develops in the milk of some cows during cold storage without being subjected to any physical or mechanical treatment. Lipolysis in these milks is initiated just by prompt cooling of the milk after removal from the cow. Milks from individual cows differ in their tendency to develop rancidity (Frankel & Tarassuk, 1955; Sundheim & Bengtsson-Olivecrona, 1987). This phenomenon is the least understood aspect of lipolysis. Susceptibility of this milk to produce elevated levels of FFA is highly variable and depends on biochemical changes in milk and several predisposing factors in the animal (Jellema, 1975). The main biochemical factors include the amount of lipase activity, the integrity of the MFGM and the balance of lipolysis activating and inhibiting factors (Deeth & Fitz-Gerald, 1975; Sundheim, 1988; Cartier & Chilliard, 1990). The major predisposing factors associated with spontaneous lipolysis in the cow are late stage of lactation (Chazal & Chilliard, 1986), poor quality feed (Jellema, 1980) and mastitis (Downey, 1980b).

Contribution of bacterial lipases to lipolysis

Modern farm and milk collection practices have resulted in milk being cooled rapidly to $<8^{\circ}\text{C}$ following milking and a relatively low frequency of milk collection from the farm, for example every 2 or 3 days. Moreover, cold milk is hauled over long distances and is often cold stored at the cheese plant for 1–3 days, depending on time of year and the manufacturing schedules; hence, milk can be cold stored for up 2–5 days prior to processing. Psychrotrophic bacteria grow during refrigerated storage of milk and produce lipase enzyme, which can have a major effect on the quality of products (Shah, 1994; Sorhaug & Stepaniak, 1997). These lipases are heat-stable enzymes and generally survive pasteurisation and ultra-high temperature treatments (Cogan, 1977; Shipe and Senyk, 1981). Even though the bacterial lipase is not inactivated by pasteurisation (unlike indigenous LPL), the psychrotrophic bacteria that produced them are destroyed. This has implications in that the bacterial lipase may be carried through to the manufactured cheese where they contribute to off-flavours (rancidity, soapiness, bitterness) during advanced maturation, especially when large populations ($>1 \times 10^6 - 1 \times 10^7$ cfu mL⁻¹) are present in the milk (Chapman *et al.*, 1976; Cousin & Marth, 1977; Law *et al.*, 1979).

Occurrence of lipolysis in the dairy industry and minimisation of the problem

Lipolysis in milk and milk products is a persistent concern in the dairy industry. The effect of agitation/pumping on the rate of lipolysis depends on the nature and severity of the mechanical treatment, temperature during activation and characteristics of the milk. The design, installation and operating characteristics of the milking machine can strongly influence mechanically induced lipolysis. The agitation or pumping of milk, particularly when incorporating air entrainment and when milk temperature is relatively high at $>30^{\circ}\text{C}$, are major predisposing factors to lipolysis. Milking equipment on the farm should be designed and maintained to minimise frothing, foaming or agitation, thereby reducing physical damage to the milk fat and the development of FFA. It is important that laminar flow conditions prevail in the milk line and that pumps do not run in a 'starved' condition so as to minimise the increase in FFA. The height of the milk line can be a significant factor, particularly in the presence of air leaks (O'Brien *et al.* 1998). In addition, the bulk tank design should promote

gentle movement and handling, thus minimising FFA development. Although rapid cooling of milk is important to inhibit lipolysis, re-warming and re-cooling are very conducive to lipolysis and this can occur normally twice *per* day as fresh milks at $\sim 35^{\circ}\text{C}$ are added to the bulk tank containing milk at 4°C , bringing the blend temperature to $\sim 15^{\circ}\text{C}$. This effect may be minimised if the bulk tank is capable of rapid cooling to 4°C . However, care must be taken to avoid freezing of the milk onto the tank surface when small volumes of milk are being cooled rapidly (e.g. in direct expansion tanks). The most effective means of reducing lipolysis due to psychrotrophic bacteria is firstly by hygienic milk production to reduce bacterial numbers and secondly by minimising milk storage time between milking and processing. The processing plant also has a responsibility in ensuring minimal agitation, cavitation effects and temperature changes in milk during collection/transport and distribution to storage silos and cheese vats, and in avoiding contact between homogenised and raw milks (Reuter, 1978).

A number of studies (Deeth & Fitz-Gerald, 1976; Sapru *et al.*, 1997; O'Brien *et al.*, 2006) have reported that milk from cows in late lactation has a higher FFA level than that from cows in early lactation. This may be due to changes in milk and MFGM integrity at that time or mechanical damage of the MFGM due to excessive mixing of air into relatively small volumes of milk, particularly at evening milkings, within a seasonal milk production system. Cow diet also impacts on lipolysis, with cows under nutritional stress producing milk with relatively high FFA levels (Jellema, 1980).

1.5.5 Chemical residues

The presence of chemical residues and contaminants in milk is of public health concern and a cause of economic loss in the dairy industry. Milk is quite susceptible to contamination for many reasons. A range of veterinary drugs including antibiotics are commonly administered to animals to combat various diseases, the most prevalent being mastitis. Furthermore, other sources of contaminants to milk include cleaning and disinfecting agents (trichloromethane (TCM), iodine) and compounded animal feeds (mycotoxins).

Antibiotics

Antimicrobial drugs are administered to treat bacterial infections or employed prophylactically to prevent spread of disease. All antimicrobial drugs administered to dairy cows enter the milk to a certain degree, and each drug is given a certain withdrawal period, during which time the concentration in the tissues declines and the drug is excreted by the animal. The most frequently and commonly used antimicrobials are antibiotics, employed to combat mastitis-causing pathogens. Other infectious diseases such as laminitis and respiratory diseases are also treated with antimicrobial agents, but are of relatively minor importance (Fisher *et al.*, 2003).

The occurrence of residues of antimicrobials in milk has both economical and technological impact on the dairy industry. Antimicrobial residues can lead to partial or complete inhibition of acid production by starter cultures, inadequate ripening and ageing of cheese and cause defects of flavour and texture of these products (Honkanen-Buzalski & Reybroeck, 1997). A general concern linked to the widespread usage of antimicrobials at farm level is the potential development of antibiotic-resistant pathogens. This may complicate human

treatment and possibly cause selection of antibiotic-resistant strains of bacteria in the gut. Further concern exists that sensitive individuals may exhibit allergic reactions to antibiotic residues (Lee *et al.*, 2001). A survey in the United States of America (USA) between 1993 and 1994 reported that ~6% of milk samples (~2495) tested positive for antibiotics (Anonymous, 2005). Since mastitis is quite a common disease within dairy herds, it is likely that a high incidence of antibiotic residues arises from the use of lactating and dry cow intramammary formulations. In addition, failure to discard the milk from such treated cows for the recommended period is the principal cause of antibiotic residues in milk. Contamination of milking equipment after milking a treated cow also causes antibiotic residues in milk. Antibiotic residues on the milking equipment can be avoided by milking treated cows last or by flushing contaminated parts of the equipment before it is used on subsequent cows. Thus, both the withdrawal period of milk (from sale) and the separation of equipment (surfaces) with residues of antibiotic-contaminated milk from those that do not are critical in eliminating antibiotic residues in milk.

Milk indicated as positive for antibiotic residues on receipt at dairy companies is discarded, the incident is investigated and the implicated producer may be fined and not allowed to sell milk for a period of time. Thus, the challenge to the dairy industry has been to develop an approach that eliminates the incidence of antibiotic-contaminated milk. This approach may differ in the detail of application in different countries, but the international principles are similar. The control strategy for antibiotics in milk normally includes monitoring of milk supplies on a routine basis, imposition of penalties for the delivery of contaminated milk and veterinary supervision of antibiotic treatment of cows.

Mycotoxins

Mycotoxins are metabolites of moulds, which can result in pathological changes in humans or animals. Their presence in food products can induce a toxic response (deterioration in kidney or liver function) in humans and other animals (O'Brien *et al.*, 2004), and for this reason is undesirable. The EU maximum level of aflatoxin M1 in milk is $0.5 \mu\text{g kg}^{-1}$ (van Egmond & Dekker, 1995). Mycotoxins occur in cheese (Sengun *et al.*, 2008; Rahimi *et al.*, 2009) as a result of transfer from the milk or due to production by moulds (*Penicillium* spp. and *Aspergillus* spp.) (Erdogan & Sert, 2004; O'Brien *et al.*, 2004; Sengun *et al.*, 2008).

The presence of mycotoxins in milk normally occurs by indirect contamination through the feedstuffs consumed by dairy cattle. Of major importance in this respect is aflatoxin M1, the milk metabolite of aflatoxin B1. Aflatoxin M1 appears in milk and milk products as the direct result of the intake of aflatoxin B1-contaminated feed by dairy cows. Aflatoxin B1 can be present in feeds due to poor storage and favourable climatic conditions suitable for fungal growth. Aflatoxin B1 can be produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* under certain conditions of temperature, water activity and availability of nutrients. Mycotoxins produced by fungal species other than *Aspergillus* and *Penicillium* are of minor concern for dairy products. While there has been concern in recent years over the presence of aflatoxin M1 in milk, bovine milk normally contains extremely low levels of aflatoxin M1 (Blanco *et al.*, 1988). The efficiency of aflatoxin conversion in cows is poor; Frobish *et al.* (1986) reported that <2% of aflatoxin B1 deliberately added to feed offered to lactating animals was converted to the hydroxylated form (M1).

Other residues

Targeted or desired limits for other milk residues are becoming evident in specifications by retailers in some countries for some dairy products, for example 0.03 mg kg⁻¹ of TCM in lactic butter and 250 µg kg⁻¹ iodine in milk for infant feed formulation. TCM, otherwise known as chloroform, is classed as a Group 2B carcinogen, and has been shown to cause cancer in laboratory animals (International Agency for Research in Cancer, 1999), while excess iodine in the human diet causes alterations in thyroid activity (Castillo *et al.*, 2003). The formation of TCM in milk is a consequence of the reaction between the organic matter in milk and active chlorine in the detergent solvent used to clean the milk contact surfaces (Resch & Guthy, 2000). The TCM is formed in the detergent solvent and is then transferred to milk as a consequence of solvent residues on surfaces that come in contact with the milk, for example milk pipelines. TCM development is minimised by sufficient rinsing of milking equipment both before and after detergent washing and correct use of cleaning products having the appropriate chlorine content.

Excess iodine in milk results from either transfer from animal feeds containing high iodine levels or teat disinfection of cows pre- or post-milking. Thus, monitoring of iodine content in animal feed and reducing the carry-over of teat disinfectants (containing iodine) from the teat to the milk would minimise the level of iodine in milk.

1.6 Strategy for quality milk production

Cheese is a concentrated gelled product that structurally consists of a casein/*para*-casein matrix, enclosing fat and moisture. It is essentially formed by controlled gelation (aggregation) of the milk protein (in particular casein) and dehydration of the gel to the desired degree by subjecting it to various operations (such as gel cutting, stirring, heating) and drainage of the expressed whey. Gelation is induced by enzymatic treatment of the milk with rennet (e.g. chymosin) in rennet-curd cheeses and by acidification (to pH 4.8–4.6) in acid-curd cheeses. In both cases, the basic building blocks of the gel are aggregates (of *para*-casein in rennet-curd cheese and of casein in acid-curd cheese), comprising interacted casein micelles. The aggregates subsequently fuse together to form a constrained, periodic-repeating structural continuum of protein throughout the milk. On defining the formation of cheese using this approach, the most important milk quality characteristics for cheese manufacture are those that enhance:

- aggregation of the casein to form a gel that is sufficiently firm to cut within an acceptable time frame (typically 30–50 min for rennet-curd cheeses and 4–14 h for acid-curd cheeses);
- continued aggregation together with whey expulsion during the remaining cheesemaking operations post-gelation; and
- development of a gel structure and curd rheology, which at all stages of manufacture provides a robustness that maximises the retention of fat and casein in the curd and curd yield.

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These attributes are a prerequisite to the formation of a fresh curd with desired composition, structure, texture and yield. Characteristics of the milk that are generally positively correlated with enhanced rennet coagulation include:

- high values for casein number, intact casein content, contents of total casein, individual (α_{s1} -, β - and κ -) caseins, calcium-to-casein ratio, and ratios of κ -casein to total casein and to individual caseins (α_{s2} - and β -caseins); and
- low values for serum casein, micelle size and degree of κ -casein glycosylation.

These characteristics are conditional on a number of factors including the breed, health status, age, plane of nutrition and stage of lactation of the cow, the SCC and bacterial count of the milk, associated enzymatic activities, season and milk production practices. Apart from these characteristics, it is also important that the milk characteristics are conducive to the development of a finished cheese that has satisfactory sensory properties, such as desired flavour by having a clean bland taste free from off-flavours, such as rancidity, taints and chemical tastes of residues, and complies with the safety and wholesomeness expected by the consumer/user. Some recommended attributes of good quality milk for cheese manufacture are listed in Table 1.4.

The factors affecting the overall quality of milk produced on the farm are summarised in Fig. 1.5. Some of these can be controlled short term (implementing proper cleaning protocols prior to milking) or longer term (implementing selection/breeding programmes for desired composition characteristics – protein content, frequency of genetic variants); others cannot (weather, environment). The optimum ‘designer’ milk for cheesemaking is more naturally and cheaply arrived at through ‘best farm and cow management practices’. Some technological interventions within the milk-processing factory just before the cheesemaking process can modify some milk characteristics to make it more suitable for cheesemaking (e.g. casein content by ultrafiltration of milk; cf. Chapter 8). However, some other milk characteristics, such as effects of SCC, microbial and enzymatic activity, cannot be modified at this point. The quality of the raw material leaving the farm (and being purchased by the dairy processor) is of ultimate importance and is most difficult to control. Thus, it is critical that optimum production methods for ‘designer’ milk for cheesemaking be employed. The key elements of good milk production management are outlined as follows:

- breeding/selecting for target cheesemaking properties;
- maintaining a high plane of animal nutrition;
- minimising bacterial count of milk;
- maintaining a low SCC in milk;
- minimising enzymatic activity associated with somatic cells and contaminating bacteria;
- minimising chemical residues and contaminants; and
- minimising fat damage and levels of FFA.

The details of implementation of each of these steps are outlined in Appendix 1.1.

In summary, milk for cheesemaking should be of optimum quality, produced on-farm following guiding principles of optimum animal health, milking hygiene, animal feeding, animal welfare and environment. This approach should also incorporate record keeping for various

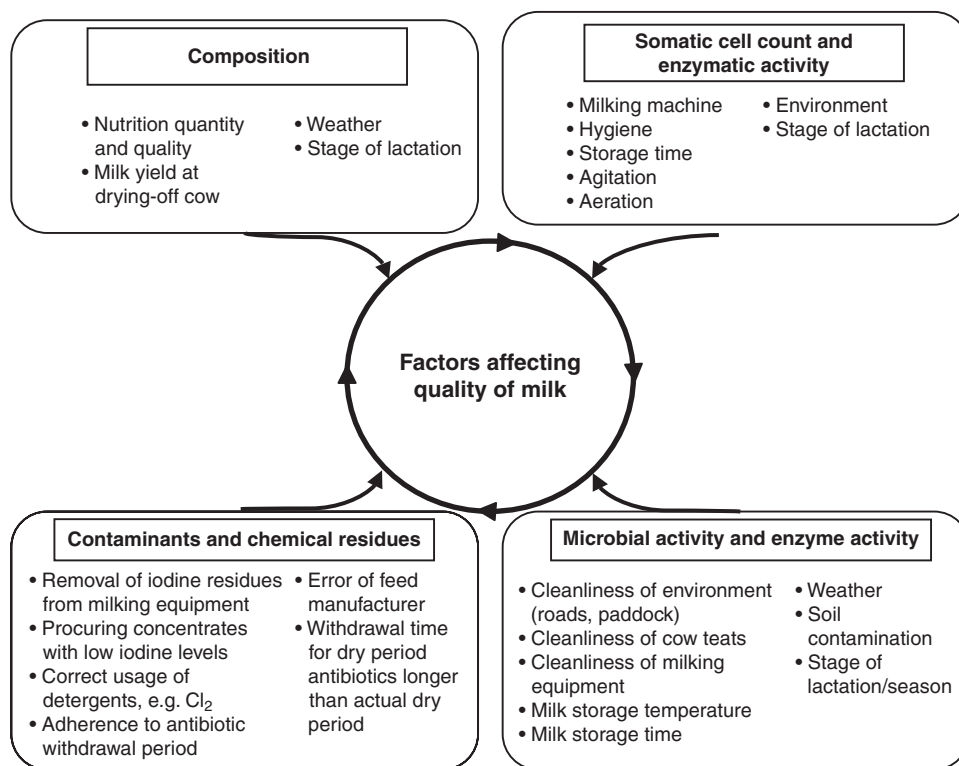


Fig. 1.5 Factors affecting the quality of milk produced on the farm. Note: There are four key quality parameters that influence quality of milk for cheese manufacture directly or indirectly: (a) composition, (b) somatic cell count and enzymatic activity, (c) microbial activity and (d) residues/contaminants. For each parameter, there are factors which can or cannot be controlled by the farmer, and these are represented by the bullet points on the left- and right-hand sides of the parameter boxes, respectively.

procedures (milk storage temperatures and veterinary or medication treatments of individual animals). Additional everyday guidelines to achieve milking excellence on-farm include setting performance goals, rapidly identifying mastitis problems, standardising the milking routine, training of staff (having standard operating protocols for each of these) and allotting adequate time to manage milk quality issues.

1.7 Conclusions

The efficient manufacture of high-quality cheese consistently is a highly complex biotechnological process involving controlled destabilisation and gelation of the milk protein, fermentation of the milk sugar lactose to lactic acid, dehydration of the gel to obtain cheese curd and maturation of the curd to a ripened cheese with the desired quality attributes (sensory, aesthetic, usage, safety, convenience, wholesomeness, value for money) required by the consumer (cf. Chapter 8). A critical prerequisite for the manufacture of quality cheese is to start with milk of the highest quality. This chapter has examined the factors affecting the

quality of milk for generic manufacture of cheese. These include the composition, state of the components (ratio of globular to free fat; degree of hydrolysis of casein or fat), the levels of indigenous and contaminating enzyme activity (from bacteria, somatic cells) and levels of contaminants and chemical residues. The quality of milk for cheese manufacture has greatly improved in recent years as a direct consequence of: (a) greater scientific knowledge of the factors affecting milk composition and how these are affected by animal breeding, husbandry and milk handling, and (b) the quality control measures that have been implemented at farm level, for example training and education of farmers, improved hygiene, measurement systems, documentation and traceability. However, the milk quality concept is a dynamic entity, and a continuous quality improvement approach is required to meet the requirements of different stakeholders including the cheese manufacturer and the consumer. Currently, the demands of the consumer appear to be increasing in importance and it is likely that this will continue as a consequence of the increasing awareness of food, health and security concerns on the part of the consumer, who consequently requires more assurance about food quality. Further improvement in the quality of milk for cheese manufacture will be assisted by:

- developing a better understanding of the relationships between milk composition and chemistry and various aspects of cheese quality and manufacturing efficiency, from manufacture through to the final characteristics of the finished cheese (for example effects of lipolysis and FFA in milk on the levels of FFA and sensory aspects of cheese; effects of degree of glycosylation of κ -casein on the rate of hydrolysis by rennets and on the flocculation/gelation of *para*- κ -casein, for example by comparison of the behaviour of native casein micelles with casein micelles treated *in situ* with glycosidases to remove sialic acid and other glycans from the κ -casein; effects of various factors such as pH, ionic strength, whey protein type and concentration on the interaction of κ -casein with denatured whey protein in high-temperature-treated milks and the assimilation of the resultant aggregates into the rennet-induced milk gel, and their impact on the physical properties of the final cheese; effects of feed on flavour and physical properties of milk fat and the properties of the resultant cheese);
- breeding and selection of cows for the most desired quality attributes in milk (protein level and protein genetic variants);
- ongoing developments in proteomics, i.e. as a means of elucidating the molecular basis for inter-cow variations in milk gelation properties (Tyriseva *et al.*, 2008);
- improvements in analytical capability, such as high-pressure liquid chromatography has contributed to quantifying individual proteins and assisting our understanding of protein interactions of importance to cheese (Donato & Guyomarc'h, 2009), or the aggregation of micelles varying in sialic acid content; and
- progressing quality milk production on farms via an integrated education programme covering all rudimentary aspects (breeding, husbandry, hygiene, milk handling/storage, relationship between milk and product, consumer requirements, traceability/documentation) of quality milk production for products such as cheese (see Appendix 1.1).

In addition, the application of a quality management programme on-farm where risk identification and prevention would play a role is important. This programme should be similar to a hazard analysis critical control points programme as operated in the cheese

manufacturing industry. This structure would allow measurement of critical points throughout the milk production chain (feed, cow, milk, milk tank, with regard to pathogens, indicator organisms of contamination, antibiotics, toxins, chemical contaminants).

Appendix 1.1 strategy for quality milk production

- Breeding/selecting for target cheesemaking properties
 - select cow breeds for high casein and/or fat contents, or for BB genetic variants of κ -casein and β -lactoglobulin
- Maintaining a high plane of animal nutrition
 - offering a high pasture diet can support efficient production of quality milk when adequate grass is available
 - supplementing a pasture-based system with concentrates to increase the energy supply to cows in periods of inadequate grass availability
 - implementing a good cow management system during the late stage of lactation, such as maintenance of milk yield (through supplementation of pasture and subsequently silage, with concentrates and drying off cows at milk yields of 8–9 kg day⁻¹)
- Minimising bacterial count of milk
 - maintaining good hygiene standards at all stages of milk production
 - providing an environment in which cows are maintained in a clean condition and one in which bacterial challenge to the udder is minimised (good grazing conditions or bedding material)
 - carrying out a complete pre-milking routine that ensures minimal bacteria on the udder and teat skin is necessary
 - practicing an effective cleaning routine for the milking plant after each milking
 - rapidly cooling milk to below 4°C
- Maintaining a low SCC in milk
 - reducing the risk of bacterial contamination of the cows' teats and udder through the maintenance of clean cows and post-milking teat disinfection
 - providing winter accommodation that is clean, dry and comfortable for the cow
 - preventing the transfer of mastitis-causing organisms from cow to cow or from one quarter to other quarters of the same animal during the milking process
 - ensuring proper sizing of the milking equipment for individual herds, thus allowing sufficient time for cow preparation prior to milking and avoidance of overmilking
 - ensuring that the milking machine is properly installed, regularly maintained, tested and serviced routinely, and generally functioning properly
 - setting time aside to manage milk SCC and mastitis incidence in terms of the collection, recording, checking and interpretation of herd and individual cow SCC data as well as clinical mastitis incidences
 - buying cows of known SCC from a healthy herd, e.g. a milk recording herd with a normal SCC of $<150 \times 10^3$ cells mL⁻¹
 - application of dry cow therapy
 - culling of cows with persistent high SCC and/or clinical mastitis incidence

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- Minimising enzymatic activity associated with somatic cells and contaminating bacteria
 - maintaining cow milk yields towards the end of lactation through maintaining a good cow diet
 - minimising the occurrence of mastitis and high SCC levels in milk
 - reducing the effect of proteolytic activities contributed by psychrotrophic bacteria by reducing the bacterial level in the milk, degree of microbial growth and the duration of storage time
 - maintaining excellent hygiene (low bacterial levels) together with fast milk cooling (to minimise microbial growth) and minimum storage time
- Minimising fat damage and levels of FFA
 - ensuring that milk transfer equipment is designed and maintained to minimise cavitation, frothing, foaming or agitation and to promote laminar milk flow conditions
 - ensuring bulk tank design that allows gentle movement and handling of milk
 - avoidance of freezing of the initial milk in the tank onto the surface in very fast cooling tanks
 - ensuring minimal agitation and temperature changes as milk is transferred into storage silos and vats together with avoiding contact between homogenised and raw milks within the processing plant
 - maintaining a good nutritional cow diet to prevent nutritional stress particularly in late lactation
- Minimising chemical residues and contaminants
 - discarding milk from antibiotic-treated cows for the recommended withdrawal period
 - flushing milking equipment after milking an antibiotic-treated cow to prevent contamination of the main milk pool
 - ensuring the quality, traceability and storage conditions of feed

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