Part III

Cheese manufacture

19

Acceleration of cheese ripening

V. K. Upadhyay and P. L. H. McSweeney, University College Cork, Ireland

19.1 Introduction

Most rennet-coagulated cheeses are ripened after manufacture for periods ranging from *ca*. two weeks (e.g., Mozzarella) to more than two years (e.g., Parmigiano-Reggiano or extra-mature Cheddar). Cheese ripening involves a complex series of microbiological and biochemical events which result in the development of the flavour and texture characteristic of each variety. Biochemical changes which occur during ripening include metabolism of residual lactose and of lactate and citrate (often, although incorrectly, referred to as 'glycolysis'), lipolysis and metabolism of free fatty acids and proteolysis and metabolism of free amino acids. The biochemistry of cheese ripening, which has been reviewed by Fox *et al.* (1993, 1996a), Fox and McSweeney (1997) and McSweeney and Sousa (2000), is summarized below.

Cheese ripening is a slow, and consequently an expensive, process. The expense of cheese ripening originates principally from the inventory cost associated with holding a large amount of cheese in storage and the capital cost of providing a ripening facility adequate to hold sufficient cheese for its ripening time. The temperature and, in certain cases, the relative humidity of ripening rooms must also be controlled, adding to the cost of cheese ripening. Ripening costs have been estimated at approximately €500–800 per tonne of cheese matured for nine months. Thus, acceleration of cheese ripening has received considerable attention in the scientific literature. This topic has been reviewed by Fox (1988/89), El Soda and Pandian (1991), Wilkinson (1993) and Fox *et al.* (1996b).

Overview of cheese ripening

Most of the lactose in milk is lost in the whey during cheesemaking. Residual lactose is metabolized quickly to lactate early in ripening by the action of the starter or non-starter flora of the cheese. Lactate is an important precursor compound for certain reactions which occur during ripening. Lactate metabolism is most significant in surface mould-ripened cheeses (e.g., Brie and Camembert) where oxidative metabolism of lactate at the surface by *Penicillium camemberti* causes pH gradients across the cheese mass, migration of Ca^{2+} and lactate to the surface with a concomitant softening of the cheese (see McSweeney and Sousa, 2000). Lactate metabolism is also of great importance to Swiss cheese where *Propionibacterium freudenreichii* ssp. *shermanii* metabolizes it to propionate, acetate, H_2O and CO_2, contributing to the flavour of the cheese and causing its characteristic eyes. Other pathways for lactate metabolism include racemization of L- to DL-lactate, resulting in white crystals of calcium-D-lactate on many mature cheeses. Late gas blowing is a defect resulting from the anaerobic metabolism of lactate to butyrate and H_2 gas by *Clostridium* spp.

Milk contains low levels of citrate (*ca.* 8 mmol L^{-1}) most of which is lost in the whey. However, the low level of citrate in cheese curd is an important substrate for citrate-positive (Cit^+) microorganisms (*Leuconostoc* spp. and Cit^+ strains of *Lactococcus*), the metabolism of which produces important flavour compounds (e.g., diacetyl, 2,3-butanediol, acetoin) in Dutch-type cheese.

The fat fraction of cheese also acts as an important source of flavour compounds; cheeses made with a reduced fat level or in which milk fat has been replaced with other lipids do not develop satisfactory flavour (see McSweeney and Sousa, 2000). In foods in general, fats can undergo degradation by either oxidative or hydrolytic mechanisms; the former is not important in cheese due to its low oxidation–reduction potential. Milk fat is hydrolysed in cheese during ripening by lipases originating from the milk, the coagulant or the cheese microflora. Indigenous lipoprotein lipase is of particular importance for lipolysis in raw milk cheese. Lactic acid bacteria are weakly lipolytic, but secondary starters (particularly *Penicillium* spp. in mould-ripened cheeses and coryneform bacteria in smear cheeses) may cause extensive lipolysis in certain varieties. Free fatty acids, particularly short chain acids, contribute directly to cheese flavour. In addition, they also act as precursor compounds for a range of catabolic reactions, including the formation of lactones by intramolecular esterification of hydroxyacids. Esters (principally ethyl esters) are important flavour compounds, imparting a fruity note to cheese. Recent research has indicated that esters may be formed during cheese ripening by transesterification of partial glycerides (Holland *et al.*, 2002). Free fatty acids can form thioesters by reaction with sulphydryl compounds. However, the most important example of fatty acid catabolism occurs in blue cheese where the mould *P. roqueforti* converts fatty acids via incomplete β-oxidation to alkan-2-ones (*n*-methyl ketones), which are the characteristic flavour compounds of these varieties.

Proteolysis is the principal biochemical event which occurs during the ripening of most hard cheese varieties. The initial breakdown of the caseins is

catalysed by residual coagulant (usually chymosin) and the principal indigenous proteinase in milk, plasmin. A range of enzymes from the starter bacteria degrades the resulting peptides. The cell envelope-associated proteinase (lactocepin, PrtP) of lactic acid bacteria (LAB) is particularly important in degrading intermediate-sized peptides produced by chymosin and plasmin to a range of shorter peptides. Short peptides are substrates for a range of intracellular exopeptidases produced by LAB and released into the cheese curd on lysis of the cells, which occurs after cell death. The action of peptidases from the starter and non-starter microflora of the cheese results in the production of free amino acids which, in addition to being important flavour compounds *per se*, act as precursors for a wide range of catabolic reactions which produce volatile compounds characteristic of the flavour of many cheese varieties.

Various approaches have been used to accelerate the ripening process of cheese including:

- Use of elevated ripening temperatures
- Addition of exogenous enzymes or attenuated starters
- Use of adjunct cultures
- Genetic modification of starter bacteria
- High-pressure treatment.

Certain approaches (in particular the use of attenuated starters and adjunct cultures) are also used commercially in hard cheese to modify flavour, without necessarily reducing ripening time. Although not strictly acceleration of cheese ripening, the technology of enzyme-modified cheeses, products which rapidly develop a cheese-like flavour, is also discussed briefly.

19.2 Accelerating cheese ripening: elevated temperature

Cheese ripening at an elevated temperature is technically the simplest method for accelerating ripening and the lower refrigeration costs may provide overall savings to the producer. The drawbacks of this approach are an increased risk of microbial spoilage and non-specific increases in ripening reactions, possibly leading to unbalanced flavour or off-flavours (Wilkinson, 1993).

Although ripening temperature is the most important single factor determining flavour intensity (Law *et al.*, 1979), relatively few studies have been conducted on the effect of elevated temperatures on cheese ripening (El Soda, 1993). Studies in which the effects of different time–temperature combinations on cheese ripening were used to accelerate ripening were reviewed by Wilkinson (1993). Ripening temperature influences the rate of proteolysis (Aston *et al.*, 1983a, b; Fedrick *et al.*, 1983; Folkertsma *et al.*, 1996), lipolysis (Folkertsma *et al.*, 1996; O'Mahony and McSweeney, unpublished), cheese microflora (Cromie *et al.*, 1987; Folkertsma *et al.*, 1996), texture (Fedrick and Dulley, 1984) and quality (Aston *et al.*, 1985) of cheese. Ripening at elevated temperature to 15ºC has been recommended to accelerate the ripening of cheese

of good chemical and microbiological quality (Fedrick, 1987; Folkertsma *et al*., 1996).

El Soda and Pandian (1991) concluded that the use of elevated temperature to accelerate ripening was likely to be limited to large cheese factories where very hygienic procedures are adopted during manufacture and ripening. Cheddar and certain other hard cheeses are now generally produced from pasteurized milk in large, highly automated plants with high hygienic standards and thus ripening of Cheddar and other cheeses at elevated temperatures is feasible and is practised in industry, particularly for cheese intended for ingredient use. However, successful ripening at elevated temperatures requires careful control of cheese composition and microflora.

19.3 Addition of exogenous enzymes or attenuated starters

Rennet and plasmin are mainly responsible for primary proteolysis while proteinases and peptidases from starter and non-starter bacteria convert the larger peptides, produced by action of rennet on caseins to intermediate and smaller peptides, which are precursors for flavour compounds. Addition of exogenous enzymes to cheese increases the enzyme pool, which eventually helps to accelerate the rate of certain reactions in cheese in contrast to elevated temperature, which results in the increase in rate of all reactions, some of which may impart an off-flavour to the cheese. However, there are certain limitations to enzyme addition as a method for accelerating ripening. Legal barriers in many countries restrict the use of exogenous enzymes in cheesemaking

Addition to cheesemilk appears to be the best stage for enzyme incorporation due to the homogeneous mixing of the enzyme with the milk and its subsequent transfer to cheese curd. However, most of the enzyme added to the milk (~95%) is lost in the whey and proteolytic enzymes degrade caseins to peptides, which are lost in the whey, resulting in a reduction in cheese yield. Moreover, early breakdown of the caseins results in disruption of the casein matrix, leading to poor gel strength and difficulty in working the curd at later stages of the cheese manufacture (Law, 2001). Due to loss of expensive enzyme preparations in cheese whey and an additional cost to inactivate enzymes in whey before its use as an ingredient in food preparations, alternative stages or techniques of addition of enzyme during the cheesemaking process need to be explored. Figure 19.1 indicates stages at which enzymes may be added during cheesemaking

Microencapsulation of the enzyme before addition to cheesemilk helps to protect casein from degradation and facilitate more enzyme to be entrapped physically in the curd, hence reducing the loss of the enzyme in the whey (El Soda *et al.*, 1989). Law and King (1985) and Kirby *et al.* (1987) developed a type of phospholipid liposome, which degraded in the cheese matrix after the whey separation. Due to the high cost of pure phospholipids to prepare high capacity and stable liposomes, application of this technique on a large scale is not feasible.

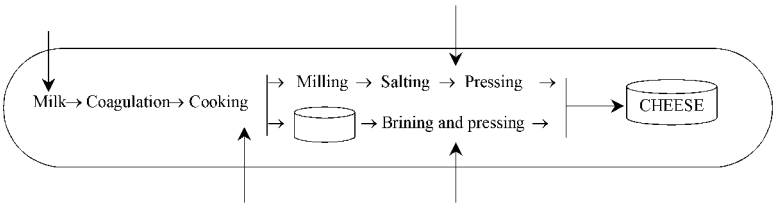

Fig. 19.1 Possible points for addition of enzymes to cheese curd (modified from Law, 2001). Arrows indicate the possible points of enzyme addition.

Certain semi-hard cheeses such as Edam and Gouda have a characteristic 'curd washing' step in the manufacturing protocol, where part of cheese whey is replaced by hot water in an attempt to reduce the acidity. This is another stage where addition of enzyme to soft curd is a possibility, although the flux of whey from the curd would impede uptake of enzymes, which in any case diffuse only very slowly through the protein matrix of curd. Thus, enzyme addition at this stage of manufacture is not usually practised. Likewise, addition of the enzyme at the brining stage is difficult due to the close texture of the cheese block. Kosikowski (1976) first suggested the addition of enzymes to dry-salted cheese varieties by mixing enzymes with salt. Addition of granulated enzyme with salt after milling in Cheddar-type cheeses is also an option for enzyme addition but is difficult to adopt at an industrial level. Also, since diffusion of enzymes through curd is extremely slow, localized high concentrations of enzymes can lead to uneven ripening throughout the cheese ('hot spots').

The proteinase(s) from the coagulant cause primary proteolysis in most cheese varieties (Fox and McSweeney, 1997); hence, increasing the level of coagulant might be expected to accelerate proteolysis. Research indicates that chymosin is the limiting proteolytic agent in the initial hydrolysis of the caseins during ripening. However, increasing the levels of chymosin often results in bitter cheese (Fox and Tobin, 1999).

Plasmin plays an important role in proteolysis in many cheese varieties, especially in high-cooked cheese varieties where the high cooking temperature during cheese manufacture results in increased plasmin activity and in extensive denaturation of chymosin. Since casein in milk can bind at least 10 times the amount of plasmin normally present, plasmin added to cheesemilk is retained in the curd. Plasmin, when added to cheesemilk, accelerated proteolysis in Cheddar cheese (Farkye and Fox, 1992). However, exogenous plasmin is expensive and, due to its scarce availability on the market, activation of its zymogen, plasminogen, by means of various plasminogen activators (PA) could provide an alternative to adding exogenous plasmin to accelerate the ripening of cheese. This approach was used by Barrett *et al.* (1999) using exogenous urokinase for plasminogen activation to accelerate proteolysis in Cheddar cheese. A similar concept, using a semi-purified preparation of streptokinase, a bacterial PA secreted by *S. uberis*, was used by Upadhyay *et al.* (unpublished) to accelerate proteolysis in Cheddar cheese. Streptokinase from *S. uberis* is able to activate

bovine plasminogen to plasmin, whereas streptokinases from Lancefield C or Lancefield E streptococci do not activate bovine plasminogen (Leigh, 1993). In both studies, plasmin activity in cheese was increased, levels of plasminogen decreased, and proteolysis, as determined by levels of water-soluble N and peptide profiles by urea-polyacrylamide gel electrophoresis and reversed-phase HPLC, increased. Increased plasmin activity had little effect on levels of free amino acids in the latter study while individual free amino acids increased with increasing levels of urokinase in the former.

Use of enzyme mixtures has an advantage over the use of a single enzyme as they enhance the rates of multiple reactions aimed at enhancing or modifying particular aspects of cheese ripening. Since proteolysis is the major biochemical process occurring during the ripening of Cheddar, Gouda and Italian-type cheeses, most enzyme preparations used to accelerate ripening contain proteinases and peptidases; lipases may also be present in certain preparations. Some commercially available enzymes or enzyme preparations are listed in Table 19.1.

Accelase™, an enzyme preparation developed based on research on the role of starter enzymes and cell lysis in flavour development (Law *et al.*, 1974; Law and Wigmore, 1983) by IBT Ltd (now Rhodia Foods, UK), has been evaluated for acceleration of cheese ripening. Experimental results on cheeses made with Accelase™ suggest that cheeses reach the equivalent of 9 months' maturity after 5 months of ripening. It is also claimed that Accelase™ reduces the bitterness in cheese and there is an increase in flavour notes including 'sulphur', 'acid' and 'Cheddar' (Law, 2001). A number of studies have used Neutrase®, a neutral proteinase from *Bacillus subtilis*, as exogenous enzyme to accelerate ripening in cheese (see Wilkinson, 1993). Guinee *et al.* (1991) added Neutrase®, FlavorAge™-FR (a lipase proteinase preparation from *Aspergillus oryzae*) and extra rennet to Cheddar cheese curd at salting. Addition of these enzymes accelerated the ripening in Cheddar cheeses, when ripened at 5ºC for 4–5 months. Longer storage of the cheeses resulted in excessive proteolysis and flavour defects in cheeses. When using FlavorAge™-FR and DCA 50 (a proteinase–peptidase blend; IBT, London), these authors reported acceleration of flavour development.

Since lipases play a role in the ripening of Cheddar cheese, many researchers have focused work on accelerating lipolysis in Cheddar cheese by the use of exogenous lipases or lipase preparations. FlavorAge™ is a commercially available enzyme preparation containing a lipase, which has very high specificity for C_6–C_8 acids. Arbige *et al.* (1986) used FlavorAge™ in Cheddar cheese manufacture and found increased formation of short-chain fatty acids and acceleration in the development of flavour intensity. O'Connell (2002), when investigating the effects of addition of low levels of two commercially available enzyme preparations (Lipase M 'Amano' 10, Amano Enzymes and Palatase® 20000L, NOVO) on ripening and sensory characteristics of Cheddar cheese, found higher lipolysis in cheeses with added enzymes as indicated by increased levels of free fatty acids. Enzyme addition had a profound effect on the sensory characteristics of cheese; the highest level of addition gave attributes (pungent,

Table 19.1 Enzyme preparations, other than rennets, commercially available for cheese or enzyme-modified cheese (modified from Wilkinson and Kilcawley, 2002)

Principal enzymatic activity	Trade name	Host organism/source	Company
Aminopeptidase	Accelase™[a], Savorase®, Debitrase®	*Lactococcus lactis* *Rhizopus oryzae*	Rhodia Food
Aminopeptidase	Acid Protease A	*Aspergillus niger*	Amano Enzymes
Prote(in)ase	Acid Protease II	*Rhizomucor niveus*	Amano Enzymes
	Bioprotease A conc	*Aspergillus niger*	Quest International
	Bioprotease N 100	*Bacillus subtilis*	Quest International
	Bioprotease P conc	*Aspergillus oryzae*	Quest International
	Fermizyme® B 500	*Bacillus subtilis*	DSM
	FlavorAge[b]	*Aspergillus* sp.	Chr Hansen
	Flavorpro 192	*Aspergillus* var. strains	Biocatalysts
	Flavourzyme	*Aspergillus oryzae*	NOVO
	Neutrase	*Bacillus subtilis*	NOVO
	Peptidase 'R' Amano	*Rhizomucor oryzae*	Amano Enzymes
	Promod 24L	*Bacillus subtilis*	Biocatalysts
	Promod 215P	*Aspergillus sojae*	Biocatalysts
	Protease 'A' Amano 2	*Aspergillus oryzae*	Amano Enzymes
	Protease M	*Aspergillus oryzae*	Amano Enzymes
	Protease N	*Bacillus subtilis*	Amano Enzymes
	Prozyme 6	*Aspergillus melleus*	Amano Enzymes
	Sternzyme B5021	*Aspergillus niger*	Stern-Enzyme
	Sternzyme B5026	*Aspergillus oryzae*	Stern-Enzyme
Lipase	Capalase®	Animal	Degussa Bioactives
	Italase®	Animal	Degussa Bioactives
	Kid Lipase	Animal	Chr Hansen
	Lipase M 'Amano' 10	*Rhizomucor javanicus*	Amano Enzymes
	Palatase® 20000 L	*Rhizomucor miehei*	NOVO

[a] Can also be mixed with enzymes from other sources.
[b] Proteinase-lipase preparation.

rancid, sweaty, vomit) that are used to describe enzyme-modified cheeses (EMCs) or mould-ripened cheese.

19.3.1 Attenuated starters

As discussed above, enzymes play very important roles in the ripening of cheese. The use of attenuated starters provides an alternative means of increasing the enzyme pool in the cheese curd without the drawbacks of exogenous enzyme addition (enzyme loss in cheese whey, additional cost of processing the whey, legal barriers in many countries and difficulties of incorporation of enzymes with cheese curd in combination with high cost). An advantage of the use of attenuated starters compared with exogenous enzymes is that the former are largely trapped within the curd on whey drainage and release their enzymes directly into the curd on lysis of the cells. In addition to accelerating ripening, recent work has shown the potential of attenuated starters in modifying flavour.

Attenuated starters can be defined as LAB which are unable to produce significant levels of acid during cheesemaking, but which provide active starter enzymes that are important for cheese ripening and flavour development. Attenuated starter is added to cheesemilk together with the primary starter. Attenuation can be achieved by heat treatment, freezing and thawing, freeze or spray-drying, lysozyme treatment, use of solvents, and natural and induced genetic modification. The use of attenuated starters and methods of attenuation were discussed by Klein and Lortal (1999).

Petterson and Sjöström (1975) proposed the use of attenuated starters in cheesemaking to accelerate proteolysis and reduce ripening time. They attenuated the cells by heat treatment, which is the most widely studied method of attenuation. Heat treatment of LAB retards acid production but excessively severe treatment results in denaturation of starter enzymes. Hence, the temperature–time combination of heat treatment is critical; combinations are chosen to ensure greatest reduction of acid production by LAB but with least denaturation of starter enzymes. Petterson and Sjöström (1975) suggested treatment at 59ºC for 15 s for mesophilic starters and at 69ºC for 15 s for thermophilic starters. However, the treatment parameters vary between studies (see Klein and Lortal, 1999). Attenuated preparations of various LAB strains (e.g., mixed lactococcal strains; Exterkate *et al.*, 1987) have been studied, but the most studied species is *Lb. helveticus* owing to its high peptidase activity compared to other LAB. Inoculation level for heat-shocked cells varies; an estimated 5×10^6 to 5×10^7 cells ml^{-1} were added to milk in most trials. Retention of the added cells in the curd varies depending on cell morphology; retention of rods is higher than that of cocci (Petterson and Sjöström, 1975). Retention of cells in curd (as a percentage of cells added to milk) decreases with increasing addition of attenuated cells to the milk. Many of the studies conducted on attenuated starter using heat treatment have shown acceleration in proteolysis of cheese, improvement of flavour and reduction in levels of bitterness (Petterson and Sjöström, 1975; Skeie *et al.*, 1997; Salomskiene, 1998).

Development of flavour is a challenge in low-fat cheese, cheese made from ultrafiltered milk and cheeses made on a large industrial scale from pasteurized milk. Based on the research of Ardö and Petterson (1988), a commercial preparation, ENZOBACT^{TM} (composed of heat-shocked *Lb. helveticus*), is available from Medipharm, Sweden, which has been shown to accelerate the ripening in reduced-fat Swedish hard cheese.

Attenuation by freezing and thawing was first proposed by Petterson and Sjöström (1975), and was studied in cheese trials by Bartels *et al.* (1987). The authors subjected concentrated bacterial cells to freezing at −20ºC overnight or longer and rapidly thawing the cells to 40ºC, resulting in attenuation of LAB. During the process of freezing and thawing, physical, chemical and biochemical changes occur within the cell. Attenuation is achieved more easily by this method than by heat treatment, where accurate time–temperature control is essential to minimize denaturation of starter enzymes. Freeze-drying of the attenuated starter can be used to reduce the storage volume and facilitate its use. A comparative study on the effectiveness of freezing, freeze-drying and spray-drying treatments for the production of attenuated *Lb. helveticus* CNRZ 32 by Johnson and Etzel (1995) indicated that spray-drying at an outlet temperature of 120ºC delayed acid production but decreased enzyme activity substantially, while cells spray-dried at an outlet temperature of 82ºC had higher enzyme activity compared to cells treated by other methods, but acid production was not delayed. El Soda *et al.* (2000a) studied the effects of heat-shocking and freeze-shocking on cell viability, autolytic properties, aminopeptidase and esterase activities and acid production of *Lb. helveticus* and *Lb. casei* in buffer and cheese slurry systems. Heat-shocking was very effective in reducing cell viability but at the cost of enzymatic activity.

Treatment of bacterial cells with lysozyme results in greater reduction in acid production while preserving more enzyme activity than heat treatment and hence can be used as a means to attenuate LAB. However, the high cost of lysozyme treatment limits its application for attenuation. Use of solvents such as *n*-butanol has been attempted to prepare LAB for use as attenuated starters. However, legal barriers and risk of health hazards has hampered the use of solvents to achieve attenuation. A method for attenuation using the surfactant sodium dodecyl sulphate has been patented by Smith *et al.* (2000).

Use of lactase-negative (Lac^-), proteinase-negative (Prt^-) or Lac^- proteinase positive (Prt^+) mutants has added a new dimension to use of attenuated starter cultures in cheesemaking. Culture variants that spontaneously lose their ability to ferment lactose (Lac^-) can be classified as attenuated cultures as they can no longer produce acid in milk and hence do not acidify milk or curds during manufacture. Dulley *et al.* (1978) isolated Lac^- and Prt^- variants from *Lc. lactis* ssp. *lactis* C2 which were used by Grieve and Dulley (1983) to accelerate proteolysis in Cheddar cheese. They added concentrated cells (approximately $10^{11}\ g^{-1}$) to the cheesemilk, and ripening of the cheese made from this milk at 20ºC for 1 month resulted in increased proteolysis and flavour development. Besides deriving these mutants naturally, chemicals have been used to induce

modifications to obtain Lac^- Prt^+ mutants. Nakajima *et al.* (1991) used nitroguanidine while Birkeland *et al.* (1992) used ethidium bromide to induce Lac^- Prt^+ mutants. A commercially available system based on natural genetic variants of lactococci, named Flavour Control (FC^{TM}) CR cultures, is available from Chr Hansen A/S, Hørsholm, Denmark. These cultures are effective in flavour enhancement of full-fat hard and semi-hard cheeses and improving flavour in reduced-fat Cheddar (Banks *et al.*, 1993). Tobin (1999) assessed a selection of Lac^- cultures from Chr Hansen's Laboratories (Reading, UK) in Cheddar cheese. Inoculation of these cultures individually or in combination increased the flavour of experimental Cheddar cheeses and resulted in higher production of amino acids. All the studies done so far with Lac^- mutants have used mutants of lactococci; use of similar mutants from other genera may be interesting.

19.3.2 Increased rate of lysis of starter cells

Immediately after manufacture, cheese curd generally contains *ca.* 10^8–10^9 viable starter cells g^{-1} cheese. As ripening progresses, the starter cells die and lyse. Starter lactococci contain proteinases and peptidases, which degrade large peptides derived from casein to small peptides and amino acids (Fox *et al.*, 1996a). Acceleration of autolysis of starter bacteria is seen as a possible mechanism to accelerate cheese ripening, owing to the fact that lysis of the starter cells releases intracellular peptidases into the matrix of the cheese. Release of these intracellular enzymes into the cheese matrix results in higher levels of free amino acids and a reduction in bitterness due to the breakdown of hydrophobic peptides by peptidases.

Autolysins are involved in breaking the peptidoglycan structure during the growth of the cell, in order to separate cells during division. The rate of lysis of cells in the cheese matrix is strain-dependent and factors such as difference in cell wall structure and autolysins affect lysis of cells. In Gram-positive bacteria, five types of enzymes with lytic activity against peptidoglycan have been identified. Figure 19.2 illustrates the structure of the peptidoglycan in *Lc. lactis* together with the action of peptidoglycan-degrading enzymes. Among these peptidoglycan-degrading enzymes, only endo-β-*N*-acetylmuramidase (AcmA) has been isolated biochemically from *Lc. lactis* ssp. *cremoris* AM2 (Mou *et al.*, 1976). The gene for AcmA was cloned and studied at molecular level by Buist *et al.* (1995), which is the only autolysin gene cloned to date from *Lactococcus*. However, recent findings suggest that AcmA is not solely responsible for autolysis in *Lc. lactis* (Pillidge *et al.*, 1998, 2002).

Autolysis can be monitored in cheese by electron microscopy or measurement of the release of intracellular enzymes (Crow *et al.*, 1995a). Measurement of starter cell numbers by viable cell count does not give an accurate indication of the extent of autolysis, as cells which have died may not have lysed. Measurement of intracellular enzymes gives a good indication of lysis as they are released into the curd upon lysis. Wilkinson *et al.* (1994a)

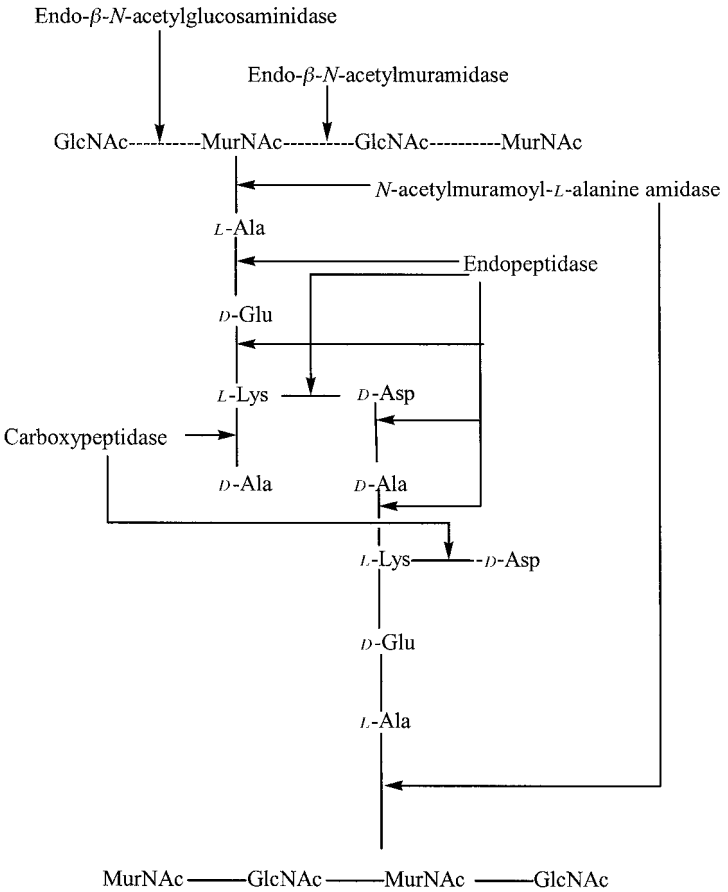

Fig. 19.2 A representation of the lactococcal peptidoglycan structure showing specificities of the different peptidoglycan hydrolases (adapted from Crow *et al.*, 1995a).

studied autolysis of starter bacteria in Cheddar cheese by monitoring the release of lactate dehydrogenase (LDH), glucose-6–phosphate dehydrogenase (G6PDH) and X-prolyl dipeptidylaminopeptidase (PepX) over time. Precisely monitoring autolysis in cheese is difficult due to possible instability of marker enzymes. Wilkinson *et al.* (1994b), when evaluating factors that influence the determination of autolysis, found that, under cheese-like conditions, LDH activity appeared to be the most stable (40% of the original activity survived after 500 h at 4ºC) compared to G6PDH and PepX, the activity of which disappeared after 72 and 24 h, respectively. The extraction method (hypertonic extraction) influences marker activity. The authors prepared cheese juice by subjecting the cheese to high pressure to extract the aqueous phase and analysing the activity of the marker in cheese juice. However, the effect of pressure treatment on the stability of these marker enzymes needs further research.

Autolysis varies greatly among strains of starters. Wilkinson *et al*. (1994a) monitored autolysis in cheese made using *Lc. lactis* ssp. *cremoris* G11/C25 (non-bitter), HP (bitter) or AM2 (non-bitter). Viability of the cells was in the order G11/C25 > HP > AM2, while autolysis in the cheeses, measured by the activity of the above markers, was in the order AM2 > G11/C25 > HP. Levels of free amino acids in the cheeses were related to the rate of lysis of strains. Bitterness in cheeses made using HP may be attributed to slow lysis of the starter cells, making the substrate inaccessible to the peptidases and resulting in slow degradation of some of the bitter casein-derived peptides. O'Donovan *et al.* (1996) showed similar trends when using starters AM2, HP and 303, where the trend for viability was in the order 303 > HP > AM2 and autolysis in the order AM2 > 303 > HP. Strain 303 is a commercial starter that maintains high viability and lyses slowly.

Other factors which affect the autolysis of starter cells include environmental factors such as temperature during cheese manufacture, salt concentration and lactose depletion (El Soda *et al.*, 2000b). Wilkinson *et al.* (1994b) showed that increasing salt concentration results in an increase in the activity of marker enzymes. Differences in the salting procedure may affect autolysis of the starter. Crow *et al.* (1995a) found a more rapid decrease in starter cell densities in Cheddar cheeses when using trummel salting, compared to a normal salting belt.

'Thermolytic' *Lc. lactis* strains such as SK11 and US3 contain temperate phage, inducible by UV light, mitomycin C or temperature shock (Pillidge *et al.*, 2002). A higher cooking temperature (38–39ºC) induces the temperature-sensitive prophage in *Lc. lactis* ssp. *cremoris* SK11 (Feirtag and McKay, 1987), resulting in cell lysis. Controlled infection of starter cells with bacteriophage is also a means for lysing cells. Crow *et al.* (1995b) found increased lysis of *Lc. lactis* ssp. *lactis* ML8 in Cheddar cheese when a homologous phage was added with increased rennet levels compared to *Lc. lactis* ssp. *lactis* ML8 alone, resulting in increased levels of free amino acids (FAAs). However, the use of phage in cheesemaking is not advisable (Morgan *et al.*, 1995).

The use of bacteriocins or bacteriocin-producing starters in cheesemaking is a novel approach for accelerating lysis. Plasmids encoding genes for bacteriocin production isolated from strains of LAB can be cloned into other LAB which can then be used as adjuncts. The use of bacteriocins in cheesemaking carries the risk of delay in acid production, hence affecting the cheesemaking schedule. However, the use of a bacteriocin-producing starter immune to the bacteriocin (Imm^+) provides a remedy for any delay in acidification during cheesemaking (Martínez-Cuesta *et al.*, 2001). These authors transferred a 46 kb plasmid, encoding the production of lacticin 3147 from *Lc. lactis* IFPL105 to *Lc. lactis* IFPL359, which was used for the manufacture of goats' milk cheese. Cheesemaking with *Lc. lactis* IFPL3593 (Lac^+ Imm^+) allowed proper acidification without any delay, and increased lysis was observed, resulting in increased levels of amino nitrogen. O'Sullivan *et al.* (2002) isolated a lantibiotic (lacticin 481)-producing strain, *Lc. lactis* DPC5552, from raw milk. Lacticin 481 resulted in a slower growth rate of the target strain along with release of

intracellular enzymes such as LDH or PepX, unlike lacticin 3147 or lactococcins A, B and M which kill the target strain. Use of the strain *Lc. lactis* DPC5552 in laboratory-scale cheesemaking resulted in increased release of LDH from starter *Lc. lactis* HP strain, without severely delaying acid production. Morgan *et al.* (1997) used a citrate-utilizing *Lc. lactis* ssp. *lactis* DPC3286 modified to contain a plasmid that encodes production of lactococcins A, B and M which have a lytic effect against the lactococcal starter cultures, as an adjunct with a cheesemaking strain *Lc. lactis* ssp. *cremoris* HP for manufacture of Cheddar cheese. Cheddar cheese manufactured with the adjunct bacteriocin-producing strain had increased cell lysis, higher levels of free amino acids and higher sensory score compared to the cheeses manufactured either by starter culture alone or with adjunct that did not produce bacteriocin.

19.4 Use of adjunct cultures

Several studies have shown that non-starter lactic acid bacteria (NSLAB) play an important role in cheese flavour development (Fox *et al.*, 1998; Shakeel-Ur-Rehman *et al.*, 1999, 2000). The NSLAB, which are present in numbers below $100\,\text{cfu}\,\text{g}^{-1}$ cheese at day 1, grow rapidly in the hostile environment of cheese (low pH, low E_h, low a_w, low lactose concentration, anaerobic) to reach counts of 10^7–$10^8\,\text{cfu}\,\text{g}^{-1}$ cheese within 2 months, the rate of growth depending on temperature. NSLAB gain entry to milk from various sources. Post-processing contamination of pasteurized milk results in an increase in NSLAB numbers in milk. NSLAB include facultatively heterofermentative (mesophilic) lactobacilli, micrococci, pediococci and enterococci; mesophilic lactobacilli (e.g., *Lactobacillus casei*, *Lb. paracasei*, *Lb. plantarum*, *Lb. curvatus*) usually dominate the NSLAB microflora. Cheeses manufactured from raw milk develop a more intense flavour than cheeses manufactured from pasteurized milk, which may be attributed to killing of the indigenous microflora by pasteurization. The role of the indigenous microflora in cheese ripening was investigated by McSweeney *et al.* (1993), who manufactured Cheddar cheese from raw, pasteurized or microfiltered milk. Cheeses made from raw milk had a more intense flavour than cheese produced from pasteurized or microfiltered milk. Cheeses made from pasteurized milk or microfiltered milk were similar in terms of proteolysis, lipolysis and microflora. Similar observations on the difference in flavour of raw and pasteurized milk cheeses were reported by Bouton and Grappin (1995) and Beuvier *et al.* (1997). A study by Ryan *et al.* (1996) also demonstrated the importance of NSLAB in cheese ripening and flavour development.

Several cheese varieties contain a secondary microflora which does not produce acid during manufacture but is responsible for development of a particular characteristic of the cheese, e.g., eye formation in Swiss cheese caused by *Pr. freudenreichii* ssp. *shermanii*, blue veins in Blue cheese (*P. roqueforti*) or the red smear in smear-ripened cheese (a complex Gram-positive microflora).

Cheddar cheese generally does not contain a deliberately added secondary flora. However, owing to the importance of NSLAB in flavour development, the use of NSLAB as adjuncts has opened a new area of research as a means for manipulating chemical and sensory characteristics of Cheddar cheese.

Many studies have used mesophilic lactobacilli to accelerate cheese ripening. The inoculation level of mesophilic lactobacilli in cheesemilk varies from 10^2 to 10^5 cfu ml^{-1}. A higher inoculation level (~10^5 ml^{-1}) has been used for pasteurized cheesemilk in many studies to resemble the count of NSLAB in raw cheesemilk. Mesophilic lactobacilli, which have been studied as adjuncts either singly or in combination, include strains of *Lb. casei*, *Lb. paracasei*, *Lb. plantarum*, *Lb. brevis*, *Lb. curvatus* and *Lb. rhamnosus.* Lynch *et al.* (1996) studied the effects of added mesophilic lactobacilli on proteolysis and flavour development in Cheddar cheese made under controlled microbiological conditions. The *Lactobacillus* counts in experimental cheeses ranged from 10^4 to 10^5 cfu g^{-1} cheese at milling, which increased to 5×10^7 cfu g^{-1} after 4 weeks. The control cheese remained free from lactobacilli for up to 100 days and did not exceed 5×10^5 cfu g^{-1}. Addition of adjuncts to the cheesemilk increased the levels of free amino acids in experimental cheeses compared to control cheeses. However, little differences were observed in other indices of proteolysis. Lane and Fox (1996) found that addition of adjunct lactobacilli increased proteolysis in starter-free cheese (acidified using gluconic acid-δ-lactone), but this increase was not apparent when a starter was used together with the adjunct.

Recently, many investigations have reported use of adjuncts for improving flavour in low fat cheese varieties. Katsiari *et al.* (2002) investigated the effect of two commercial adjuncts, LBC 80 (*Lb. casei* ssp. *rhamnosus)* and CR-213 (containing *Lc. lactis* ssp. *cremoris* and *Lc. lactis* ssp. *lactis*), on the compositional, sensory and textural characteristics of low-fat Kefalograviera-type cheese. Low-fat cheese with adjuncts received higher scores for flavour intensity, texture and body than low-fat cheese without adjuncts. The aminopeptidase activity of the adjunct culture can reduce bitterness, which is a defect encountered commonly in low-fat cheese.

Thermophilic lactobacilli such as *Lb. helveticus* do not constitute part of NSLAB of Cheddar-type cheeses but are commonly used in the production of high-cooked cheese varieties and yogurt. Compared to mesophilic lactobacilli, few studies have been done on the use of thermophilic lactobacilli as adjunct, although our experience is that they improve quality substantially. Tobin (1999) found beneficial effects of the use of *S. thermophilus* TS3 and *Lb. helveticus* HL3 as adjuncts on the quality of Cheddar cheese. Hannon *et al.* (2003) manufactured Cheddar cheese using blends of two *Lc. lactis* strains (223, 227) as a starter with or without *Lb. helveticus* DPC4571 as adjunct or using *Lb. helveticus* DPC4571 as the only starter. *Lb. helveticus* DPC4571 lysed rapidly and cheeses made using *Lb. helveticus* DPC4571 as starter contained greater levels of free LDH than the other cheeses. Higher levels of proteolysis and flavour scores were obtained in cheeses made using *Lb. helveticus* DPC4571 as

starter or as adjunct compared to cheese made only using *Lc. lactis* strains 223 and 227.

19.5 Genetic modification of starter bacteria

In recent years, attention has been focused on genetic engineering as a means of producing starters expressing proteinases and many intracellular enzymes (e.g., PepC, PepI, PepN, PepO, PepW, PepX) which play an important role in secondary proteolysis in cheese during ripening.

Due to their commercial significance, intensive research has been carried out to understand the genetics of LAB. The complete genomic sequence of *Lc. lactis* ssp. *lactis* IL1403 (Bolotin *et al.*, 2001) is now known and those of a number of other LABs are expected in the near future. Hence, targeted modifications to starter *Lactococci* used in cheesemaking are possible.

McKay and Baldwin (1976) found that lactococci contain extrachromosomal elements called plasmids. Most lactococcal strains contain multiple copies of four to seven plasmids with molecular masses of 2 to 100 kDa. The importance of plasmids can be highlighted from the fact that many important industrial traits such as proteinase production and lactose metabolism (and hence the ability to grow in milk), citrate metabolism, phage resistance and bacteriocin production are encoded on plasmids. The information that needs to be expressed is coded on a plasmid vector and then inserted into a plasmid-free host. When the host multiplies, the inserted plasmid vector also multiplies and expresses the characteristics encoded on the plasmid. Plasmids, and thus the traits they encode, can be lost easily on subculturing.

Based on the huge potential of genetic engineering in the area of cheese ripening, efforts have been made to accelerate ripening of cheeses by cloning and inserting genes for certain enzymes into starter bacteria. McGarry *et al.* (1995) made Cheddar cheese using a strain of *Lactococcus* containing the cloned gene for the neutral proteinase from *Bacillus subtilis* (Neutrase). Cheeses manufactured with the modified starter showed extensive proteolysis and their texture became very soft after 2 weeks of ripening at 8ºC. A blend of Neutrase-producing lactococci and wild-type strains was used as a starter for Cheddar cheese manufacture by McGarry *et al.* (1994); a blend of 80:20 gave the best results and controlled and accelerated proteolysis was observed. Upadhyay *et al.* (unpublished) observed an increase in plasmin activity and increased proteolysis in miniature Cheddar-type cheese made using a strain of *Lactococcus* modified to express the plasminogen activator streptokinase from *S. uberis*. The production of streptokinase during cheesemaking resulted in activation of plasminogen to active plasmin as indicated by increased degradation of β-casein and concomitant increase in the concentration of γ_1-, γ_2- and γ_3-caseins.

Owing to the importance of starter peptidases in secondary proteolysis, peptidases from LAB have been characterized biochemically and genetically. Among LAB, *Lc. lactis* and *Lb. delbruechi* ssp. *bulgaricus* are the most

extensively studied organisms (Christensen *et al.*, 1999). Several investigations have attempted to clone various peptidases into *Lactococcus*. Courtin *et al.* (2002) cloned five different peptidases of *Lactobacillus* into strains of *Lc. lactis* in order to assess their effect on ripening in a model cheese system. The authors found an increase in the levels of PepQ, PepX and PepW, which led to a threefold increase in production of amino acids in a model curd system. Joutsjoki *et al.* (2002) cloned genes for PepN, PepC, PepX and PepI from the proteolytic *Lb. helveticus* into *Lc. lactis* using a food grade cloning system. Peptidase activity, assayed under conditions similar to that of cheese, was higher in the recombinant strain than in the control strain. Similarly Anastasiou *et al.* (2002) cloned the gene for PepX from *S. thermophilus* ACA-DC4 into a lactococcal strain, while Christensson *et al.* (2002) cloned the gene for an oligopeptidase, PepO, from *Lb. rhamnosus* HN001 (DR 20) and over-expressed it into *Lc. lactis* ssp. *cremoris* NZ9000. The purified PepO enzyme demonstrated specificity for cleavage of α_{s1}-casein fragment 1-23.

Genetic modification (GM) of starter cultures seems a very promising method for accelerating cheese ripening by increasing the pool of enzymes with uniform distribution through the cheese curd matrix. This approach for accelerating cheese ripening is relatively novel and attention to date has focused primarily on the proteolytic system of *Lactococcus*. Recent work on cheese ripening has suggested that the proteinases and peptidases of *Lactococcus*, while very important for proteolysis in cheese during ripening, play only an indirect role in flavour development. Much attention has been paid recently to the importance of amino acid catabolic enzymes to flavour (Yvon and Rijnen, 2001) and exciting developments are expected in the form of starter cultures genetically modified to enhance their amino acid catabolic abilities. However, besides legal barriers to the use of GM starters in industrial cheesemaking, consumer concerns and lack of knowledge of the importance of key or limiting lactococcal enzymes in cheese ripening are stumbling blocks to the successful adoption of GM starters in industrial cheesemaking.

19.6 High-pressure technology

The application of high-pressure treatment (HPT) of milk dates back to the end of the nineteenth century when it was first investigated by Hite (1899). Since then, research in the area of HPT at the industrial level was limited until the advances in ceramics and metallurgy made the utilization of HPT techniques practical in 1970s and 1980s. The success of HPT at an industrial level has drawn the attention of many researchers to the application of HPT to the dairy and other food sectors. A range of pressure-treated food products, including fruit preparations, fruit juices, oysters, raw squid and rice cakes, are available on the French, Japanese and US markets (Trujillo *et al.*, 2000).

According to Le Chatelier's principle, when a disturbance is imposed on a system at equilibrium, the equilibrium shifts in such a way as to minimize the

effect of the disturbance. In the case of HPT, an increase in pressure tends to result in a decrease in volume, which enhances chemical reactions, phase transitions and changes in molecular configuration. Irrespective of size and geometry, the pressure is instantaneously and uniformly distributed throughout the food. The increased pressure affects the environment of bacterial cell and many biochemical reactions in cells. HPT can cause conformational change in proteins but small macromolecules (such as those responsible for flavour and odour) and vitamins are not affected.

The application of HPT to cheese is limited. Studies have been carried out to evaluate the effect of HPT on rennet coagulation time (RCT), curd firmness, acid production by starter cultures, salt uptake, proteolysis, lipolysis and the rheological characteristics of cheese, in addition to the growth and survival of spoilage and pathogenic bacteria. The effects and application of HPT to milk and cheese were reviewed by Trujillo *et al.* (2000), O'Reilly *et al.* (2001) and Huppertz *et al.* (2002).

HPT of cheesemilk affects the coagulation process and its cheesemaking properties. Many studies have shown that HPT of cheesemilk results in improvement of RCT and gel strength. In most studies, pressure up to 200 MPa reduces the RCT compared to untreated milk, while treatment at a pressure in the range 200 to 400 MPa results in reduction in RCT compared to untreated milk but RCT values are higher than those of milk treated at 200 MPa (O'Reilly *et al*, 2001). HPT gives better curd firmness compared to untreated milk. Needs *et al.* (2000) reported that treatment of milk at 200–600 MPa yielded curd with a higher firmness than untreated milk, while López-Fandino *et al.* (1996, 1997) found the pressure treatment below 200 MPa increased curd firming rate, but that this parameter decreased following treatment at 200–400 MPa, though in all cases the rates were higher than in untreated milk. When cheesemilk is treated at a high pressure (above 100 MPa), whey proteins, particularly β-lactoglobulin, are denatured and interact with κ-casein, which leads to smaller losses of whey protein in the whey and increase in moisture retention, hence concomitant increase in the cheese yield.

Similar to thermal treatment, HPT causes destruction of microorganisms but, unlike thermal treatment, it does not inactivate certain enzymes that play an important role in cheese ripening, e.g. indigenous lipoprotein lipase. This characteristic of HPT may allow use of milk without thermal treatment in cheese manufacture. Trujillo *et al.* (1999) found higher production of free fatty acids in HP-treated goats' milk compared to pasteurized milk, indicating higher lipoprotein lipase activity in HP-treated milk than in pasteurized milk. Buffa *et al.* (2001) found that the levels of free fatty acids were higher in cheeses made from HP-treated goats' milk than in cheeses made from pasteurized milk; levels were similar to those in cheeses made from raw milk.

The application of HPT to cheese results in an increase in moisture content and pH and causes changes to the cheese matrix and lysis of cells, which contribute to ripening. HPT of cheeses affects the pattern of proteolysis during ripening, the effect of which is dependent on the type of cheese, magnitude,

duration and temperature of pressure treatment and the age of the cheese. Yokoyama *et al.* (1992) applied pressure of 10 to 250 MPa at 25ºC for 3 days to Cheddar cheese, made using a 10-fold higher level of a proteolytic starter. The authors claimed that the flavour of the treated cheese was equivalent to that of six-month-old Cheddar. The treated cheese had higher levels of free amino acids than control cheeses, but this may have been due to the use of a more proteolytic starter in cheese manufacture. The authors also treated cheese at 50 MPa at 25ºC for 3 days in combination with addition of lipase and protease at salting and claimed that the resultant cheese developed a Parmesan-type flavour equivalent to a commercial control in terms of flavour scores and levels of FAA. O'Reilly *et al.* (2000) studied the effect of 50 MPa at 25ºC for 3 days on Cheddar cheese ripening. Results indicated an immediate increase in the levels of pH 4.6-soluble nitrogen and free amino acids at 2 days of ripening, although the effect decreased with cheese age. Higher breakdown of α_{s1}-casein was observed by urea-polyacrylamide gel electrophoresis in the pressure-treated cheese compared to the untreated cheese. The effect of HPT on the degradation of α_{s1}-casein observed by O'Reilly *et al.* (2000) may have been due to pressure-induced modification of the substrate or to the high temperature (25ºC) used for HPT. These authors also suggested that HPT is more effective in increasing proteolysis if applied to young cheese than to cheese that had been ripened for a few months. Plasmin activity remained unaffected by the above HPT and no differences were observed in the levels of α_{s2}-caseins and β-caseins between HPT cheeses and controls.

Goats' milk cheese was pressure treated at 50 MPa for 72 h or at 400 MPa for 5 min by Saldo *et al.* (2000). Treatment at 400 MPa for 5 min reduced starter counts by ~3 log cycles, while treatment at 50 MPa for 72 h decreased the starter counts slightly. As the ripening progressed (after 3 weeks), the starter population recovered. Lysis of starter by HPT is important in cheese ripening, as it releases intracellular enzymes into the cheese matrix, which play an important role in the breakdown of large and intermediate size peptides to small peptides and amino acids. The treatment resulted in an increase in pH and in levels of proteolysis. Saldo *et al.* (2002) pressure treated hard caprine milk cheese at 50 MPa for 72 h or at 400 MPa for 5 min. Pressure treatment at 50 MPa for 72 h resulted in slight differences compared to the control which became less apparent at the end of the ripening, while pressure treatment at 400 MPa for 5 min resulted in qualitative and quantitative differences that persisted throughout the ripening.

Messens *et al.* (1999) found no increases in indices of proteolysis when Gouda cheese was HP treated. However, a pH shift was observed between pressurized and non-pressurized cheese. HPT at 50 MPa for 8 h of Père Joseph, a French semi-hard smear-ripened cheese, resulted in a higher pH, which enhanced the proteolytic activity of the enzymes of *Brevibacterium linens* and peptidases of the starter bacteria (Messens *et al.*, 2000). Similar effects on proteolysis were observed when Paillardin, a white-mould ripened cheese, was HP treated at 50 MPa for 8 h by Messens *et al.* (2001).

19.7 Enzyme-modified cheeses as flavourings

The use of cheese flavours in various food preparations has increased significantly in recent times. Natural cheese can be used to obtain a characteristic cheese flavour in food products. However, variations in the flavour of natural cheeses due to variations in milk composition, degree of ripening, cost and other factors have led to the development of alternative sources of cheese flavours

Table 19.2 Differences between enzyme-modified cheeses (EMC) and natural cheeses (modified from Wilkinson and Kilcawley, 2002)

Parameter	EMC	Natural cheese variety (Cheddar, Gouda, Swiss, blue)
Raw material	Cheese curd, natural cheese, caseinate, butterfat	Milk, cream (standardized to protein to fat ratio)
Additives	Water, emulsifiers, enzymes (proteinases/peptidases, lipases/esterases or combination thereof), potentiators (monosodium glutamate, yeast extract, diacetyl)	Starter culture, adjuncts, $CaCl_2$, rennet, salt
Fermentation	Time 24–72 h Temperature 30–45ºC	Acidification during manufacture (5–24 h) or shortly thereafter
Heat treatments	Pasteurization of curd-water slurry to inactivate bacteria or other contaminants. Heat treatment of enzyme treated slurry at high temperatures (~85ºC for 30 min) to terminate enzymatic reactions	Cooking treatment of curd–whey mixture Up to 36ºC for Gouda, 39ºC for Cheddar, 55ºC for Swiss
Ripening	Not deliberately ripened after manufacture	Ripening time 0.5 to >48 months Ripening temperature 4–23ºC
Flavour genesis	Exogenous enzymes (contribute to extensive proteolytic and lipolytic changes)	Proteolysis, lipolysis, 'glycolysis' (metabolism of residual lactose and of lactate and citrate) by agents from the milk and coagulant, and from starter and non-starter microorganisms
Final product	In paste or powder form; high in flavour intensity with long shelf-life	Solid; lower flavour intensity with relatively short shelf-life after ripening
Legal parameters	Undefined legal parameters	Often defined legal parameters for cheese (pH, salt levels, fat-in-dry-matter, moisture, manufacturing technology)

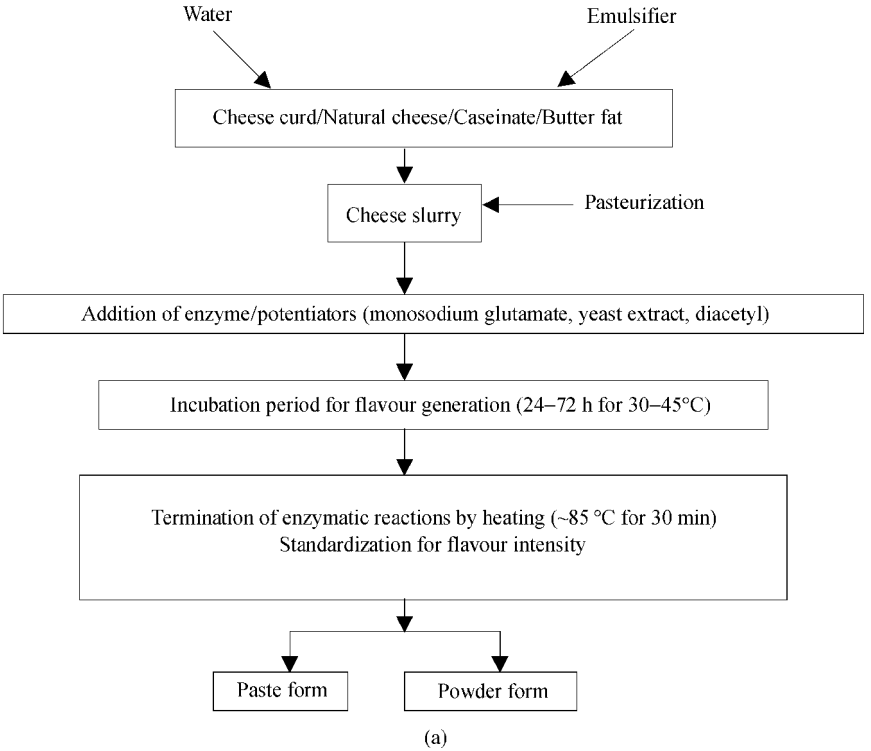

Fig. 19.3 Manufacture of EMC (modified from Wilkinson and Kilcawley, 2002): (a) one-step approach; (b) component approach.

(Kilcawley *et al.*, 1998). One such source is enzyme-modified cheeses (EMCs). EMCs are categorized as flavour preparations (EU, 1988) and have GRAS (generally regarded as safe) status under US regulations. EMCs have the advantage of cost effectiveness, ease of production, enhanced product stability, consistency in flavour and ease of handling over natural cheeses made by traditional processes (Kilcawley *et al.*, 1998). EMCs differ from traditional natural cheese in many different aspects. A comparison of EMC and natural cheeses is given in Table 19.2.

EMCs have approximately 5–25 times the flavour intensity of natural cheese (Moskowitz and Noelck, 1987). EMC flavours that are commercially available include Blue, Brick, Cheddar, Colby, Emmental, Feta, Gouda, Gruyère, Mozzarella, Parmesan, Provolone and Romano. EMCs have a wide range of application in various food preparations and are generally added to these preparations at levels of 0.1–2.0%, although they can be used up to 5% (Moskowitz and Noelck, 1987). The use of EMCs is ideal for frozen cheese-type products in which the proteins of natural cheese tend to produce a grainy texture (Kilcawley *et al.*, 1998). Because of their high flavour intensity, a given cheese flavour intensity can be obtained in a product using lower levels

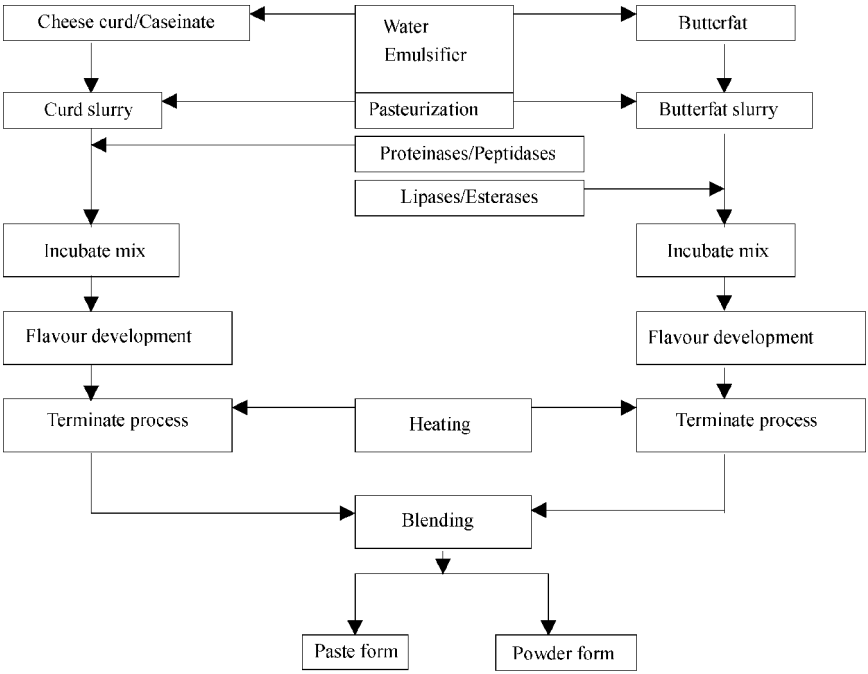

Fig. 19.3 continued

of EMCs than with natural cheese. Thus, low-fat cheese flavoured food products can be made using EMCs which are attractive to health-conscious consumers.

EMCs can be manufactured in two different ways, namely a one-step process (Fig. 19.3a) or a component approach process (Fig. 19.3b). The one-step process involves simultaneous hydrolysis of fat and protein, while in the component approach process, several flavour components are created separately and, at the end of hydrolysis, components are blended to give the final EMC product.

Companies that produce EMCs usually use different starting materials and enzyme preparations. The base material for the manufacture of EMC is usually immature cheese curd, although caseinates are used occasionally. In order to obtain the most authentic flavour, immature cheese curd of the target cheese may be used as the starting material. Small amounts of mature cheese may also be included in the blend (Wilkinson and Kilcawley, 2002).

Emulsifying agents, usually calcium sequestrants, are used in the production of EMCs. These salts solubilize protein by chelating Ca^{2+} and the solubilized proteins act as emulsifiers for the fat. The emulsified state of fat facilitates lipase action. The fat fraction is freely emulsified in EMC, increasing the area of the oil/water interface and providing improved conditions for the action of lipases (Villeneuve and Foglia, 1997).

Flavour enhancers (potentiators) such as monosodium glutamate, yeast extract or diacetyl can be used in the production of EMCs to enhance the flavour perception and savoury attributes of the product. The use of natural, nature-identical or artificial flavours is permitted for a specific flavour note but has to be declared on the label of the final product into which the EMC is added.

Selection of the enzyme or enzyme blend is very important in the production of EMC as it influences the flavour development directly. Generally, use of broad specificity proteinases ensures rapid proteolysis for flavour development. Most of the proteinases used in EMCs are derived from *Bacillus* spp. or *Aspergillus* spp. and often result in the production of bitter peptides from the caseins. Hence, it becomes necessary to include peptidases in the enzyme mix to break down these bitter peptides. Generally, the peptidases used are aminopeptidases from *Aspergillus oryzae* or from LAB. Use of lipases, which cause intense flavour development, in the enzyme mix can reduce reliance on proteinases and peptidases (Godfrey and Hawkins, 1991). Lipases in the enzyme mix are very important when producing Romano and blue cheese-type flavours as lipolysis contributes strongly to flavour development in these varieties.

After the preparation of the cheese slurry, it is necessary to pasteurize it so as to kill pathogenic or spoilage bacteria, as the incubation conditions used in EMC manufacture are favourable for their growth. Care should be taken to avoid subsequent contamination, and sterility of the equipment must be ensured. Heat treatment also inactivates many enzymes in the base material (e.g., residual coagulant and starter proteinases and peptidases) and thus helps to standardize the starting material. During the incubation period, efficient control of time, temperature, stirring and pH is necessary in order to obtain consistency in the final product. When the desired flavour is achieved, the enzymes are inactivated by heat treatment. The extent of heat treatment is very critical so as to inactivate enzyme without affecting the developed flavour. It is necessary to inactivate the enzymes, as continued activity would lead to instability in the EMC product, or residual enzyme activity may lead to the development of off-flavours in the food preparations in which EMCs are used. Hence, it is necessary to check EMCs for the residual proteinase and lipase activity after manufacture. Some processes used industrially in EMC manufacture were discussed by Kilcawley *et al.* (1998).

19.8 Future trends

Because of the cost of ripening hard cheeses, research into acceleration of ripening will continue in the future. Of the approaches discussed above, some have been restricted to date to academic research (e.g., high-pressure treatment) while others are used commercially to a lesser or greater extent (e.g., elevated temperatures and attenuated or adjunct cultures). As novel processing technologies become available, it is likely that they will find application in the area of acceleration of cheese ripening. The simplest and most successful

approach to accelerate ripening studied to date is elevated temperature. Modification of the ripening temperature is used to control the rate of flavour development in hard cheese, and high temperature ripening (e.g., *ca.* 16ºC) results in the rapid development of flavour, although problems can occur with texture but this is not a serious drawback if the cheese is to be used in certain ingredient applications. Finally, recent advances in the genetics of LAB and a greater understanding of the role of specific enzymes in the generation of volatile flavour compounds in cheese during ripening will facilitate the development of starter strains genetically modified to enhance flavour development.

19.9 Acknowledgement

The authors wish to thank Professor P. F. Fox for valuable comments and suggestions.

19.10 Sources of further information and advice

EL SODA M and PANDIAN S (1991), 'Recent developments in accelerated cheese ripening', *J Dairy Sci*, 74, 2317–2335.

FOX P F (1988/9), 'Acceleration of cheese ripening', *Food Biotechnol*, 2, 133–185.

FOX P F and TOBIN J (1999), 'Acceleration and modification of cheese ripening', *Proc 36th Annual Marschall Cheese Seminars*, 3, Santa Clara, CA.

FOX P F, WALLACE J M, MORGAN S, LYNCH C M, NILAND E J and TOBIN J (1996), 'Acceleration of cheese ripening', *Antonie van Leeuwenhoek*, 70, 271–297.

KLEIN N and LORTAL S (1999), 'Attenuated starters: an efficient means to influence cheese ripening – a review', *Int Dairy J*, 9, 751–762.

LAW B A (2001), 'Controlled and accelerated cheese ripening: the research base for new technologies', *Int Dairy J*, 11, 383–398.

WILKINSON M G (1993), 'Acceleration of cheese ripening', in Fox P F (ed.), *Cheese: Chemistry, Physics and Microbiology*, 2nd edn, Vol. 1, London, Chapman & Hall, pp. 523–555.

19.11 References

ANASTASIOU R, PAPADELLI M, GEORGALAKI M D, KALANTZOPOULOS G and TSAKALIDOU E (2002), 'Cloning and sequencing of the gene encoding X-prolyl-dipeptidyl aminopeptidase (Pep X) from *Streptococcus thermophilus* strain ACA-DC 4', *J Appl Microbiol*, 93, 52–59.

ARBIGE M V, FREUND P R, SILVER S C and ZELRO J T (1986), 'Novel lipase for Cheddar cheese flavor development', *Food Technol*, 40, 91–98.

ARDÖ Y and PETTERSON H E (1988), 'Accelerated ripening with heat-shocked *Lb. helveticus* and a commercial proteinase', *J Dairy Res*, 55, 239–245.

ASTON J W, FEDRICK I A, DURWARD I G and DULLEY J R (1983a), 'The effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese. 1. Higher initial storage temperatures', *NZ J Dairy Sci Technol*, 18, 143–151.

ASTON J W, GRIEVE P A, DURWARD I G and DULLEY J R (1983b), 'Proteolysis and flavour development in Cheddar cheeses subjected to accelerated ripening treatments', *Aust J Dairy Technol*, 38, 59–65.

ASTON J W, GILES J E, DURWARD I G and DULLEY J R (1985), 'Effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese', *J Dairy Res*, 52, 565–572.

BANKS J, HUNTER E and MUIR D (1993), 'Sensory properties of low-fat Cheddar cheese: effects of salt content and adjunct culture', *J Soc Dairy Technol*, 46, 119–123.

BARRETT F M, KELLY A L, MCSWEENEY P L H and FOX P F (1999), 'Use of exogenous urokinase to accelerate proteolysis in Cheddar cheese during ripening', *Int Dairy J*, 9, 421–427.

BARTELS H J, JOHNSON M E and OLSON N F (1987), 'Accelerated ripening of Gouda cheese. II. Effect of freeze shocked *Lb. helveticus* on proteolysis and flavour development', *Milchwissenschaft*, 42, 139–144.

BEUVIER E, BERTHAUD K, CEGARRA S, DASEN A, POCHET S, BUCHIN S and DUBOZ G (1997), 'Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltered milk', *Int Dairy J*, 7, 311–323.

BIRKELAND S E, ABRAHAMSEN R K and LANGSRUD T (1992), 'Accelerated cheese ripening: use of a Lac^- mutants of lactococci', *J Dairy Res*, 59, 389–400.

BOLOTIN A, WINCKER P, MAUGER S, JAILLON O, MALARME K, WEISSENBACH J, EHRLICH S D and SOROKIN A (2001), 'The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403', *Genome Res*, 11, 731–753.

BOUTON Y and GRAPPIN R (1995), 'Comparaison de la qualité de fromages à pâte pressée cuite fabriqués à partir de lait cru ou microfiltré', *Lait*, 75**,** 31–44.

BUFFA M, GUAMIS B, PAVIA M and TRUJILLO A J (2001), 'Lipolysis in cheese made from raw, pasteurized or high-pressure treated goats' milk', *Int Dairy J*, 11, 175–179.

BUIST G, KOK J, LEENHOUTS K J, DABROWSKA M, VENEMA G and HAANDRIKMAN A J (1995), 'Molecular cloning and nucleotide sequence of the gene encoding the major peptidoglycan hydrolase of *Lactococcus lactis*, a muramidase needed for cell separation', *J Bacteriol*, 177, 1554–1563.

CHRISTENSEN J E, DUDLEY E G, PEDERSON J A and STEELE J L (1999), 'Peptidases and amino acid catabolism in lactic acid bacteria', *Antonie van Leeuwenhoek*, 76, 217–246.

CHRISTENSSON C, HENRIK B, COLLINS L J, COOLBEAR T, HOLLAND R, LUBBERS M W, O'TOOLE P W and REID J R (2002), 'Cloning and expression of an oligopeptidase, Pep O, with novel specificity from *Lactobacillus rhamnosus* HN001 (DR20)', *Appl Environ Microbiol*, 68, 254–262.

COURTIN P, NARDI M, WEGMANN U, JOUTSJOKI V, OGIER J C, GRIPON J C, PALVA A, HENRICH B and MONNET V (2002), 'Accelerating cheese proteolysis by enriching *Lactococcus lactis* proteolytic system with lactobacilli peptidases', *Int Dairy J*, 12, 447–454.

CROMIE S J, GILES J E and DULLEY J R (1987), 'Effect of elevated ripening temperatures on the microflora of Cheddar cheese', *J Dairy Res*, 54, 69–76.

CROW V L, COOLBEAR T, GOPAL P K, MARTLEY F, MCKAY L L and RIEPE H (1995a), 'The role of autolysis of lactic acid bacteria in the ripening of cheese', *Int Dairy J*, 5, 855–875.

CROW V L, MARTLEY G M, COOLBEAR T and ROUNDHILL S J (1995b), 'The influence of phage assisted-lysis of *Lactococcus lactis* ssp. *lactis* ML8 on Cheddar cheese ripening', *Int Dairy J*, 5, 451–472.

DULLEY J R, BROOKS D E J and GRIEVE P A (1978), 'The possible use of Lac$^-$ starter strains to accelerate cheese ripening and a method for their detection in cheese', *Proc XX International Dairy Congress*, Paris, p 485.

EL SODA M (1993), 'Accelerated maturation of cheese', *Int Dairy J*, 3, 531–544.

EL SODA M and PANDIAN S (1991), 'Recent developments in accelerated cheese ripening', *J Dairy Sci*, 74, 2317–2335.

EL SODA M, PANNELL L and OLSON N (1989), 'Microencapsulated enzyme systems for the acceleration of cheese ripening', *J Microencapsul*, 6, 319–326

EL SODA M, MADKOR S A and TONG P S (2000a), 'Evaluation of commercial adjuncts for the use in cheese ripening: 4. Comparision between attenuated and non attenuated lactobacilli', *Milchwissenschaft*, 55, 260–263.

EL SODA M, MADKOR S A and TONG P S (2000b), 'Adjunct cultures: recent developments and potential significance to the cheese industry', *J Dairy Sci*, 83, 609–619.

EUROPEAN UNION (1988), *Official Journal of the European Communities*, 31 (L184), 61–66. Completed by Directive No. 91/71/EEC of 16 January 1991, 34 (L42), 25.

EXTERKATE F A, DE VEER G J and STADHOUDERS J (1987), 'Acceleration of the ripening process of Gouda cheese by using heat-treated mixed-strain starter cells', *Neth Milk Dairy J*, 41, 307–320.

FARKYE N Y and FOX P F (1992), 'Contribution of plasmin to Cheddar cheese ripening: effect of added plasmin', *J Dairy Res*, 59, 209–216.

FEDRICK I A (1987), 'Technology and economics of the accelerated ripening of Cheddar cheese', *Proc Ann Conf Victorian Div Dairy Ind Assoc Aust*, Melbourne.

FEDRICK I A and DULLEY J R (1984), 'The effect of elevated storage temperatures on the rheology of Cheddar cheese', *NZ J Dairy Sci Technol*, 19, 141–150.

FEDRICK I A, ASTON J W, DURWARD I F and DULLEY J R (1983), 'The effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese. I. High temperature storage midway during ripening', *NZ J Dairy Sci Technol*, 18, 253–260.

FEIRTAG M J and MCKAY L L (1987), 'Thermoinducible lysis of temperature-sensitive *Streptococcus cremoris* strains', *J Dairy Sci*, 70, 1779–1784.

FOLKERTSMA B, FOX P F and MCSWEENEY P L H (1996), 'Accelerated ripening of Cheddar cheese at elevated temperatures', *Int Dairy J*, 6, 1117–1134.

FOX P F (1988/9), 'Acceleration of cheese ripening', *Food Biotechnol*, 2, 133–185.

FOX P F and MCSWEENEY P L H (1997), 'Rennets: their role in milk coagulation and cheese ripening', in Law B A (ed.), *The Microbiology and Biochemistry of Cheese and Fermented Milk*, 2nd edn, London, Chapman & Hall, pp 1–49.

FOX P F and TOBIN J (1999), 'Acceleration and modification of cheese ripening', *Proc 36th Annual Marschall Cheese Seminars*, 3, Santa Clara, USA.

FOX P F, LAW J, MCSWEENEY P L H and WALLACE J (1993), 'Biochemistry of cheese ripening', in Fox P F (ed.), *Cheese: Chemistry, Physics and Microbiology*, Vol 1, London, Chapman & Hall, pp 389–438.

FOX P F, O'CONNOR T P, MCSWEENEY P L H, GUINEE T P and O'BRIEN N M (1996a), 'Cheese: physical, chemical, biochemical and nutritional aspects', *Adv Food Nutr Res*, 39, 163–328.

FOX P F, WALLACE J M, MORGAN S, LYNCH C M, NILAND E J and TOBIN J (1996b), 'Acceleration of cheese ripening', *Antonie van Leeuwenhoek*, 70, 271–297.

FOX P F, MCSWEENEY P L H and LYNCH C M (1998), 'Significance of non-starter lactic acid bacteria in Cheddar cheese', *Aust J Dairy Technol*, 53, 83–89.

GODFREY T and HAWKINS D (1991), 'Enzymatic modification of fats for flavour', *Eur Food*

Drink Rev, Autumn, 103–107.

GRIEVE P A and DULLEY J R (1983), 'Use of *Streptococcus lactis* Lac$^-$ mutants for accelerating Cheddar cheese ripening. 2. Their effect on rate of proteolysis and flavour development', *Aust J Dairy Tech*, 38, 49–54.

GUINEE T P, WILKINSON M G, MULLHOLLAND E and FOX P F (1991), 'Influence of ripening temperature, added commercial enzyme preparations and attenuated, mutant (lac$^-$) *Lactococcus lactis* starter on the proteolysis and maturation of Cheddar cheese', *Ir J Food Sci Technol*, 15, 27–51.

HANNON J, WILKINSON M G, DELAHUNTY C M, WALLACE J M, MORRISSEY P A and BERESFORD T P (2003), 'The use of autolytic starter systems to accelerate ripening of Cheddar cheese', *Int Dairy J*, 13, 313–323.

HITE B H (1899), 'The effect of pressure in the preservation of milk', *West Virginia Agric Exp Stat Bull*, 58, 15–35.

HOLLAND R, LIU S Q, WANG T, BENNETT M, NORRIS G, DELABRE M L, LUBBERS M W, DEKKER J W and CROW V L (2002), 'Esterases of lactic acid bacteria', *Aust J Dairy Technol*, 57, 116.

HUPPERTZ T, KELLY A L and FOX P F (2002), **'**Effects of high pressure on constituents and properties of milk', *Int Dairy J*, 12, 561–572.

JOHNSON J A C and ETZEL M R (1995), 'Properties of *Lb. helveticus* CNRZ-32 attenuated by spray-drying, freeze drying or freezing', *J Dairy Sci*, 78, 761–768.

JOUTSJOKI V, LOUMA S, TAMMINEN M, KILPI M, JOHANSEN E and PALVA A (2002), 'Recombinant *Lactococcus* starters as a potential source of additional peptidolytic activity in cheese ripening', *J Appl Microbiol*, 92, 1159–1166.

KATSIARI M C, VOUTSINAS L P and KONDYLI E (2002), 'Improvement of sensory quality of low-fat Kefalograviera-type cheese with commercial adjunct cultures', *Int Dairy J*, 12, 757–764.

KILCAWLEY K N, WILKINSON M G and FOX P F (1998), 'Enzyme-modified cheese', *Int Dairy J*, 8, 1–10.

KIRBY C J, BROOKER B E and LAW B A (1987), 'Accelerated ripening of cheese using liposome-encapsulated enzyme', *Int J Food Sci Technol*, 22, 355–375.

KLEIN N and LORTAL S (1999), 'Attenuated starters: an efficient means to influence cheese ripening – a review', *Int Dairy J*, 9, 751–762.

KOSIKOWSKI F V (1976), 'Flavour development by enzyme preparations in natural and processed cheese', US Patent 3, 975,544.

LANE C N and FOX P F (1996), 'Contribution of starter and adjunct lactobacilli to proteolysis in Cheddar cheese during ripening', *Int Dairy J*, 6, 715–728.

LAW B A (2001), 'Controlled and accelerated cheese ripening: the research base for new technologies', *Int Dairy J*, 11, 383–398.

LAW B A and KING J C (1985), 'The use of liposomes for the addition of enzymes to cheese', *J Dairy Res*, 52, 183–188.

LAW B A and WIGMORE A S (1983), 'Accelerated cheese ripening of Cheddar cheese with commercial proteinase and intracellular enzymes from starter streptococci', *J Dairy Res*, 50, 519–525.

LAW B A, SHARPE M E and REITER B (1974), 'The release of intracellular dipeptidases from starter streptococci during Cheddar cheese ripening', *J Dairy Res*, 41, 137–146.

LAW B A, HOSKING Z D and CHAPMAN H R (1979), 'The effect of some manufacturing conditions on the development of flavour in Cheddar cheese', *J Soc Dairy Technol*, 32, 87–90.

LEIGH J A (1993), 'Activation of bovine plasminogen by *S. uberis*', *FEMS Microbiol Lett*,

114, 67–72.

LÓPEZ-FANDINO R, CARRASCOSA A V and OLANO A (1996), 'The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk', *J Dairy Sci*, 79, 929–936.

LÓPEZ-FANDINO R, RAMOS M and OLANO A (1997), 'Rennet coagulation of milk subjected to high pressures', *J Agric Food Chem*, 45, 3233–3237.

LYNCH C M, MCSWEENEY P L H, FOX P F, COGAN T M and DRINAN F D (1996), 'Manufacture of Cheddar cheese with and without adjunct lactobacilli under controlled microbiological conditions', *Int Dairy J*, 6, 851–867.

MARTÍNEZ-CUESTA M C, REQUENA T and PELÁEZ C (2001), 'Use of a bacteriocin producing transconjugant as starter in acceleration of cheese ripening', *Int J Food Microbiol*, 70, 79–88.

MCGARRY A, EL-KHOLI A, LAW J, COFFEY A, DALY C, FOX P F and FITZGERALD G F (1994), 'Impact of manipulating the lactococcal proteolytic system on ripening and flavour development in Cheddar cheese', *Proc 4th Meeting BRIDGE T-Project*, Oviedo, Spain, p 32 (abstr.).

MCGARRY A, LAW J, COFFEY A, DALY C, FOX P F and FITZGERALD G F (1995), 'Effect of genetically modifying the lactococcal proteolytic system on ripening and flavour development in Cheddar cheese', *Appl Environ Microbiol*, 60, 4226–4233.

MCKAY L L and BALDWIN K A (1976), 'Plasmid distribution and evidence for a proteinase plasmid in *S. lactis*', *Appl Microbiol*, 29, 546–548.

MCSWEENEY P L H and SOUSA M J (2000), 'Biochemical pathways for the production of flavour compounds in cheese during ripening', *Lait*, 80, 293–324.

MCSWEENEY P L H, FOX P F, LUCEY J A, JORDAN K N and COGAN T M (1993), 'Contribution of the indigenous microflora to the maturation of Cheddar cheese', *Int Dairy J*, 3, 613–634.

MESSENS W, ESTEPAR-GARCIA J, DEWETTINCK K and HUYGHEBAERT A (1999), 'Proteolysis of high-pressure treated Gouda cheese', *Int Dairy J*, 9, 775–782.

MESSENS W, FOUBERT I, DEWETTINCK K and HUYGHEBAERT A (2000), 'Proteolysis of a high-pressure treated smear ripened cheese', *Milchwissenschaft*, 55, 328–332.

MESSENS W, FOUBERT I, DEWETTINCK K and HUYGHEBAERT A (2001), 'Proteolysis of a high-pressure treated mould ripened cheese', *Milchwissenschaft*, 56, 201–204.

MORGAN S, O'DONOVAN C, ROSS R P, HILL C and FOX P F (1995), 'Significance of autolysis and bacteriocin-induced lysis of starter cultures in Cheddar cheese ripening', *Proc 4th Cheese Symp*, Moorepark, Fermoy, Co. Cork, Ireland, pp. 51–60.

MORGAN S, ROSS R P and HILL C (1997), 'Increasing starter cell lysis in Cheddar cheese using a bacteriocin-producing adjunct', *J Dairy Sci*, 80, 1–10.

MOSKOWITZ G J and NOELCK S S (1987), 'Enzyme modified cheese technology', *J Dairy Sci*, 70, 1761–1769.

MOU L, SULLIVAN J J and JAGO G R (1976), 'Autolysis of *Streptococcus cremoris*', *J Dairy Res*, 43, 275–282.

NAKAJIMA H, TOYODA S, KITAMURA K and AHOKI K (1991), 'Accelerated ripening of Gouda cheese: direct inoculation of a lactose-negative mutant of *Lactococcus lactis* subsp. *cremoris* into cheesemilk', *Milchwissenschaft*, 46, 8–10.

NEEDS E C, STENNING R A, GILL A L, FERRAGUT V and RICH G T (2000), 'High-pressure treatment of milk: effects of casein micelle structure and on enzymatic coagulation', *J Dairy Res*, 67, 31–42.

O'CONNELL P B (2002), 'Studies in lipolysis in cheese during ripening', MSc thesis, National University of Ireland, Cork.

O'DONOVAN C M, WILKINSON M G, GUINEE T P and FOX P F (1996), 'An investigation of the autolytic properties of three lactococcal strains during cheese ripening', *Int Dairy J*, 6, 1149–1165.

O'REILLY C E, O'CONNOR P M, MURPHY P M, KELLY A L and BERESFORD T P (2000), 'The effect of exposure to pressure of 50 MPa on Cheddar cheese ripening', *Innov Food Sci Emer Technol*, 1, 109–117.

O'REILLY C E, KELLY A L, MURPHY P M and BERESFORD T P (2001), 'High pressure treatment: application in cheese manufacture and ripening', *Trends Food Sci Tech*, 12, 51–59.

O'SULLIVAN L, MORGAN S M, ROSS R P and HILL C (2002), 'Elevated enzyme release from lactococcal starter cultures on exposure to the lantibiotic lacticin 481, produced by *Lactococcus lactis* DPC5552', *J Dairy Sci*, 85, 2130–2140.

PETTERSON H E and SJÖSTRÖM G (1975), 'Accelerated cheese ripening: a method for increasing the number of lactic starter bacteria in cheese without detrimental effect to the cheese-making process, and its effect on the cheese ripening', *J Dairy Res*, 42, 313–326.

PILLIDGE C J, GOVINDASAMY-LUCEY R, GOPAL P K and CROW V L (1998), 'The major lactococcal cell wall autolysin AcmA does not detemine the rate of autolysis of *Lactococcus lactis* ssp. *cremoris* 2250 in Cheddar cheese', *Int Dairy J*, 8, 843–850.

PILLIDGE C J, RALLABHANDI P S V, TONG X, GOPAL P K, FARLEY P C and SULLIVAN P A (2002), 'Autolysis of *Lactococcus lactis*', *Int Dairy J*, 12, 133–140.

RYAN M P, REA M C, HILL C and ROSS R P (1996), 'An application in Cheddar cheese manufacture for a strain of *Lactococcus lactis* producing a novel broad-spectrum bacteriocin, Lacticin 3147', *Appl Environ Microbiol*, 62, 612–619.

SALDO J, MCSWEENEY P L H, SENDRA E, KELLY A L and GUAMIS B (2000), 'Changes in curd acidification caused by high pressure treatment', *Ir J Agric Food Res*, 39, 169.

SALDO J, MCSWEENEY P L H, SENDRA E, KELLY A L and GUAMIS B (2002), 'Proteolysis in caprine milk cheese treated by high pressure to accelerate cheese ripening', *Int Dairy J*, 12, 35–44.

SALOMSKIENE J (1998), 'Use of heat-treated starter for the intensification of cheese ripening', *Milchwissenschaft*, 53, 28–30.

SHAKEEL-UR-REHMAN, MCSWEENEY P L H and FOX P F (1999), 'A study on the role of the indigenous microflora of raw milk on the ripening of Cheddar cheese', *Milchwissenschaft*, 54, 388–392.

SHAKEEL-UR-REHMAN, BANKS J M, MCSWEENEY P L H and FOX P F (2000), 'Effect of ripening temperature on the growth and significance of non-starter lactic acid bacteria in Cheddar cheese made from raw or pasteurized milk', *Int Dairy J*, 10, 45–53.

SKEIE S, NARVHUS J A, ARDÖ Y, THORVALDSEN K and ABRAHAMSEN R K (1997), 'The effect of reduced salt content on the function of liposome-encapsulated neutrase and heat-treated lactobacilli in rindless low-fat cheese', *Lait*, 77, 575–585.

SMITH M R, BROWNING P D and PAWLETT D (2000), 'Cheese ripening process', World Patent 00000037.

TOBIN J (1999), 'Effects of adjunct cultures and starter blends on the quality of Cheddar cheese', PhD thesis, National University of Ireland, Cork.

TRUJILLO A J, ROYO C, GUAMIS B and FERRAGUT V (1999), 'Influence of pressurization on goat milk and cheese composition and yield', *Milchwissenschaft*, 54, 197–199.

TRUJILLO A J, CAPELLAS M, BUFFA M, ROYO C, GERVILLA R, FELIPE X, SENDRA E, SALDO J, FERRAGUT V and GUAMIS B (2000), 'Application of high pressure treatment for cheese production', *Food Res Int*, 33, 311–316.

VILLENEUVE P and FOGLIA T A (1997), 'Lipase specificities: potential application in lipid bioconversions', *INFORM*, 8, 640–650.

WILKINSON M G (1993), 'Acceleration of cheese ripening', in Fox P F (ed.), *Cheese: Chemistry, Physics and Microbiology*, 2nd edn, Vol 1, London, Chapman & Hall, pp 523–555.

WILKINSON M G and KILCAWLEY K N (2002), 'Technology of enzyme-modified cheese and natural cheese', Bulletin 371, Brussels, International Dairy Federation, pp 10–15.

WILKINSON M G, GUINEE T P, O'CALLAGHAN D M and FOX P F (1994a), 'Autolysis and proteolysis in different strains of starter bacteria during Cheddar cheese ripening', *J Dairy Res*, 61, 249–262.

WILKINSON M G, GUINEE T P and FOX P F (1994b), 'Factors which may influence the determination of autolysis of starter bacteria during Cheddar cheese ripening', *Int Dairy J*, 4, 141–160.

YOKOYAMA H, SAWAMURA N and MOTOBAYASHI N (1992), 'Method for accelerating cheese ripening', *Eur Patent* 0 469 587 A1.

YVON M and RIJNEN L (2001), 'Cheese flavour formation by amino acid catabolism', *Int Dairy J*, 11, 185–201.