ADVANCES IN FOOD AND NUTRITION RESEARCH, VOL. 39

CHEESE: PHYSICAL, BIOCHEMICAL, AND NUTRITIONAL ASPECTS

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I. INTRODUCTION

A. HISTORICAL

Cheese is the generic name for a diverse group of fermented milk-based foods produced in at least 500 varieties throughout the world. Cheese evolved in the "Fertile Crescent" between the Tigris and Euphratres rivers, in Iraq, some 8000 years ago. From a very early stage in the Agricultural Revolution, Man consumed milk from domesticated animals but since milk is a rich source of nutrients for contaminating bacteria, it has a short shelf life, especially in warm climates.

Certain bacteria (lactic acid bacteria, LAB) ferment milk sugar, lactose, as a source of energy, producing lactic acid as a by-product. When sufficient

acid is produced, the principal proteins of milk, the caseins, coagulate, i.e., at their isoelectric points (~pH 4.6), to form a gel, entrapping the fat. Acid-coagulated (fermented) milks are produced throughout the world and are increasing in popularity. The ability of LAB to produce just enough acid to coagulate the caseins is quite fortuitous: neither the LAB nor the caseins were designed for this function. The caseins were "designed" to be enzymatically coagulated in the stomachs of neonatal mammals at ca. pH 6. The ability of LAB to ferment lactose, a sugar specific to milk, is encoded on plasmids, suggesting that this characteristic was acquired relatively recently. Their natural habitats are vegetation, from which they colonized the intestinal tract and the teats of mammals, contaminated with milk; through evolutionary pressure, these bacteria probably acquired the ability to ferment lactose.

When an acid-coagulated milk gel is broken, the pieces synerese, expressing whey. Acid-coagulated casein is the starting material for a family of cheeses that are usually consumed fresh, i.e., are not matured (ripened); major examples are Cottage and cream cheeses, Quarg, and fromage frais.

An alternative mechanism for milk coagulation exploits the ability of many proteinases, referred to as rennets, to modify the milk protein system, leading to coagulation. Rennets from bacteria, moulds, and plant and animal tissues may be used, but the principal traditional source was stomachs of neonatal mammals, the principal proteinase in which is chymosin. The properties of rennet-coagulated curds are very different from those of acid-coagulated curds, e.g., they exhibit better syneretic properties which make it possible to produce low-moisture cheese curd without hardening, thus permitting the production of more stable products than is possible from acid-coagulated curds. Therefore, rennet coagulation is exploited in the manufacture of most cheese varieties, which are normally ripened.

Cheese manufacture accompanied the spread of civilization throughout the Middle East, Egypt, and Greece and was well established in the Roman Empire. Cheese making remained a localized, essentially farmstead, enterprise until the mid-19th century. Due to particular local circumstances, certain varieties of cheese evolved in specific regions and remained localized due to limited communications. Hence, several hundred varieties of cheese evolved, most of which are still produced locally although the principal varieties, Cheddar, Dutch, Swiss, Camembert, and fromage frais types, are now produced internationally. Information on the history of cheese can be found in Davis (1965), R. Scott (1986), and Fox (1993b).

B. CHEESE PRODUCTION AND CONSUMPTION

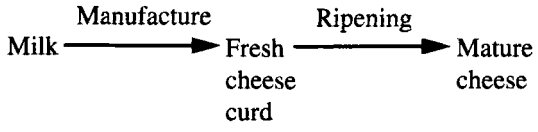
World production of cheese in 1990 was ~14×10^6 tonnes [Food and Agriculture Organization (FAO), 1994] and is increasing at an average

annual rate of ~4%. Europe, with a production of ~6×10^6 tonnes per annum, is the largest producing region (Table I). Cheese consumption varies widely, as shown in Table II for countries for which up-to-date information is available. Some form of cheese is produced throughout the world and some interesting minor varieties are produced in "nondairying" countries.

C. CHEESE SCIENCE AND TECHNOLOGY

Cheese is the most diverse, most scientifically interesting, and most challenging group of dairy products. While most dairy products, if properly manufactured and stored, are biologically, biochemically, and chemically very stable, cheeses are biologically and biochemically dynamic and, consequently, inherently unstable. Cheese manufacture and ripening involves a complex series of consecutive and concomitant microbiological, biochemical, and chemical events which, if synchronized and balanced, lead to products with highly desirable flavors but when unbalanced, result in off-flavors. Considering that a basically similar raw material (milks from a very limited number of species) is subjected to a generally common manufacturing protocol, it is fascinating that such a diverse range of products can be produced.

Acid-coagulated cheeses are consumed fresh but the production of rennet-coagulated cheese can be subdivided into two phases, manufacture and ripening:



The manufacturing phase comprises those operations performed during the first 24 hr and although the protocols for the various varieties differ in detail, the basic steps are common to most varieties, i.e., (1) acidification, (2) coagulation, (3) dehydration (cutting the coagulum, cooking, stirring, pressing, salting, and other operations that promote gel syneresis), (4) shaping (kneading, moulding, pressing) and (5) salting.

Cheese manufacture is essentially a dehydration process in which the fat and casein of milk are concentrated 6- to 12-fold, depending on the variety. The degree of hydration is regulated by the extent and combination of the above operations and, in addition, by the chemical composition of the milk. In turn, the levels of moisture and salt, the pH, and the cheese microflora regulate and control the biochemical changes that occur during ripening and hence determine the taste, aroma, and texture of the finished product. Thus, the nature and quality of the finished cheese are determined largely

TABLE I

CHEESE PRODUCTION IN THE LEADING COUNTRIES IN 1993[a]

Regions	Cheese production (tonnes $\times 10^3$)
Europe	
France	1,528.7
Germany	1,282.9
Italy	885.1
Netherlands	632.3
United Kingdom	324.4
Denmark	323.3
Poland	293.3
Russia	280.0
Greece	211.6
Spain	147.0
Switzerland	134.6
Czech Republic	115.5
Sweden	115.0
Hungary	96.8
Irish Republic	92.3
Austria	90.6
Finland	89.0
Norway[b]	77.7
Belgium/Luxembourg	69.0
Portugal	57.5
Slovakia	34.5
Iceland	2.1
Americas	
U.S.A.	3,268.5
Argentina	315.0
Canada	291.1
Mexico	116.4
Brasil	60.2
Africa	
Egypt	325.0
Sudan	70.0
South Africa	38.0
Uruguay	19.8
Zimbabwe	4.2
Others	
Australia	210.7
China	156.1
New Zealand	145.5
Japan	100.4
Israel	84.9
Total	12,089

[a] Food and Agriculture Organization (FAO) (1994).
[b] From The International Dairy Federation (IDF) (1988).

TABLE II

CONSUMPTION OF CHEESE (KG PER CAPUT) 1991[a]

Country	Hard and processed cheeses	Fresh cheese	Total
France	15.3	7.5	22.8
Greece	21.8	0.8	22.6
Italy	14.5	5.5	20.0
Belgium	13.7	4.3	18.0
Federal Republic of Germany	9.7	7.8	17.5
Iceland	10.3	6.0	16.3
Luxembourg	11.3	5.0	16.3
Switzerland	13.5	2.8	16.3
Sweden	14.8	0.9	15.7
Denmark	14.0	1.3	15.3
Netherlands	13.6	1.6	15.2
Finland	11.4	2.4	13.8
Canada	12.7	1.1	13.8
Norway	12.9	0.2	13.1
USA	11.4	1.5	12.9
Austria	6.2	5.3	11.5
Czech and Slovak Republics	6.6	4.0	10.6
Australia	—	—	8.8
United Kingdom	7.4	0.8	8.2
New Zealand	—	—	8.0
Russia	4.9	2.8	7.7
Hungary	3.4	4.2	7.6
Spain	—	—	7.0
Ireland	—	—	5.6
South Africa	1.7	0.1	1.8
Japan	1.2	0.04	1.24
India	—	0.2	0.2

[a] From International Dairy Federation (IDF) (1993).

by the manufacturing steps. However, it is during ripening that the characteristic flavor and texture of the individual cheese varieties develop.

The principal scientific aspects of cheese manufacture and ripening will be discussed in this review; more comprehensive coverage can be found in textbooks edited by Fox (1987, 1993a).

II. COMPOSITION AND CONSTITUENTS OF MILK

The coagulability of milk by acid or proteinases is due to certain characteristics of the milk proteins and is influenced by other milk constituents,

especially milk salts. The development of cheese flavor and texture is due to certain changes in the constituents of the cheese curd during ripening. Therefore, knowledge of chemistry of milk constituents is essential for a thorough understanding of cheese manufacture and ripening. However, such topics are outside the scope of this review and the interested reader is referred to textbooks on dairy chemistry by Walstra and Jenness (1984) and Fox (1982, 1983, 1985, 1989b, 1992, 1995).

III. CONVERSION OF MILK TO CHEESE CURD

The conversion of milk to cheese curd essentially involves coagulating the casein, either isoelectrically or enzymatically; if present, the milk fat is occluded in the curd. The mechanisms of rennet and acid coagulation of casein and the subsequent manipulation of the coagula to produce cheese curd are described in the following sections.

A. RENNET COAGULATION OF MILK

The rennet coagulation of milk is a two-stage process: the primary phase involves the enzymatic production of *para*-casein and TCA-soluble peptides [(glyco) macropeptides] while the secondary phase involves the aggregation or gelation of *para*-casein Ca^{2+} at temperatures >20°C; the two stages overlap somewhat. The subject has been reviewed by Fox (1984) and Dalgleish (1992, 1993).

1. Primary Phase of Rennet Action

κ-Casein was first isolated by Waugh and von Hippel (1956), who showed that this protein is responsible for the stability of the casein micelles and that its micelle-stabilizing properties are lost on renneting. Only κ-casein is hydrolyzed to a significant extent during the primary phase of rennet action. The primary cleavage site is Phe_{105}–Met_{106} (Delfour *et al.*, 1965), which is many times more susceptible to hydrolysis by acid proteinases (all commercial rennets are acid proteinases) than any other peptide bond in the milk protein system.

The unique sensitivity of the Phe–Met bond has aroused interest. The dipeptide, H–Phe–Met–OH, is not hydrolyzed nor are tri- or tetrapeptides containing a Phe–Met bond. However, this bond is hydrolyzed in the pentapeptide, H–Ser–Leu–Phe–Met–Ala–OMe (Hill, 1968, 1969) and reversing the positions of serine and leucine in this pentapeptide, to give the correct sequence for κ-casein, increases the susceptibility of the Phe–Met bond to

hydrolysis by chymosin (Schattenkerk and Kerling, 1973). Both the length of the peptide and the sequence around the sectile bond are important determinants of enzyme–substrate interaction. Ser_{104} appears to be particularly important (Hill, 1968, 1969) and its replacement by Ala in the above pentapeptide renders the Phe–Met bond very resistant to hydrolysis by chymosin (Raymond *et al.*, 1972) but not by pepsins (Raymond and Bricas, 1979); even substituting D-Ser for L-Ser markedly reduces its sensitivity (Raymond and Bricas, 1979). Extension of the pentapeptide H–Ser–Phe–Met–Ala–Ile–OH (i.e., κ-CN f104–108) from the N- and/or C-terminal to reproduce the sequence of κ-casein around the chymosin-susceptible bond increases the efficiency with which the Phe–Met bond is hydrolyzed by chymosin (Visser *et al.*, 1976, 1977). Taking the pentapeptide κ-CN f104–108 as a standard, studies at pH 4.7 showed that extending the peptide toward the C-terminal by three residues, i.e., κ-CN f104–111, caused a 6-fold increase in the catalytic ratio, k_{cat}/K_m, while addition of Leu_{103} to a pentapeptide, i.e., to give κ-CN f103–108, increased the ratio 600-fold. Addition of His_{102} and Pro_{101} (i.e., κ-CN f101–108) increased k_{cat}/K_m a further 5-fold. The sequence κ-CN f98–111 includes all the residues necessary to render the Phe–Met bond as susceptible to hydrolysis by chymosin as it is in intact κ-casein; it is hydrolyzed ~66,000 times faster than the parent pentapeptide (κ-CN f104–108), with a k_{cat}/K_m of ~2 $m^{-1}sec^{-1}$, which is similar to that for intact κ-casein (Visser *et al.*, 1980). κ-Casein and the peptide κ-CN f98–111 are also readily hydrolyzed at pH 6.6 but smaller peptides are not.

The residues Phe and Met are not intrinsically essential for chymosin action. Replacement of Phe by Phe (NO_2) or cyclohexylamine reduces k_{cat}/K_m 3- and 50-fold, respectively (Visser *et al.*, 1977). Oxidation of Met_{106} reduces k_{cat}/K_m 10-fold but substitution of norleucine for Met increases this ratio 3-fold. Neither porcine nor human κ-casein possesses a Phe–Met bond [both have a Phe–Ile bond at this position (Brignon *et al.*, 1985; Chobert *et al.*, 1976; Fiat *et al.*, 1977)], yet both are readily hydrolyzed by calf chymosin, although more slowly than bovine κ-casein; in contrast, porcine milk is coagulated more effectively than bovine milk by porcine chymosin (Foltmann, 1987). Thus, the sequence around the Phe–Met bond, rather than the bond itself, contains the important determinants for hydrolysis. The particularly important residues are Ser_{104}, the hydrophobic residues Leu_{103} and Ile_{108}, at least one of the three histidines (residues 98, 100, or 102), some or all of the four prolines (residues 99, 101, 109, and 110), and Lys_{111}.

Visser *et al.* (1987), using chemical and enzymatic modifications of the peptide κ-CN f98–112, attempted to identify the relative importance of residues in the regions of 98–102 and 111–112. They suggest that the sequence Leu_{103}–Ile_{108}, which probably exists as an extended β-structure

(Loucheux-Lefebvre *et al.*, 1978; Raap *et al.*, 1983), fits into the active site cleft of acid proteinases. The hydrophobic residues, Phe_{105}, Met_{106}, Leu_{103}, and Ile_{108}, are directed toward hydrophobic pockets along the active site cleft while the hydroxyl group of Ser_{104} forms a hydrogen bridge with some counterpart on the enzyme. The sequences 98–102 and 109–111 form β-turns (Loucheux-Lefebvre *et al.*, 1978; Raap *et al.*, 1983) around the edges of the active site cleft and are stabilized by Pro residues at positions 99, 101, 109, and 110. The three His residues, at positions 98, 100, 102, and Lys_{111} are probably involved in electrostatic bonding between enzyme and substrate.

Small peptides mimicking or identical to the sequence of κ-casein around the Phe–Met bond, especially chromogenic peptides, are very useful for determining the activity of rennets, independent of variations in the nonenzymatic phase (e.g., Hill, 1969; Raymond *et al.*, 1973; de Koning *et al.*, 1978; Martin *et al.*, 1981; Salesse and Garnier, 1976; Visser and Rollema, 1986). Since the specific activity of different rennets on these peptides varies, methods for quantifying the proportions of different acid proteinase in commercial rennets have been proposed (e.g., de Koning *et al.*, 1978; Martin *et al.*, 1981; Salesse and Garnier, 1976).

a. Rennets. The rennets used in cheesemaking are crude preparations of selected proteinases. Many proteinases will coagulate milk under suitable conditions but most are too proteolytic relative to their milk clotting activity and cause excessive proteolysis during coagulation and subsequent ripening, leading to reduced cheese yields and/or inferior cheese quality. Traditionally, the most widely used rennets were extracts of the stomachs of young calves, kids, or lambs in which the principal proteinase is chymosin. Extracts of certain plants have been used since ancient times and extracts of certain species of thistle are still used for certain cheeses, e.g., Serra de Estrela in Portugal.

Chymosins are aspartyl (acid) proteinases produced by neonatal mammals for the specific purpose of coagulating milk in the stomach, presumably to delay its discharge into the intestine, and thereby improve the efficiency of digestion. The general proteolytic activity of chymosins is low in comparison with their specific action on κ-casein and they probably play little or no role in the general digestion of proteins. The chymosins are well characterized at the enzymatic and molecular levels (see reviews by Foltmann, 1987, 1993). As the young animal ages, chymosin is gradually replaced by pepsin as the principal gastric proteinase at a rate which is affected by the animal's feed, being slow on a milk diet.

Owing to increasing world cheese production (~4% p.a. over the past 30 years), concomitant with a reduced supply of calf rennet (due to a

reduced number of calves and a tendency to slaughter more mature calves), the supply of calf rennet has been inadequate for many years and has led to a search for rennet substitutes. Although many proteinases can coagulate milk, only six rennet substitutes have been found to be more or less acceptable: bovine, porcine, and chicken pepsins and the acid proteinases from *Rhizomucor miehei, R. pusillus,* and *Cryphonectria parasitica.*

Chicken pepsin is the least suitable of these and is used widely only in Israel. Bovine pepsin is probably the most satisfactory and many commercial "calf rennets" contain up to 50% bovine pepsin; its proteolytic specificity is generally similar to that of calf chymosin. The proteolytic specificities of the three principal fungal rennets are considerably different from that of calf chymosin but the acceptability of most cheese varieties made using fungal rennets is fairly good. Microbial rennets are widely used in the United States but to only a limited extent in Europe. The extensive literature on rennet substitutes has been reviewed by Sardinas (1972), Ernström and Wong (1974), Nelson (1975), Green (1977), and Phelan (1985).

Although rennets are relatively cheap, they represent the largest single industrial application of enzymes with a world market of ca. 30×10^6 liters of standard rennet per annum (worth US$250–350 $\times 10^6$). The gene for calf chymosin has been cloned in *Kluyveromyces marxiaus* var. *lactis* (Gist Brocades), *Escherichia coli* (Pfizer), and *A. nidulans* (Hansen's). Genetically engineered chymosins, which have been cleared by Food and Drug Administrations in many but not all countries, have given very satisfactory results in large-scale cheesemaking trials on several varieties and are now widely used commercially in several countries (see Teuber, 1990).

b. Principal Factors Affecting the Hydrolysis of κ-Casein. The optimum pH for chymosin on κ-CN f98–112 is 5.3–5.5 (Visser *et al.*, 1987) and for the first stage of rennet action in milk is ~6.0 (Van Hooydonk *et al.*, 1986b). The pH optimum for the general proteolytic activity of chymosin is ~4.

The optimum temperature for the coagulation of milk by calf rennet at pH 6.6 is ~45°C. The temperature coefficient (Q_{10}) for the hydrolysis of κ-casein in Na caseinate is ~1.8, activation energy, E_a, is ~10,000 cal/mol, and activation entropy, ΔS, is ~−39 cal/deg/mol (see Fox, 1988).

Severe heat treatments, especially >80°C, adversely affect the rennet coagulation of milk. Although changes in calcium phosphate equilibria are contributory factors, complexation of β-lactoglobulin and/or α-lactalbumin with κ-casein via intermolecular disulfide bond formation is the principal factor responsible (see Fox, 1988). Most authors agree that the primary and, especially, the seconday (nonenzymatic) phase of rennet coagulation are adversely affected by severe heat treatments, as is the strength of the

resulting gel. The adverse affects of heating on the rennetability of milk can be reversed by acidification before or after heating or by addition of $CaCl_2$ (see Fox, 1988).

2. *Secondary (Nonenzymatic) Phase of Coagulation*

Hydrolysis of κ-casein during the primary phase of rennet action releases highly charged, hydrophilic macropeptides, representing the C-terminal ⅓ of κ-casein, which protrudes from the micelle surfaces, thereby reducing the zeta potential of the casein micelles from ca. −20 to ca. −10 mV and destroying their steric stabilizing layer. When ca. 85% of the total κ-casein has been hydrolyzed, the micelles begin to aggregate; reducing the pH or increasing the temperature from the normal values (~6.6 and ~31°C, respectively) permits coagulation at a lower degree of κ-casein hydrolysis (see Fox, 1984, 1988; Dalgleish, 1992, 1993).

Coagulation of rennet-altered micelles depends on a critical concentration of Ca^{2+}, which may act by crosslinking micelles via serine phosphate residues or by charge neutralization. Colloidal calcium phosphate is also essential for coagulation, but its role can be partially fulfilled by increased $[Ca^{2+}]$. Partial enzymatic dephosphorylation of casein impairs rennet coagulability. Cationic species predispose casein micelles to coagulate and may even coagulate unrenneted micelles. Chemical modification of histidine, lysine, or arginine residues inhibits coagulation, presumably by reducing the positive charge on the micelles. It has been suggested that coagulation occurs via electrostatic interaction between a positively charged cluster toward the C-terminal of *para*-κ-casein, which is exposed on removal of the macropeptide, and an unidentified, negatively charged cluster on neighboring micelles.

The coagulation of renneted micelles is very temperature-dependent (Q_{10} ~16) and normal bovine milk does not coagulate <18°C unless $[Ca^{2+}]$ is increased. The marked difference between the temperature dependence of the enzymatic and nonenzymatic phases of rennet coagulation has been exploited in the study of the effect of various factors on rennet coagulation, in attempts to develop a system for the continuous production of cheese or casein curd and in the application of immobilized rennets. The very high dependence on temperature of rennet coagulation suggests that hydrophobic interactions play a major role.

3. *Gel Assembly and Strength*

The assembly of renneted micelles into a gel has been studied using various forms of viscometry, electron microscopy, and light scattering. The

micelles remain discrete until ~60% of the visual coagulation time has elapsed, after which they begin to aggregate steadily, without sudden changes, into chain-like structures which eventually link up to form a network (for reviews, see Fox, 1984; Dalgleish, 1992, 1993; Green and Grandison, 1993). Aggregation of rennet-altered micelles can be described by the Smoluchowski theory for diffusion-controlled aggregation of hydrophobic colloids when allowance is made for the need to produce, enzymatically, a sufficient concentration of particles capable of aggregating. Gel assembly is poorly understood and will not be discussed further.

The strength of renneted milk gels (curd tension, CT) is very important, especially from the viewpoint of cheese yield. This subject has been reviewed (Fox, 1984; Green and Grandison, 1993). Suffice it to say here that curd tension is positively affected by protein concentration, $[Ca^{2+}]$, and reduced pH to ~5.9 and adversely by high heat treatments. Thus, CT is affected by the same variables that affect rennet coagulability.

4. Curd Syneresis

When the renneted milk gel has reached the desired degree of firmness, usually assessed subjectively by the cheesemaker but for which objective instrumental methods are sought, the gel is cut or broken. If left undisturbed, the gel is stable over a long time period but when broken, the curd particles syneresis, expressing whey. The tendency of renneted casein gels to synerese enables the cheesemaker to control the extent of dehydration and thereby the composition of the resulting cheese curd, which in turn strongly affects cheese texture and the various biochemical reactions that occur during ripening. The individuality of cheese varieties could be said to commence with the syneresis process.

The mechanism of syneresis at the molecular level is poorly understood but the physical and operational aspects have been well described and reviewed (van den Bijgaart, 1988; Pearse and MacKinlay, 1989; Akkerman, 1992; Walstra, 1993); the subject will not be reviewed here.

Syneresis is promoted by increasing temperature, decreasing pH, addition of $CaCl_2$, fine cutting of the gel, vigorous stirring, and high casein concentrations and retarded by high heat treatment of the milk and increasing fat content. Curd for many cheese varieties is pressed to shape and then consolidated to remove some whey. Additional whey is removed by salting (for hard and semihard cheeses, ~2 kg H_2O are lost per kilogram NaCl absorbed).

5. Acidification during Cheese Manufacture

One of the primary events in the manufacture of most, if not all, cheese varieties is the fermentation of lactose to lactic acid by selected lactic acid

bacteria added as a culture (starter) or, in traditional cheesemaking, by the indigenous microflora. The rate and point in the process at which lactic acid is principally produced are characteristic of the variety; e.g., in Cheddar-type cheeses, most of the acid is produced prior to moulding while in most other varieties it occurs mainly after moulding. For rennet-coagulated cheeses, the pH reaches ca. 5 within 5 to 12 hr.

Acid production affects almost all facets of cheese manufacture and is probably the most important factor affecting cheese quality. Among the most important consequences of acid development are:

1. Activity of the coagulant during manufacture.
2. Retention of coagulant in cheese curd.
3. Activity and perhaps specificity of the coagulant during ripening.
4. Activity of plasmin (indigenous milk alkaline proteinase).
5. Curd tension.
6. Curd syneresis.
7. Solubilization of colloidal calcium phosphate, which, among other factors, affects curd (cheese) texture, stretchability, and meltability.
8. Shelf-life. The spectrum of cheese varieties exhibits a range of storage stability from low (e.g., Cottage, Camembert) to high (e.g., Parmesean); pH and a_w play complementary roles in determining cheese stability and rate of ripening.
9. Growth and/or survival of pathogens.
10. The taste of acid-coagulated and young rennet-coagulated cheeses is strongly affected by the concentration of acid in the cheese.
11. Lactate serves as substrate for the production of propionic acid, acetic acid, and CO_2 during the ripening of Swiss-type cheeses.

The significance of some of these functions is discussed further below. The metabolism of lactose by lactic acid bacteria is well understood but will not be discussed here; the interested reader is referred to reviews by Cogan and Daly (1987), Fox *et al.* (1990), and Cogan and Hill (1993).

a. Buffering Capacity. The pH of cheese curd is determined directly by the amount of lactic acid produced and indirectly by the buffering capacity of the curd which is determined primarily by the casein with contributions from phosphate and citrate. Cheese curd has a relatively low buffering capacity in the pH range 6.8 to 5.5, but buffers strongly at pH 5.5 to 4.5. Due to the high buffering capacity below pH 5.5, even extensive acid production in Cheddar curd after salting has little effect on the final pH of the cheese and explains why the pH of highly acidic cheese, e.g., Cheshire (pH 5.0), increases little during ripening. The higher the moisture content of the curd, the higher its lactose content and hence the higher ratio of lactate to buffering substances and the lower the pH. Lawrence

and Gilles (1982) suggested that the buffering capacity of curd is determined largely at whey drainage since 85 to 90% of the total calcium, phosphate, and citrate removed during cheesemaking are removed at this stage.

b. Structure and Texture. The pH at whey draining determines the mineral content of cheese curd. The loss of calcium and phosphate from the casein micelles determines the extent to which they are disrupted and largely determines the basic structure and texture of a cheese (see Lawrence *et al.*, 1983; Fox *et al.*, 1990, for references). Curds with a low pH tend to have a crumbly texture, e.g., Cheshire, while high pH curds tend to be elastic, e.g., Emmental. Scanning electron microscopy showed that the aggregates in Swiss or Gouda (high pH, high calcium) are globular, like the original submicelles in milk, whereas in Cheshire (low pH, low calcium), the protein aggregates are smaller and less well organized and occur as strands or chains. The aggregates in Cheddar (intermediate pH) are immediate between those in Gouda and Cheshire. Other cheesemaking variables, e.g., the direct effect of pH on protein charge, curd composition (levels of water, fat, and protein), also influence the nature of the protein aggregates.

The texture of Cheddar cheese is considered to be more dependent on pH than on any other factor (see Lawrence and Gilles, 1982; Fox *et al.*, 1990, for references); for the same calcium content, the texture of Cheddar varies from "curdy" (pH $\geq$ 5.3), to "waxy" (pH 5.3 >5.1), to "mealy" (pH <5.1). Suggested explanations for this pH dependence include micelle hydration, especially in the presence of NaCl, and the extent to which colloidal calcium phosphate (CCP) is solubilized.

Proteolysis during ripening modifies cheese texture. The casein in low-pH Cheddar is hydrolyzed more rapidly than in normal pH cheese partly because solubilization of CCP causes micellar dissociation and renders the caseins more susceptible to proteolysis (O'Keeffe *et al.*, 1975) and partly because more chymosin is retained, and is more active, at low pH (Holmes *et al.*, 1977; Creamer *et al.*, 1985).

Cheddaring involves the development of a fibrous curd structure, but this occurs only at a curd pH $\leq$ 5.8 and is a consequence of the loss of calcium from the protein matrix (Lawrence and Gilles, 1982). Optimum stretchability of Mozzarella occurs at pH 5.2–5.4.

c. Enzyme Retention and Activity. The retention and activity of proteinases in cheese are influenced by the pH of the curd and cheese. Thus, other conditions being equal, cheese pH can be expected to influence the rate and extent of proteolysis during ripening and therefore cheese texture and flavor.

As discussed in Section IV E, the proteolytic activity of residual rennet is responsible for primary proteolysis in cheese. Most (>90%) of the rennet is lost in the whey but the level retained depends on pH and cooking temperature. The chymosin content of Gouda is inversely related to the pH at renneting and to cooking temperature (Stadhouders and Hup, 1975). Progressively more chymosin and pepsin is retained in Cheddar curd as the pH at coagulation is reduced from 6.6 to 5.2 but the retention of microbial rennets is not affected (Holmes *et al.*, 1977; Creamer *et al.*, 1985). On renneting at pH 6.6, ~6% of the original chymosin activity is retained in Cheddar cheese after pressing, while only 2–3% of microbial rennets and no detectable pepsin activity remain. Pepsin is denatured rapidly at pH 6.6 but becomes more stable at lower pH. Why microbial rennets behave differently from chymosin is not obvious. The activity of residual chymosin and other rennets, which are acid proteinases, is likely to be increased at low pH.

Chymosin is partly or totally inactivated in high-cooked cheeses, e.g., Swiss (Creamer, 1976b; Matheson, 1981; Garnot and Molle, 1987), but plasmin is stable at 85°C for 5 min (Creamer, 1976b). Plasmin is associated with the casein micelles in milk, but is released ≤pH 4.6 (see Grufferty and Fox, 1988). Its activity in Swiss and Dutch-type cheeses is considerably higher than that in Cheddar (Richardson and Pearce, 1981); the high plasmin activity in high-cook cheeses appears to be due to inactivation of plasmin inhibitors or the inhibitors of plasminogen activators (Farkye and Fox, 1990). Cheese contains high levels of plasminogen, which may be activated to plasmin (Ollikainen and Nyberg, 1988), especially if the inhibitors of the plasminogen activator are inactivated. In the case of washed-curd cheeses, e.g., Gouda, high plasmin activity may be due to the removal of inhibitors on washing.

6. *Salting of Cheese*

All cheese are salted but the concentration varies markedly, as does the method of salt application (for review, see Guinee and Fox, 1987, 1993). Typical concentrations are: Emmental, 0.5%; Cheddar, 1.7%; Gouda, 2.0%; Blue, 4.0%; Feta, 7%. Converted to salt-in-moisture, these values range from 1.5 to 12%. Salting is usually performed by immersion in brine for a period ranging from 30 min to several weeks, depending on the size of the cheese, its moisture content, and the desired salt content. Initially, there is a steep salt gradient from the surface to the center, which affects biological and biochemical events in the different regions of the cheese. Salt diffuses inward and, eventually, equilibrium is established throughout the cheese if time permits. Some cheeses, e.g., Blue, are salted by the application of

dry salt on the surface of the cheese. The salt dissolves in the surface moisture, causing the outward movement of moisture. Salt diffuses into the cheese, in effect as in brine-salted cheeses.

Cheddar and other British cheeses are salted by mixing dry salt with milled or broken curd. Each curd piece then behaves as a minicheese, with salt diffusing from the surface to the center. There is no overall gradient throughout the cheese and salt distribution should be uniform from the start. However, salt distribution is frequently uneven and in such cases equilibrium is not established, probably due to the lack of an overall gradient. Since the salt concentration in Cheddar cheese is sufficient to inhibit or retard acid production, the pH of Cheddar curd is close (~5.3) to its final value (~5.1) at salting and prior to moulding, whereas acidification of surface-salted (brined or dry) occurs mainly after moulding.

Salt plays several important roles in cheese ripening:

1. Controls the growth and survival of microorganisms, including food poisoning and pathogenic bacteria.
2. Influences the activity of enzymes.
3. Regulates water activity (a_w), especially of young cheese.
4. Affects cheese composition due to loss of water (2 kg H_2O lost per 1 kg NaCl absorbed).
5. Affects cheese flavor directly and indirectly via its effect on bacteria and enzymes.
6. Affects cheese texture via dehydrating effects on the caseins, cheese composition, and its effect on proteolysis.
7. Nutritional: high dietary intakes of NaCl are considered undersirable; however, in most cases, cheese is a relatively small contributor to dietary NaCl.

B. FRESH ACID-CURD CHEESE VARIETIES

Fresh acid-curd cheeses refer to those varieties produced by the coagulation of milk, cream, or whey via acidification or a combination of acid and heat and which are ready for consumption directly after manufacture (Fig. 1). They differ from rennet-curd cheeses, where coagulation is induced by the action of rennet at a pH value of 6.4–6.6, in that coagulation occurs close to the isoelectric point of casein, i.e., pH 4.6, at 30°C or at higher values when a higher temperature is used, e.g., Ricotta (pH 6.0, 80°C). A very small amount of rennet may be used in the production of Quarg, Cottage, and Fromage frais to provide firmer coagulum and minimize casein losses on whey separation, but it is not essential. Annual world production of fresh, acid-curd cheeses amounts to about 3.2 million tonnes, ca. 25% of

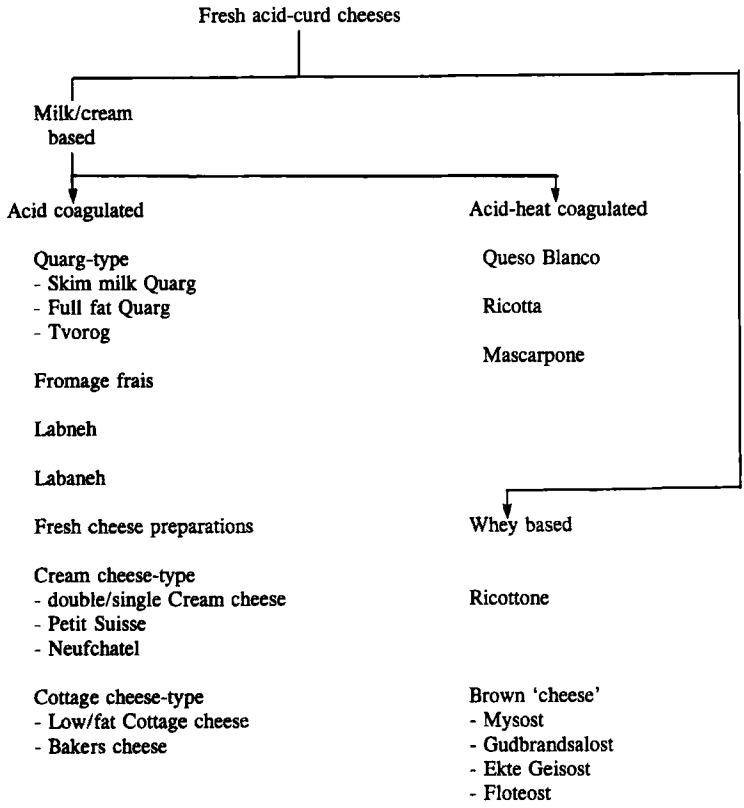


FIG. 1. Fresh acid-curd cheese varieties (from Guinee *et al.*, 1993).

total cheese [International Dairy Federation (IDF), 1986; Milk Marketing Board (MMB), 1991]; Quarg, Cottage, Cream, Fromage frais, and Ricotta are the most important types. Consumption has grown by ca. 4% per annum during the past decade. Factors contributing to this growth include:

(i) The large variety available in terms of consistency and flavor as effected by variations in processing parameters, blending of different cheese types to create new products and the addition of sugars, fruit purees, spices, or condiments.
(ii) Their soft, ingestable consistency, which makes them safe and attractive to very young children.
(iii) Their healthy perception by diet-conscious consumers. In general, their fat content is lower than that of rennet-curd cheeses; double cream cheese, an exception in the group, has a fat content similar to that of

Cheddar. However, acid-curd cheeses are relatively low in calcium compared to rennet-curd cheeses such as Cheddar (ca. 0.75% Ca) and Swiss (ca. 0.95% Ca) (Table III).

This review will concentrate on those varieties produced by coagulation at pH values close to the isoelectric point of casein at 20–40°C (e.g., Quarg, Fromage frais, cream cheese); acid–heat coagulated fresh cheese varieties (e.g., Ricotta) were reviewed by Torres and Chandan (1981a,b).

1. Production

Production generally involves pretreatment of milk (standardization, pasteurization, and/or homogenization), slow quiescent acidification, gelation,

TABLE III

APPROXIMATE COMPOSITION OF VARIOUS FRESH CHEESES[a]

Variety	% (w/w)						
	Dry matter	Fat	Protein	Lactose (lactate)	Salt	Ca (mg/100 g)	pH
Cream cheese							
Double	40	30.0	8–10	2–3	0.75	80	4.6
Single	30	14.0	12	3.5	0.75	100	4.6
Neufchatel	35	20.0	10–12	2–3	0.75	75	4.6
Labneh	25	11.6	8.4	4.3	—	—	4.2
Quarg							
Skim milk	18	0.5	13	3–4	—	120	4.5
Full fat	27	12.0	10	2–3	—	100	4.6
Cottage cheese							
Low fat	21	2.0	14	—	—	90	4.8
Creamed	21	5.0	13	—	—	60	4.8
Fromage frais							
Skim milk	14	1.0	8	3.5	—	0.15	4.4
Queso blanco	49	15.0	23	1.8	3.9	—	5.4
Ricotta							
Whole milk	28	13.0	11.5	3.0	—	200	5.8
Part skim	25	8.0	12	3.6	—	280	5.8
Ricottone	18	0.5	11	5.2	—	400	5.3
Mysost							
Gudbrandsalost	82	30.0	11	38	—	400	—
Floteost	80	19.0	11	46	—	—	—

[a] Compiled from data by USDA (Posati and Orr, 1976), Kosikowski (1982), Winwood (1983), Oterholm (1984), Patel *et al.* (1986), Sohal *et al.* (1988), Tamime *et al.* (1989), and Jelen and Renz-Schauen (1989).

whey separation, and curd treatments (pasteurization, shearing, addition of salt, condiments, and stabilizers, and/or homogenization) (Fig. 2). Acidification is generally slow, 12–16 hr at 21–23°C (long set) or 4–6 hr at 30°C (short set), via the *in situ* production of lactic acid by a *Lactococcus* starter culture; less frequently, an acidogen, e.g., glucono-δ-lactone, is used.

Many processing factors (e.g., pasteurization, rate and temperature of acidification, level of gel-forming protein, gel pH) influence coagulum structure and hence the textural/organoleptic attributes and the physicochemical

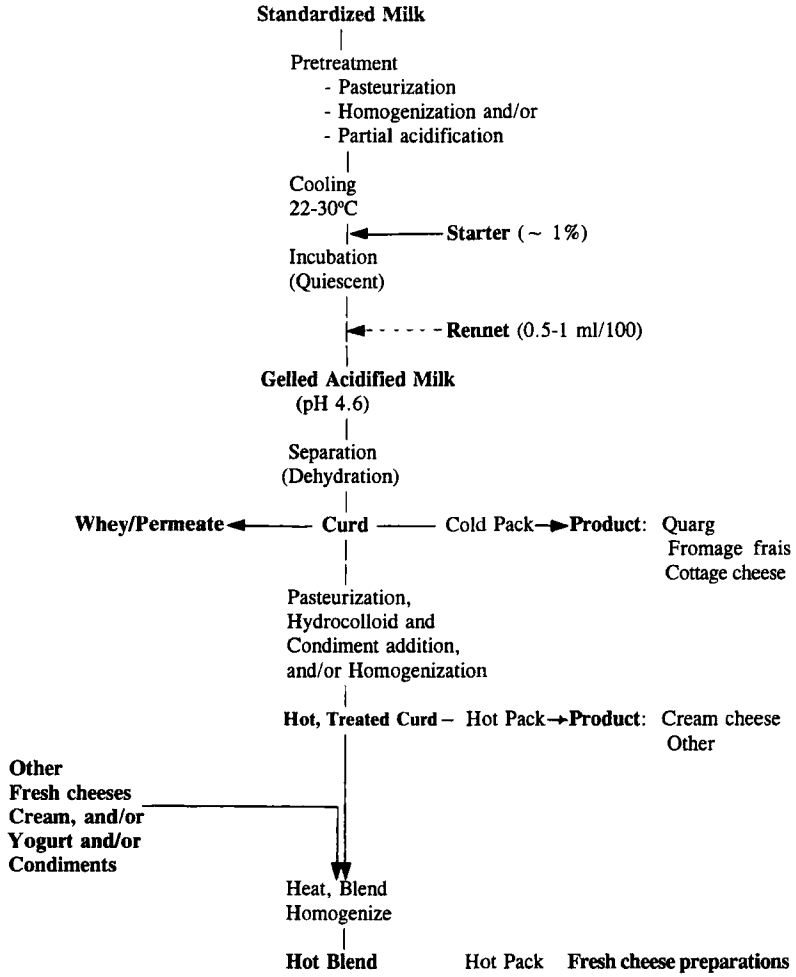


FIG. 2. Generalized scheme for the production of fresh cheese products (from Guinee *et al.*, 1993).

stability of the end product. This is especially true for cold-pack products where the curd, following whey separation and concentration, is not treated further. In hot-pack products, curd treatments (i.e., pasteurization, homogenization, hydrocolloid addition) have a major impact on the quality of the final product (Guinee *et al.*, 1993).

2. *Principles of Acid Gel Formation*

Slow acidification of milk under quiescent conditions is accompanied by two opposing sets of physicochemical changes:

1. A tendency toward disaggregation of the casein micelles to a more disordered system as a result of: (a) solubilization of CCP, which, at 20–30°C, is fully soluble at pH ~ 5.2–5.3 (Creamer, 1985; van Hooydonk *et al.*, 1986a); (b) a pH- and temperature-dependent dissociation of individual caseins, especially β, from the micelles with a concomitant increase in the level of serum casein [(Creamer, 1985; Roefs *et al.*, 1985; Dalgleish and Law, 1989); casein dissociation decreases with decreasing pH to ~6.2, then increases to a maximum at pH 5.3–5.6 (depending on temperature), and thereafter decreases to a minimum at the isoelectric pH (Snoeren *et al.*, 1984)]; (c) An increase in micelle solvation and porosity as a consequence of a and b, over the pH range 6.7 to 5.3–5.4 (Vreeman *et al.*, 1989).
2. A tendency for the casein micelles to aggregate to a more ordered system due to: (a) The reduction of the negative surface charge on the micelles and hence intermicellar repulsive forces (Darling and Dickson, 1979; Schmidt and Poll, 1986); (b) a decrease in casein hydration in the pH range 5.4 to 4.6 (Creamer, 1985); and (c) the increase in the ionic strength of the milk serum (due to the increased concentrations of calcium and phosphate ions), which has a shrinking effect on the matrix of casein micelles (Vreeman *et al.*, 1989).

At pH values greater than that at the onset of gelation, i.e., ~5.1–5.3 at 20–30°C, disaggregating forces predominate and hence a gel is not formed. At lower pH values, forces that promote aggregation predominate and gelation occurs.

a. Structural Changes. Slow quiescent acidification of milk from pH 6.6 to 4.6 is accompanied by a number of concerted structural changes which are summarized below:

1. At pH values > 5.6, no major changes are observed; the individual micelles retain their shape, dimensions and integrity; a wide spectrum

of particles with an average diameter of ~120 nm are present (Heertje *et al.*, 1985).

2. At the pH of maximum casein dissociation (~pH 5.5), the micelles become more porous.
3. On further acidification to pH 5.2, where practically all CCP is solubilized, smaller particles, in addition to the "original" micelles, are formed; these new particles are probably formed by aggregation of dissociated caseins (J. Visser *et al.*, 1986; Roefs, 1986; Rollema and Brinkhuis, 1989).
4. At pH ~5.2, i.e., the pH at the onset of gelation, a heterogeneous distribution of casein aggregates (composed of aggregated casein micelles and new casein particles) with a range of sizes is observed.
5. Further reduction in pH is paralleled by a touching of aggregates which initiates the formation of loose, porous strands. Eventually, on close approach to the isoelectric pH, dangling strands touch and crosslink to form a three-dimensional particulate gel network which extends, more or less continuously, throughout the serum phase.

b. Prerequisites for Gel Formation. Acid casein gels may be considered as particulate network gels, i.e., they consist of overlapping, cross-linked strands which are composed of particles (i.e., casein aggregates) linked together by various types of bonding (Harwalkar and Kalab, 1980; Schellhaass and Morris, 1985; Heertje *et al.*, 1985). Aggregation and structural rearrangement of casein during quiescent acidification of milk may result in the formation of a gel, as described, or in a precipitate, depending on the extent of aggregation. Gelation occurs when aggregation forces slowly overcome repulsive forces, resulting in the formation of relatively loose, porous, hydrated aggregates with a small density gradient between them and the serum phase in which they are dispersed. Owing to the relatively low density gradient, the aggregates have sufficient time to knit together, via strand formation, to form a continuous network before sedimenting. When conditions promoting aggregation are more extreme (i.e., rapid acidification under nonquiescent conditions at high temperature), casein particles aggregate more rapidly to form smaller, less porous, and less hydrated aggregates which, owing to their relatively high density, sediment as a precipitate which lacks the matrix continuity and water-holding characteristics of a gel (Kinsella, 1984).

To obtain a gel rather than a precipitate, the number of attractive forces, and hence the surface area of contact, between the dispersed particles must be limited (Walstra *et al.*, 1985; Walstra and van Vliet, 1986). Such limited interparticle attractions are promoted by an optimum ratio of attractive to repulsive forces between the conformationally rearranged casein particles (Kinsella, 1984), which in turn is achieved by the desired rate of concerted

physicochemical changes. As the number of interparticle points of attraction increases (e.g., when the rate of acidification is increased), the resulting gel becomes less structurally organized, coarser, less voluminous, and closer to a precipitate. Alternatively, if the number of interparticle attractive sites is lower than optimum, slowly forming aggregates may have sufficient time to precipitate before fusing to form into strands of a network. An example of the latter is the defect in Cottage cheese production known as "major sludge formation," whereby phage infection of the starter, after acid development has progressed to an advanced stage (pH ~ 5.2–5.3), leads to casein precipitation rather than gelation (Grandison *et al.*, 1986) or clumping of starter bacteria by agglutinins causes localized concentration of bacterial cells and consequently excessively fast acid production in some regions of the vat and vice versa in other regions (Salih and Sandine, 1980).

3. *Structure–Quality Relationships*

Gel structure is a major determinant of quality attributes, such as mouthfeel (smoothness/chalkiness), appearance (coarseness/smoothness), and physicochemical stability (absence of wheying-off and graininess during storage) of fresh acid-curd cheese products, especially cold-pack varieties. The relationship between product quality and gel structure may be explained by reference to Fig. 3, which depicts the structural differences between fine and coarse gels in which the concentrations of gel-forming protein are equal. In the fine gel, Fig. 3A, the micelles have formed into thin strands (chains) giving a highly branched, continuous gel network. Such a structure has an even distribution of gel-forming protein, a relatively

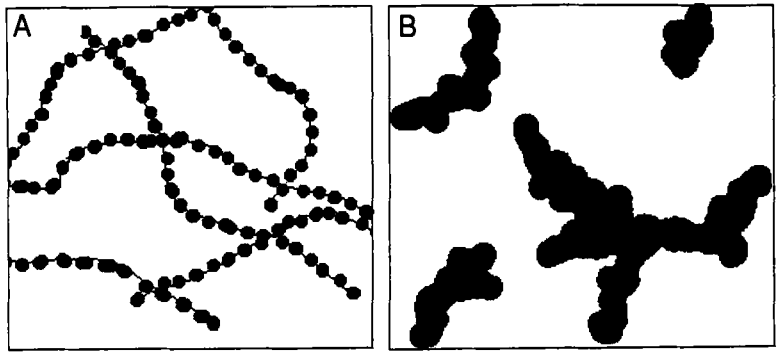


FIG. 3. Schematic diagram of a fine-structured yogurt gel (A) made from heated (90°C) milk and a coarse-structured yogurt gel (B) made from unheated milk (modified from Harwalkar and Kalab, 1980).

low porosity, a high water-holding capacity, and a low tendency to syneresis spontaneously (i.e., in the absence of external pressure) (Harwalkar and Kalab, 1980; Green, 1980). Conversely, in the coarse gel, the micelles have fused to a much greater degree, giving thicker strands and a gel which is more discontinuous and porous and more susceptible to syneresis on storage.

a. Syneresis. Syneresis requires rearrangement of the gel matrix into a more compact structure as effected by the breaking of bonds within strands and the consequent formation of new bonds (Walstra *et al.*, 1985). Rearrangement necessitates stress (for strand breakage), which can be effected by the application of external pressure, e.g., centrifugation, gravity, stirring, and/or cutting, or internally by the spontaneous breaking of gel strands due, possibly, to micelle flow and thermal motion of gel strands (Walstra *et al.*, 1985). Shrinkage of the casein particles in the network, as induced by a reduction in pH and/or increase in temperature following gel formation, may enhance both types of syneresis.

Simultaneously, the outward flow of aqueous phase is impeded by the sieve effect of the pores which becomes increasingly greater as progressive structural rearrangement leads to matrix contraction and reduced porosity. Because of the reduced syneresis for a given pressure, due to the sieve effect, and the decrease in the net pressure acting on the entrapped liquid (due to the counteraction of syneretic pressure by elastic reaction forces building up in the matrix), the rate of outward migration of aqueous phase decreases with time.

For unidimensional flow through a porous medium, such as a milk acid gel, the rate of syneresis (ν) may be expressed by Darcy's law (Walstra *et al.*, 1985; Roefs, 1986).

$$\nu = B\Delta P/hl,$$

where ν is liquid flux (i.e., volume flow rate in the direction l, divided by the cross-sectional area, perpendicular to l, through which the liquid flows) ($msec^{-1}$), B is the permeability coefficient of the matrix, which corresponds to the average cross-sectional area of the pores, h is viscosity of the serum flowing through the matrix, ΔP is the pressure gradient arising from syneretic pressure exerted on the entrapped serum by the matrix, and l is the distance over which the serum flows. The permeability coefficient, B, depends on the volume fraction of the protein matrix and the spatial distribution of the matrix strands (i.e., gel fineness/coarseness). The greater the permeability coefficient, the less is the resistance to the flow of moisture through the gel for a given syneretic pressure. Hence, fine gel struc-

tures, which have narrow pores, have lower permeability than their coarse-structured counterparts and are less susceptible to syneresis.

b. Rheology. Structure also has a major influence on the rheological properties of gels. The response of the structure to stress depends on many factors, including:

1. The number of strands per unit area. Considering a gel to which a relatively small stress (i.e., much less than the yield value) is applied in the direction x, the elastic modulus (G', i.e., ratio of stress to strain, σ/γ, in the linear viscoelastic region) can be related to the number of strands per unit area according to the equation (van Vliet and Walstra, 1985) $G' = CN\, d_2A/dx^2$, where N is number of strands per unit area in a cross section, perpendicular to x, bearing the stress, C is the characteristic length determining the geometry of the network, and dA is the change in Helmholtz energy when the particles in the strands are moved apart by a distance dx.
2. Gel homogeneity. The homogeneity of the gel determines the number of stress-bearing strands. For a coarse gel network, there are fewer stress-bearing strands than in a fine gel; however, the thickness, and hence strength, of the stress-bearing strands are, on average, greater in the coarser gel because of the greater number of attractions between the aggregates.
3. The number and type of bonds between the basic building units, i.e., aggregates, within a strand; the smaller the number and the weaker these bonds, the more susceptible the strand is to deformation. In fine gel structures, there are probably fewer intra- and interaggregate attractions/bonds than in coarser gels. Hence, products with finer gel structures may be considered to exhibit less elastic, and more viscous, behavior than those with coarser networks.

d. Sensory Attributes. Structure may also influence the sensory characteristics of gels, especially in products such as set yogurt where the gel, formed in its package, is not subjected to concentration or other treatments. In such cases, the formation of large, dense protein conglomerates (fused aggregates) causes a chalky or gritty mouthfeel (Modler *et al.*, 1989). These defects are more prevalent in products where the gel, following fermentation, is concentrated and/or heated; these conditions promote protein dehydration and hence the formation of large protein conglomerates.

4. Factors That Influence Gel Formation and Structure

a. Level of Gel-Forming Protein. For a given protein type and degree of gel fineness, higher concentrations of gel-forming protein result in a

denser [higher number of strands (of equal thickness) per unit volume], more highly branched, less porous network (Harwalkar and Kalab, 1980; Modler and Kalab, 1983; Modler *et al.,* 1983). However, for a given protein concentration, the structure is strongly influenced by the ratio of casein to whey protein (Modler and Kalab, 1983; Modler *et al.,* 1983; Tamime *et al.,* 1984). Reducing the ratio from 4.6:1 to 3.2:1 results in set yogurt with a finer, more highly- branched and less-porous structure, with a relatively low propensity to syneresis. Factors which increase the effective protein concentration include: (i) addition of milk protein supplements (e.g., skim milk powder), as practiced in yogurt production, (ii) homogenization of milk (as practiced in yogurt and Cream cheese production) which converts fat globules to pseudoprotein particles (van Vliet and Dentener-Kikkert, 1982), (iii) high-heat treatments which cause denaturation and binding of whey proteins to casein; undenatured whey proteins are soluble and do not participate in gel formation.

b. Milk Heat Treatment. It is well known that high-heat treatment of milk prior to culturing to fermented products, such as yogurt, gives a smoother, more viscous consistency although the level of the effect varies considerably with the type of preheating, i.e., whether in-vat, high-temperature–short time, or UHT treatments (Labropoulus *et al.,* 1981a,b; Parnell-Clunies *et al.,* 1986a,b). It also results in the onset of gelation at a higher pH (Kalab *et al.,* 1976). These effects may be attributed to extensive (>50%) whey protein denaturation which results in a higher effective concentration of gel-forming protein and a more finely structured gel network with reduced propensity to spontaneous wheying-off (Kalab *et al.,* 1976 Harwalkar and Kalab, 1980, 1981; Parnell-Clunies *et al.,* 1986a,b). The above effects are attributed to the formation, via disulfide interaction, of a complex between κ-casein and denatured β-lactoglobulin which results in the formation of filamentous appendages which protrude from the micelle surfaces (Harwalkar and Kalab, 1980; Heerjte *et al.,* 1985; Modler and Kalab, 1983) and prevent the close approach, and hence large-scale fusion, of micelles on subsequent acidification (Roefs, 1986).

c. Incubation Temperature. Higher temperatures (in the range 20–43°C) during acidification result in: (i) the onset of gelation at higher pH values, i.e., pH 5.5 at 43°C compared to pH 5.1 at 30°C (Heertje *et al.,* 1985) and (ii) coarser gel structures which are more prone to wheying-off on storage (Green, 1980; Schellhaass and Morris, 1985). These effects may be attributed to a higher ratio of aggregating to disaggregating forces during the early stages of acidification due to reduced casein dissociation from the

micelles, increased protein hydrophobicity (Grigorov, 1966), and a faster rate of acidification.

d. Rennet Addition. It is common practice during the manufacture of some fresh cheese products, such as Quarg and Cottage cheese, to add a small quantity of rennet (0.5–1.0 ml single-strength per 100 liters) to the milk shortly after (i.e., ~1–2 hr) culture addition when the pH is ~6.1–6.3. The rennet hydrolyzes some κ-casein with concomitant decreases in zeta-potential, casein dissociation and micelle solvation over the pH range 6.6–4.6 (Green and Crutchfield, 1971; Pearse, 1976; Darling and Dickson, 1979; Creamer, 1985; van Hooydonk *et al.*, 1986a,b). These changes contribute to an enhanced aggregation of micelles and gelation begins at a higher pH. Hence, a firmness suitable for cutting is obtained at a higher pH (i.e., 4.8–4.9); in the absence of added rennet, cutting is performed at pH 4.6–4.7 so as to prevent excessive loss of fines on whey separation.

e. Rate of Gelation. Gelation rate is faster with more rapid acid development and with increased incubation temperature in the range 20–45°C (provided starter growth is not inhibited) (Heertje *et al.*, 1985; Kim and Kinsella, 1989). Higher rates of gelation result in the onset of gelation at higher pH values and a coarser network with a greater propensity to spontaneous syneresis (Emmons *et al.*, 1959; Harwalkar and Kalab, 1980, 1981; Schellhaass and Morris, 1985). In an extreme situation, rapid acidification to pH 4.6 promotes rapid aggregation of casein with the formation of large dense aggregates which precipitate rather than gel. Gel formation by rapid acidification is, however, possible when the tendency of micelles to coagulate is reduced by acidifying to ~ pH 4.6 at low temperatures (0–4°C) and subsequently heating slowly (~0.5°C/min) under quiescent conditions to ~30°C (Harwalkar and Kalab, 1981; Roefs, 1986)

5. *Influence of Processing Parameters on Quality*

The structure and textural characteristics of fresh, acid-curd products are influenced by many factors, e.g., milk composition (levels of fat and protein), processing parameters (heat and homogenization treatments), conditions of gel formation (incubation temperature, rate of acidification, addition of rennet, final pH), and further curd treatments.

a. Texture. Increasing protein concentration, for a given rate of gelation, results in firmer and more continuous gels (Modler *et al.*, 1983; Roefs *et al.*, 1985; Roefs, 1986; Mottar *et al.*, 1989). However, for a given level of gel-forming protein, increasing the casein to whey protein ratio results in

coarser, firmer gel networks (Modler *et al.,* 1983; Tamime *et al.,* 1984). For a given level of solids, increasing the fat content gives a weaker gel because of the decrease in the level of gel-forming protein and the physical interference of fat globules with the protein network. However, if the milk (or cream) is homogenized, the fat globules become partially covered with casein and whey proteins and are converted to pseudoprotein particles, which then participate in network formation. Increasing the effective protein concentration in this way results in firmer gels (van Vliet and Dentener-Kikkert, 1982; Beyer and Kessler, 1988).

The viscosity of soft fresh cheeses, the firmness of natural, fortified yogurt, and the elastic modulus, G', of acidified caseinate and reconstituted skim milk powder gels increase with increasing protein concentration (Modler *et al.,* 1983; Tamime *et al.,* 1984; Roefs, 1986; Korolczuk and Mahaut, 1989). High-heat treatment of milk causes an increase in the viscosity and firmness of low-solids acid-curd products, such as natural yogurts (Harwalkar and Kalab, 1981; Labropoulus *et al.,* 1981a,b; Parnell-Clunies *et al.,* 1986a,b; Korolczuk and Mahaut, 1989). [However, Kim and Kinsella (1989) found that preheating (90°C × 15 min) of milk caused a decrease in the firmness of gluconic acid δ-lactone (GDL)-acidified milk gels]. The former effect is attributed to extensive whey protein denaturation and binding of denatured whey proteins to the micelles which effects an increase in the level of gel-forming protein and a finer, more highly branched continuous network. The number of stress-bearing strands (though possibly weaker) per unit volume of such a gel would be greater than in the coarser, lower-density matrix gel formed from unheated milk. For similar levels of whey protein denaturation, the type of heat treatment appears to have a significant influence on the textural parameters of fermented milks; for similar levels of whey protein denaturation, ultrahigh temperature treatment (130–150°C × 2–15 sec) gives natural yogurts of lower viscosity and firmness than high-temperature–short time (~80–90°C × 0.5–5 min) treatments, which in turn give lower values than those obtained for yogurt made using batch (63–80°C × 10–40 min) heat treatments (Labropoulus *et al.,* 1981a,b; Parnell-Clunies *et al.,* 1986a,b). While there is generally a strong positive correlation between the viscosity and firmness of yogurt as functions of whey protein denaturation, variations for similar levels of denatured whey protein may be due to the different rates of whey protein denaturation which alter their binding to casein micelles and hence gel structure (Morr, 1985).

High incubation temperatures promote coarser gel networks which are more elastic and firmer than those formed at a lower temperature (Harwalkar and Kalab, 1981; Schellhaass and Morris, 1985; Bringe and Kinsella, 1990). It is possible that in the coarser gel, the higher stress-bearing capacity

of the relatively thick strands overrides the effects of a greater gel discontinuity which would, otherwise, be expected to give a weaker more easily deformed network.

The firmness of acid milk gels increases with decreasing pH toward the isoelectric point of casein (Harwalkar *et al.*, 1977); the optimum pH for the elastic modulus, G', is ~4.5 (Walstra and Jenness, 1984; Roefs, 1986). The increase in firmness is attributed to a greater number of bonds with greater strength between the strand-forming aggregates.

b. Syneresis. In the manufacture of fresh acid-curd cheeses such as Quarg, Labneh, Cream cheese, Cottage cheese, the gel, following incubation, is concentrated (i.e., removal of whey) by cutting, stirring, cooking, whey drainage, and/or mechanical centrifugation. Further syneresis of the product may occur during storage, depending on processing conditions and product composition, which affect structure and porosity, and on the absence/presence of hydrocolloids which bind the aqueous phase. However, for other fresh cheese-type products, such as Laban, Fromage frais, set yogurt, and fresh cheeses made by recombination technology (i.e., reconstituting various dairy ingredients, e.g., skim milk powder and cream, in milk and/or water and standardizing the blend to the desired product composition prior to culturing), the gel, which is packaged, is the final product and hence whey removal is not practiced. In both classes of product, spontaneous syneresis, following packaging, is undesirable but occurs frequently because of the relatively high moisture to protein ratio compared to rennet-curd cheeses (e.g., ~17.6 g H_2O/g protein in yogurt versus ~1.44 g H_2O/g protein in Cheddar cheese).

Acid milk gels formed in the package, such as set natural yogurt, show little tendency to syneresis if left undisturbed. However, even in this situation, spontaneous syneresis may occur with time to a greater or lesser extent depending on the level of fortification and processing conditions (such as preheating of milk which affects differences in porosity and gel structure). This may be due partly to slow proteolysis of the casein, as affected by enzymes of starter bacteria, pH decrease, and temperature fluctuations. Hydrolysis of casein may be responsible for the widely different practical experience (day-to-day in-factory and interfactory inconsistencies) regarding syneresis in fermented set milk products (Walstra *et al.*, 1985). Disturbance of these products, e.g., by movement during cartoning and transport, which provides stress for bond breakage and matrix rearrangement, may initiate or accentuate syneresis.

For a given level of syneretic pressure, syneresis increases with increasing gel surface area to volume ratio. The shape of the package containing the gel may also influence syneresis; for example, in a package with sloping

walls, the gel may have a tendency to detach from the walls, leading to a stress in the gel which induces syneresis (Walstra *et al.,* 1985).

Increasing the level of total solids and gel-forming protein results in gels which are less susceptible to syneresis (Emmons *et al.,* 1959; Modler *et al.,* 1983). Harwalkar and Kalab (1983) found an inverse relationship between the level of total solids and susceptibility to syneresis for natural yogurt (10–15% DM); however, the relationship differed for yogurts from heated (90°C × 10 min) and unheated milks. As inverse relationship between gel firmness and susceptibility to syneresis has been found for natural yogurt produced from reconstituted skim milk (Modler *et al.,* 1983; Harwalkar and Kalab, 1983), Cottage cheese gels containing 8 to 15% solids (Emmons *et al.,* 1959), and chemically acidified skim milk gels (Harwalkar and Kalab, 1981). The decrease in syneresis with increasing level of gel-forming protein may be attributed to the formation of denser (greater number of strands of a given thickness per unit volume), less porous gels. However, for a given level of gel-forming protein, the susceptibility to syneresis strongly depends on the proportions of casein and whey protein (Modler *et al.,* 1983).

Spontaneous syneresis of acid skim milk-based gels decreases with increasing severity of heat treatment, homogenization, and lower gelation temperatures (Harwalkar and Kalab, 1981, 1983; Schellhaass and Morris, 1985; Parnell-Clunies *et al.,* 1986a,b). Reducing the pH of Cottage cheese gels (9–11% DM) at cutting from 4.92 to 4.59 reduced the level of syneresis on holding the cut gel for 1 hr at 32°C (Emmons *et al.,* 1959). A decrease in pH during syneresis results in greater syneresis than if the gel is brought to the same pH before cutting (i.e., before initiating external syneresis) (Walstra *et al.,* 1985). A wide variety of hydrocolloids (including gelatin, pregelatinized starch, cellulose derivatives, alginates, and carageenans) are used in practice to immobilize water and reduce syneresis in fresh acid-curd products, especially yogurts. Their effects on yogurt quality have been studied extensively (Kalab *et al.,* 1975; Modler *et al.,* 1983). The use of slime-producing cultures in yogurt has also been found to reduce syneresis considerably (Schellhaass and Morris, 1985).

c. Further Treatments of the Gel. In the production of many fresh cheese products, the gel produced following acidification is subjected to a number of further processing steps such as stirring, whey separation/concentration, heating, homogenization, agitation, and cooling (Fig. 2). Various materials, such as cream, sugar, salt, fruit purees, and/or hydrocolloids, may be added to the curd. Such treatments influence the structural, rheological, and syneretic properties of the final product, as discussed below.

Cutting of the gel into cubes, as in Cottage cheese, initiates syneresis which is enhanced by cooking and stirring, as in the manufacture of rennet-

curd cheese. Stirring of the gel (as in Quarg, Cream cheese, Fromage frais) breaks the matrix strands to an extent depending on the severity of agitation. Cooling of the gel to temperatures <20°C, to retard a further decrease in pH (before heating and whey separation), may result in greater destruction of the gel for a given degree of agitation as the strength of hydrophobic bonds, which play an important role in the structure of acid-curd cheeses, decreases with decreasing temperature (Kinsella, 1984; Hayakawa and Nakai, 1985). Increasing the temperature, in the range 25–85°C, enhances whey separation. Any factor which increases the firmness of the gel at separation (such as proximity to the isoelectric pH, rennet addition, milk homogenization, higher level of gel-forming protein, increased temperature of gelation) makes it less susceptible to breakage for a given shear. Whey separation, which may be performed by pouring the hot fluid onto cheesecloths, ultrafiltration, or centrifugation, results in concentration and aggregation of the broken pieces of gel to a greater or lesser degree. Collision during concentration may be expected to result in the formation of large irregularly shaped conglomerates (of varying thickness and length) which are forced into close proximity. The moisture content of the curd is closely related to the degree of aggregation; factors which enhance aggregation (conditions that promote a coarser gel structure, increased separating temperature, and increased syneretic force during separation) reduce the water content and increase the coarseness and firmness of the resulting curd.

Homogenization and/or shearing results in destruction, to an extent depending on the magnitude of the shear, of conglomerates and thereby yield a more homogeneous size and spatial distribution of the matrix-forming material (Kalab and Modler, 1985; Modler *et al.*, 1989). Holding hot cream cheese, while shearing, at 75 to 85°C may result in a thickening of the consistency, which is rather similar to the "creaming" process in pasteurized processed cheese products. However, while imparting a greater elastic character to the product, it may also lead to grittiness (Modler *et al.*, 1989), an effect which may be attributed to protein dehydration and the consequent formation of compact protein conglomerates. Slow cooling probably accentuates this defect. The degree of matrix reformation during cooling of the homogenized hot-packed product is uncertain; the matrix of the cooled cream cheese is more or less continuous, with the degree of continuity being governed by the size and spatial distribution of the matrix-forming material and the rate of cooling (Kalab and Modler, 1985; Modler *et al.*, 1989). A finer matrix manifests itself in a product which has a smoother appearance and mouthfeel and which is less susceptible to spontaneous wheying off on storage. The addition of hydrocolloids to the curd also minimizes syneresis; stabilizers which interact with casein, particularly κ-

carrageenan, may interrupt matrix formation and yield a smoother, softer product.

C. ULTRAFILTRATION TECHNOLOGY IN CHEESEMAKING

Ultrafiltration (UF), as a technology for cheese manufacture, was introduced in the early 1970s and has been investigated extensively and reviewed (Zall, 1985; Ernstrom, 1985; Ottosen, 1988; Lelievre and Lawrence, 1988; Lawrence, 1989; Spangler *et al.*, 1991). It has attracted the attention of cheese and equipment manufacturers, primarily because of the potential to increase yield, through the recovery of whey proteins in the cheese. Other advantages include its potential to reduce production costs and to produce new cheese varieties with different textural and functional characteristics. In this section, some of the more important aspects of UF in cheesemaking are highlighted.

The most successful commercial applications of UF in cheese manufacture to date have been in the production of cast Feta in Denmark (Tamime and Kirkegaard, 1991), fresh acid-curd varieties (Quarg, Ricotta and Cream cheeses) in Germany and other European countries (Pedersen and Ottosen, 1992), and the standardization of milk protein, to 4–5%, for the production of Camembert and other varieties (Coton, 1986; Korolczuk *et al.*, 1987; Puhan, 1992).

Based on the degree of concentration and whether whey expulsion following concentration is neccessary, UF in cheesemaking may be classified into three general areas:

(i) Low concentration factor (LCF), followed by cheesemaking and whey removal using conventional equipment. The main application of LCF-UF is the standardization of milk to a fixed protein level to obtain a more consistent end-product; variations in gel strength at cutting, buffering capacity, and rennet-to-casein ratio are minimized. However, when using conventional cheesemaking vats, concentration appears limited to a maximum CF of ~1.5, or 4–5% protein, because of difficulties in handling the curd and yield losses (Green *et al.*, 1981a; Guinee *et al.*, 1994).

(ii) Medium concentration factor (2–6×) to the final solids content of the cheese without whey expulsion. The main attraction of this type of UF technology is the increased cheese yield associated with retention of whey proteins and increased moisture when whey proteins are denatured prior to UF. The main commercial application is in the production of high-moisture cheeses which are consumed fresh (e.g., Quarg, Cream cheese) or are not very dependent on proteolysis during ripening for flavor development (e.g., Feta). Feta produced by this method (by addition of rennet to

a concentrate, i.e., precheese, without curd cutting) has a smoother more homogeneous texture than the more "curdy-textured" traditional product, hence the name "cast" Feta.

There are numerous reports on the use of UF concentration to the final cheese dry matter level for the production of soft or semihard rennet-curd cheeses, including Camembert, Blue, Havarti, and Mozzarella (Glover, 1985; Qvist *et al.*, 1987; Mohr *et al.*, 1989; Lawrence, 1989). Manufacture essentially involves preacidification, ultrafiltration/diafiltration, starter addition, rennet addition, coagulation and automated curd cutting using specialized equipment (e.g., Al-Curd or Ost Retentate coagulators), moulding, pressing, and brining. To date, uptake of UF technology by the industry for the production of the latter cheeses has been limited; apart from the uncertainties concerning the regulatory status of such cheeses and the relatively low reported increases in yield, the main drawbacks include changes in cheese texture, flavor, and functionality (i.e., meltability and stretchability).

(iii) High concentration factor, followed by whey expulsion in novel equipment. Since the upper limit of concentration by UF is ~7 : 1 for whole milk, it is not possible to achieve the dry matter levels required for hard cheeses such as Cheddar and Gouda; hence, further whey must be expelled following coagulation of the retentate and cutting the coagulum. Owing to the high curd-to-whey ratio, efficient curd handling (i.e., stirring and heat transfer) is not feasible in conventional systems. The only continuous system capable of handling such concentrates is the Siro-Curd which has been used for the production of Cheddar cheese in Australia since 1980 (Anonymous, 1986). The cheese produced by this process, which gives a yield increase of ~ 4 to 6%, is claimed to be indistinguishable from Cheddar manufactured using standard equipment.

On renneting at a fixed dosage level, increasing milk protein level results in a reduced rennet coagulation time, an increase in the level of soluble (nonaggregated) casein at the point of gelation, increased rate of curd firming, reduced set-to-cut time when cutting at a given curd strength, a decrease in the degree of aggregation at cutting, and a coarser gel network (Dalgleish, 1981; Guinee *et al.*, 1992, 1994; McMahon *et al.*, 1993). Micelles which are not modified, or aggregated, at the onset of gelation are presumably modified later and incorporated into the gel to a greater or lesser degree.

Owing to the rapid rate of curd firming, it becomes increasingly difficult, as the milk protein level is increased, to cut the curd cleanly, without tearing, before the end of the cutting cycle (Guinee *et al.*, 1994). Reflecting the tearing of curd, and consequent shattering of curd particles, fat losses in the whey are greater than those predicted on the basis of volume reduc-

tion (due to UF) for milks with protein concentrations >5%. Similar findings by Green *et al.* (1981a,b) were attributed partly to the poorer fat-retaining ability of higher protein curds which had coarser, more porous protein networks. Reduction of the setting temperature, in the range 31 to 27°C, and the level of rennet added give set-to-cut times and curd firming rates for concentrated milks closer to those of the control milk (Guinee *et al.*, 1994).

Increasing the concentration of protein in the cheesemilk also results in slower proteolysis during ripening when equal quantities of rennet on a milk volume basis are used (Green *et al.*, 1981a; Green, 1985; Creamer *et al.*, 1987; Spangler *et al.*, 1991). The slower rate of proteolysis in cheeses made from ultrafiltered milks may be attributed to a number of factors, including: (i) the lower effective rennet concentration, i.e., rennet-to-casein ratio, and hence activity in the cheese (Green *et al.*, 1981a; Creamer *et al.*, 1987); (ii) the inhibition of the indigenous milk proteinase, plasmin, by retained β-lactoglobulin in cheeses containing a significant quantity of whey proteins (Qvist *et al.*, 1987); (iii) the concentration, during UF, of proteinase/peptidase inhibitors (Hickey *et al.*, 1983b), and/or (iv) the resistance of undenatured whey proteins to degradation in cheese where they represent a substantial portion of the protein, i.e., ~18.5% (de Koning *et al.*, 1981). However, Creamer *et al.* (1987) found that at equal rennet-to-casein ratios, the level of α_{s1}-casein hydrolysis was higher in control Cheddar cheese than in that made from milk concentrated fivefold by UF. The reduced surface area-to-volume (SA/V) ratio of the protein network in cheeses made from concentrated milks, resulting from their coarser networks (Green *et al.*, 1981b), may also contribute to the observed reduction in proteolysis (Guinee *et al.*, 1992). It is conceivable that for a given level of enzyme activity in the cheese curd, casein degradation decreases as the SA/V ratio of the matrix decreases.

Cheese becomes progressively firmer (i.e., requires a higher compression force to induce fracture), more cohesive, mealier, and drier, and the structure of the protein matrix becomes coarser and more compact (fused), with increasing concentration factor. The reduced rates of protein degradation result in slower softening and flavor development during maturation.

IV. BIOCHEMISTRY OF CHEESE RIPENING

The conversion of milk to cheese curd is only the first stage in the production of most cheese varieties. Essentially all hard and most soft cheeses are ripened for periods ranging from a few weeks to 2 years or longer. During this period, cheeses undergo numerous biochemical changes which lead to the development of the appropriate flavor and aroma. The

biochemistry of cheese ripening, which is very complex, involves three primary processes, glycolysis, lipolysis, and proteolysis, the relative importance of which depends on the variety, and numerous secondary reactions which are not well understood and may be mainly responsible for the finer points of cheese flavor.

A. CHEESE RIPENING AGENTS: ASSESSMENT OF CONTRIBUTION TO RIPENING

Ripening agents in cheese generally originate from five sources: the coagulant, the milk, starter bacteria, secondary or adjunct starter bacteria, and nonstarter bacteria. The residual coagulant and enzymes from the starter, and probably the nonstarter microflora, are common to nearly all ripened cheeses. The secondary starter (i.e., microorganisms added to cheesemilk for purposes other than acidification) can exert considerable influence on maturation in cheese varieties in which they are used (e.g., *Penicillium roqueforti, P. camemberti* in mould-ripened varieties or *Brevibacterium linens* in smear-ripened cheeses). Exogenous enzymes used to accelerate ripening could be added to the above list and, when present, can be very influential.

The role of the individual ripening agents in cheese maturation has been studied using model cheese systems in which the action of one or more of the above agents is eliminated. The most comprehensive studies of this nature involved the manufacture of cheese under controlled microbiological conditions, with inactivation of the rennet and/or chemical acidification, allowing the contribution of individual ripening agents to be studied.

A milk supply of exceptionally high bacteriological quality is essential to eliminate nonstarter bacteria. Sterile teat cups, clusters, and bucket milking plant have been used by a number of authors (Perry and McGillivray, 1964; O'Keeffe *et al.*, 1976a) to obtain milk with an initial bacterial count of ca. 10^2 cfu ml^{-1}. Kleter and de Vries (1974), who included a cooling coil between the cluster and bucket, obtained counts averaging 46 CFU ml^{-1}. Reiter *et al.* (1969) withdrew milk aseptically by using a teat cannula but the quantities obtained were sufficient to produce only very small cheeses (ca. 100 g).

Heat treatment of aseptically drawn milk is necessary to further reduce bacterial counts. Perry and McGillivray (1964) used batch pasteurization (68°C × 5 min) in a steam-jacketed cheese vat. LTLT pasteurization (63°C × 30 min) was also used by Reiter *et al.* (1969) and O'Keeffe *et al.* (1976a). HTST pasteurization was used by Chapman *et al.* (1966), Visser (1976, 1977a), Kleter (1976), and Paulsen *et al.* (1980). UHT-treated milk was used by Le Bars *et al.* (1975); $CaCl_2$, more rennet, and a higher setting

temperature were used to offset the ill-effects of the high-heat treatment on the rennet coagulability of milk. Turner *et al.* (1986) concluded that a heat treatment of 83°C × 15 sec or 72°C × 58 sec is necessary to ensure a reduction of 10^8, which was deemed necessary to produce cheese with nonstarter counts <10 CFU kg^{-1} cheese from milk if the initial count is 10^3 CFU ml^{-1}; HTST pasteurization (72°C × 15 sec) is sufficient for milk with an initial count of 10 CFU ml^{-1}.

The manufacture of cheese under aseptic conditions can be achieved using enclosed cheese vats (Mabbitt *et al.*, 1959; Kleter, 1976; Visser, 1977a; Paulsen *et al.*, 1980), a sterile room with a filtered air supply (Le Bars *et al.*, 1975), or a laminar air-flow unit (O'Keeffe *et al.*, 1976a,b; McSweeney *et al.*, 1994a); the latter is probably the simplest of these techniques.

The acidifying role of starter can be simulated closely using an acidogen, usually GDL (O'Keeffe *et al.*, 1975), although the rate is faster than that which occurs in biologically acidified cheese.

To determine the role of the coagulant in cheese ripening, it is necessary to inactivate the rennet after coagulation, for which three techniques have been developed. One approach (Visser, 1976) involved separation of the first and second stages of rennet action: milk, depleted of Ca^{2+} and Mg^{2+} by treatment with an ion-exchange resin, was renneted (but did not coagulate), heated (72°C × 20 sec) to inactivate the rennet, and cooled to <5°C and $CaCl_2$ was added; the milk was then heated dielectrically and allowed to coagulated. O'Keeffe *et al.* (1977) used porcine pepsin as coagulant. This was inactivated after coagulation by adjusting the pH of the curd–whey mixture to 7.0. Mulvihill *et al.* (1979) used piglet gastric proteinase to prepare rennet-free curd in small-scale cheesemaking trials; the enzyme hydrolyzes bovine κ-casein but has little activity on α_{s1}- or β-casein.

The noncompetitive plasmin inhibitor 6-aminohexanoic acid (AHA) was used by Farkye and Fox (1991) to study the significance of plasmin in cheese ripening. It was necessary to use a high concentration of AHA which affected curd syneresis and the moisture content of the cheese; also, since AHA contains N, the background level of soluble N was increased greatly. Plasmin is inhibited by several proteins, including soybean trypsin inhibitor, which may be suitable as a plasmin inhibitor in cheese. Plasmin is also specifically inhibited by dichloroisocoumarin (Harper *et al.*, 1985), but neither it nor the inhibitory proteins have been investigated in cheesemaking. The high heat stability of plasmin [its activity is increased by high cooking temperatures (Farkye and Fox, 1990)] suggests that it may be possible to develop a model system based on aseptic curd in which the rennet is denatured by a suitable cooking temperature and acidified by GDL, in which to assess plasmin activity in isolation.

B. METABOLISM OF LACTOSE AND LACTATE DURING RIPENING

The primary glycolytic event, the conversion of lactose to lactate, is normally mediated by the starter culture during curd preparation or the early stages of cheese ripening. In cases where glycolysis has not been completed by the starter, nonstarter lactic bacteria may contribute. The metabolism of lactose was discussed in Section IIIA5.

Although ~ 98% of the lactose in milk is removed in the whey as lactose or lactate (Huffman and Kristoffersen 1984), Cheddar curd at milling typically contains 0.8 to 1.0% lactose (Turner and Thomas, 1980; Huffman and Kristoffersen, 1984). The residual lactose is fermented relatively rapidly to an extent dependent on the percentage salt-in-moisture (S/M) in the curd (Turner and Thomas, 1980; Thomas and Pearce, 1981). At low S/M concentrations and low populations of NSLAB, residual lactose is converted mainly to L-lactate by the starter. At high populations of NSLAB, e.g., at high storage temperatures, considerable amounts of D-lactate are formed, partly by fermentation of residual lactose and partly by isomerization of L-lactate (Turner and Thomas, 1980). At high S/M levels (e.g., 6%), and low NSLAB populations, the concentration of lactose decreases slowly and changes in lactate are slight. The quality of cheese is strongly influenced by the fermentation of residual lactose (O'Connor, 1974): the pH decreases after salting at S/M levels <5%, presumably due primarily to the continued action of the starter, but at higher levels of S/M, starter activity decreases abruptly, as indicated by high levels of residual lactose and high pH, accompanied by a sharp decrease in cheese quality.

Dutch-type cheese contains ~1.4% lactose at pressing but this decreases to <0.1% after pressing and to undetectable levels after brining (Raadsveld, 1957).

Typical levels of lactate in Camembert, Swiss, and Cheddar are 1.0, 1.4, and 0.5%, respectively (Karahadian and Lindsay, 1987; Turner *et al.*, 1983; Turner and Thomas, 1980). The fate of lactic acid in cheese depends on the variety. Initially, Cheddar contains only L(+) lactic acid but as the cheese matures, the concentration of D-lactate increases. The latter could be formed from residual lactose by lactobacilli (Turner and Thomas, 1980; Thomas and Pearce, 1981; Tinson *et al.*, 1982) or by racemization of L-lactate by NSLAB. Except in cases where the post-milling activity of the starter is suppressed (e.g., by S/M > 6%), racemization is likely to be the principal mechanism (Thomas and Crow, 1983). Racemization of L-lactate, which appears to occur in several cheese varieties (Thomas and Crow, 1983), is probably not significant from the flavor viewpoint. However, calcium D-lactate may crystallize on the surface of cheese, causing undesirable white specks (Pearce *et al.*, 1973; Severn *et al.*, 1986; Dybing *et al.*, 1988).

Lactate in cheese may be oxidized to acetate. Pediococci produce 1 mol of acetate and 1 mol of CO_2 and consume 1 mol of O_2 per mole of lactate utilized (Thomas *et al.*, 1985). The concentration of lactate in cheese far exceeds that required for optimal oxidation, and lactate is not oxidized until all sugars have been exhausted. The oxidation of lactate to acetate in cheese depends on the NSLAB population and on the availability of O_2, which is determined by the size of the block and the oxygen permeability of the packaging material (Thomas, 1987). Acetate, which may also be produced by starter bacteria from lactose (Thomas *et al.*, 1979) or citrate or from amino acids by starter bacteria and lactobacilli (Nakae and Elliott, 1965), is usually present at fairly high concentrations in Cheddar cheese and is considered to contribute to cheese flavor, although high concentrations may cause off-flavors (see Aston and Dulley, 1982). Thus, the oxidation of lactate to acetate probably contributes to Cheddar cheese flavor.

Presumably, the oxidation of lactate to acetate also occurs in other hard and semihard cheeses but studies are lacking. Production of lactate in Romano cheese was monitored by Mora *et al.* (1984). As with other varieties, L-lactate predominated initially, reaching a maximum of ~1.9% at 1 day (Deiana *et al.*, 1984). The concentration began to decrease at 10 days and was 0.2 to 0.6% at 150 to 240 days. Some of the decrease was accounted for by racemization to D-lactate, which reached a maximum at ~90 day (up to 0.6% in some cheeses) and then declined somewhat. In some cheeses, acetate reached very high levels (1.2%) at ~30 days, but decreased to ≥0.2% at 90 days; the agents responsible for the metabolism of acetate were not identified, but yeasts (*Debaryomyces hansenii*) may have been involved.

The metabolism of lactate is very extensive in surface mould-ripened varieties, e.g., Camembert and Brie. The concentration of lactic acid in these cheeses at 1 day is ~1.0%, produced mainly or exclusively by the mesophilic starter, and hence is L-lactate. Secondary organisms quickly colonize and dominate the surface of these cheeses—first *Geotrichum candidum* and yeasts, followed by *Penicillium caseicolum,* and, in traditional manufacture, by *Brevibacterium linens. G. candidum* and *P. caseicolum* rapidly metabolize lactate to CO_2 and H_2O, causing an increase in pH. Deacidification occurs initially at the surface, resulting in a pH gradient from the surface to the center and causing lactate to diffuse outward. When the lactate has been exhausted, *P. caseicolum* metabolizes proteins, producing NH_3, which diffuses inward, further increasing the pH. The concentration of calcium phosphate at the surface exceeds its solubility at the increased pH and it precipitates as a layer of $Ca_3(PO_4)_2$ at the surface, thereby causing a calcium phosphate gradient within the cheese. The elevated pH stimulates the action of plasmin, which, together with residual coagulant, is largely responsible for proteolysis. Although proteinases se-

creted by the surface microorganisms are very potent, they diffuse into the cheese to only a very limited extent; however, peptides produced from surface proteins may diffuse into the cheese. The combined action of increased pH, loss of calcium (necessary for the integrity of the protein network), and proteolysis are necessary for the very extensive softening of the body of Brie and Camembert. *B. linens* does not grow at pH < 5.8 and does not colonize the cheese surface until the pH has increased (Lenoir, 1984; Karahadian and Lindsay, 1987).

The metabolism of lactose and lactate in Swiss-type cheeses was described comprehensively by Turner *et al.* (1983). Typically, Emmental cheese contains ~1.7% lactose 30 min after moulding, which is rapidly metabolized by *S. thermophilus* with the production of L-lactate. Only the glucose moiety of lactose is metabolized by *S. thermophilus* and consequently galactose accumulates to a maximum of ~0.7% at ~10 hr, when the lactobacilli begin to multiply. These metabolize galactose to a mixture of D- and L-lactate, which reach ~0.35 and 1.2%, respectively, at 14 days, when the galactose is metabolized completely.

On transfer to the warm room, *Propionibacterium* metabolize lactate, preferentially the L-isomer (Crow, 1986), to propionate, acetate, and CO_2

$$3\ CH_3\text{-}\underset{\displaystyle OH}{\overset{\displaystyle H}{\underset{|}{\overset{|}{C}}}}\text{-}COOH \rightarrow 2\ CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$$

The CO_2 generated is responsible for eye development, a characteristic feature of these varieties.

The significance of the primary fermentation of lactose to L-lactate is well recognized in cheese manufacture (see Section IIIA5). However, less significance has been attached to the subsequent changes in lactose, and lactate metabolism received relatively little attention in most varieties until recently. However, these latter changes are of major proportions; they are critical in some varieties, e.g., Swiss and Camembert, and are probably of some importance in all varieties.

C. CITRATE METABOLISM

The relatively low concentration of citrate in milk (~8 m*M*) belies the importance of its metabolism in some cheeses made using mesophilic cultures (for reviews, see Cogan, 1985; Cogan and Hill, 1993). Citrate is not metabolized by *L. lactis* or *L. cremoris* but is metabolized by *L. lactis* subsp.

diacetylactis and *Leuconostoc* spp. with the production of diacetyl and CO_2. It is not metabolized by *S. thermophilus* or by thermophilic lactobacilli (Hickey *et al.*, 1983a), but several species of mesophilic lactobacilli metabolize citrate with the production of diacetyl and formate (Fryer, 1970).

Citrate is not used as an energy source by *L. lactis* subsp. *diacetylactis* or *Leuconostoc* spp., but is metabolized very rapidly in the presence of fermentable carbohydrate. CO_2 produced from citrate is responsible for the characteristic eyes of Dutch-type cheese and for the undesirable openness and floating curd in Cheddar and Cottage cheeses, respectively. Diacetyl is very significant in the aroma/flavor of Cottage cheese, Quarg, and many fermented milks. It also contributes to the flavor of Dutch-type cheeses and possibly of Cheddar (Manning, 1979a,b; McGugan, 1975; Aston and Dulley, 1982). Acetate may also contribute to cheese flavor.

Approximately 90% of the citrate in milk is soluble and is lost in the whey; however, the concentration of citrate in the aqueous phase of cheese is approximately three times that in whey (Fryer *et al.*, 1970), reflecting the concentration of colloidal citrate. Cheddar cheese contains 0.2 to 0.5% (w/w) citrate which decreases to 0.1% at 6 months (Fryer *et al.*, 1970; Thomas, 1987). Inoculation of cheesemilk with *Lb. plantarum* accelerated the depletion of citrate; pediococci did not appear to utilize citrate (Thomas, 1987).

D. LIPOLYSIS

Lipases in cheese originate from milk, rennet preparation (paste), starter, adjunct starter, or nonstarter bacteria. The degree of lipolysis in cheese varies widely between varieties, from ~6 meq free fatty acids in Gouda to ~45 meq/100 g fat in Danish Blue (Gripon, 1987, 1993). Lipolysis in internal bacterially ripened varieties, such as Gouda, Cheddar, and Swiss, is generally low but is extensive in mould-ripened and certain Italian varieties. In general, in those varieties in which extensive lipolysis occurs, lipases originate from the coagulant (rennet paste, which contains pregastric esterase, as used for some Italian varieties) or from the adjunct starter [*Penicillium* spp., which produce a number of lipases (Gripon, 1987, 1993), in mould-ripened varieties].

1. Indigenous Lipases

Milk contains substantial amounts of an indigenous lipoprotein lipase (LPL) which is well characterized (Olivecrona and Bengtsson-Olivecrona, 1991; Olivercrona *et al.*, 1992). The physiological role of LPL is in the metabolism of plasma triglycerides and, although it is generally believed that LPL occurs in milk as a result of leakage, it may have a function

in milk (see Olivecrona and Bengtsson-Olivecrona, 1991). LPL is rather nonspecific for the fatty acid but is specific for the Sn1 and Sn3 positions of mono-, di-, and triglycerides and the 1 position of glycerophospholipids. Therefore, lipolysis in milk leads to preferential release of short and medium chain acids, which in milk triglycerides are esterified predominantly at the Sn-3 position. In bovine milk, more than 80% of the LPL is associated with the casein micelles (Olivecrona *et al.*, 1992) and is incorporated into cheese curd. LPL probably causes significant lipolysis in raw milk cheese and may also contribute to lipolysis in pasteurized milk cheese as heating $\geq$78°C $\times$ 10 sec is required for complete inactivation of LPL (Driessen, 1989).

2. *Lipases from Rennet*

Rennet extract should contain no lipolytic activity. Rennet pastes used in the manufacture of hard Italian varieties (e.g., Romano, Provolone) contain a potent lipase, pregastric esterase (PGE), which is responsible for the extensive lipolysis and the characteristic "piccante" flavor in such varieties. The literature on PGE was comprehensively reviewed by Nelson *et al.* (1977).

PGE, also called lingual or oral lipase, is secreted by glands at the base of the tongue. Its secretion is stimulated by suckling and it is subsequently washed into the abomasum by milk and siliva. Rennet paste is prepared from the abomasa of calves, kids, or lambs slaughtered immediately after suckling. The abomasa are partially dried and ground into a paste which is slurried with milk or water before being added to cheesemilk. Rennet pastes are considered unhygienic and their use is not permitted in several countries; instead, partially purified PGEs are used.

Calf, kid, and lamb PGEs were partially purified from commercial preparations (Lee *et al.*, 1980) and calf PGE from oral tissue (Sweet *et al.*, 1984; Bernback *et al.*, 1985). The enzyme appears to be a glycoprotein with a p*I* of 7.0 and a mol wt of about 49 kDa. The gene for rat lingual lipase has been cloned and sequenced and the primary structure of the enzyme deduced (Docherty *et al.*, 1985). PGE is highly specific for short chain acids esterified at the Sn-3 position (Nelson *et al.*, 1977) and therefore releases high concentrations of short and medium chain acids from milk fat. Slight specificity differences between calf, lamb, and kid PGEs allow the manufacture of Italian cheeses with slightly different flavor characteristics. Most other lipases are unsuitable for the manufacture of Italian cheeses because of incorrect specificity but it has been claimed that certain fungal lipases may be acceptable alternatives (see Fox, 1988/1989; Fox and Stepaniak, 1993). The use of PGE to accelerate the ripening of other cheese varieties was discussed by Fox (1988/1989).

3. *Microbial Lipases*

Lactococcus spp. and *Lactobacillus* spp. have low lipolytic activities compared to other genera of bacteria (e.g., *Pseudomonas*). Fryer *et al.* (1967) considered that, although weakly lipolytic, lactococci will hydrolyze milk fat to a significant extent if present at high numbers for long periods (e.g., during cheese ripening). Small aseptic cheeses acidified with GDL instead of starter had lower free fatty acid (FFA) concentrations which did not increase during ripening (Reiter *et al.*, 1967). Umemoto *et al.* (1968) found that the cell-free extracts of various dairy lactic acid bacteria were most active on tributyrin at pH between 6 to 8 and at 37°C. Singh *et al.* (1973) found intracellular, but no extracellular, lipases in *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* which were more active on tributyrin than on tripalmitate or triolein. Harper *et al.* (1980) reported that *L. lactis* ssp. *lactis* had a more complex esterase system than *L. lactis* ssp. *cremoris;* the former showed two bands on a zymogram stained with α-naphtyl acetate, while the latter showed only one. In a comparative study of the lipase activity of a number of strains of lactic acid bacteria, including *L. lactis* ssp. *lactis* biovar. *diacetylactis,* Singh *et al.* (1981) found that mutants prepared by UV treatments produced more long chain acids than the parent strains. Piatkiewicz (1987) found interstrain differences in the lipolytic and esterolytic activity of *Lactococcus* spp. which were influenced by the composition of the growth medium and the physiological age of the culture; he also reported that lactococci were more lipolytic than lactobacilli. Kamaly *et al.* (1990) studied the lipolytic activity of a number of strains of *Lactococcus.* Lipases, in cell-free extracts, were most active at 37°C and at pH 7 to 8.5 (on tributyrin) or 7.0 (milk fat emulsion) and were more active on triglycerides containing short chain acids ($C_{4:0}$ to $C_{10:0}$) than long chain acids ($C_{12:0}$ to $C_{18:1}$). Starter bacteria can liberate FFA from mono- and diglycerides in milk produced by other lipases, e.g., LPL or lipases from Gram-negative bacteria (Stadhouders and Veringa, 1973). Kamaly *et al.* (1988) found quantitative interstrain differences in the esterolytic activity of lactococci assayed on *p*- and *o*-nitrophenyl esters of fatty acids.

The esterase/lipase system of *Lactococcus* has received relatively little attention in comparison with its proteolytic system. Unlike the situation with lactococcal proteinases and peptidases, little is known about the genetics of lactoccal lipase/esterase. Isolation of lipase/esterase-negative variants of *Lactococcus* would permit the significance of these enzymes in cheese ripening to be assessed.

The lipolytic/esterolytic activity of *Lactobacillus* has also received limited attention. An esterase of *Lb. plantarum* was purified by Oterholm *et al.* (1972) who found that the enzyme was maximally active at pH 6.7 and

40°C (on triacetin). The enzyme was not affected by heavy metals or low concentrations of cyanide or azide but was inhibited by higher concentrations; it preferentially hydrolyzed acetylesters and exhibited a strong preference for soluble over emulsified substrates.

The intracellular esterases of *Lb helveticus, Lb. delbrueckii* ssp. *bulgaricus,* and *Lb. lactis* were studied by El Soda *et al.* (1986) on *o*- and *p*-nitrophenyl derivatives of fatty acids. The optimum temperature for esterase production was 40 to 45°C and the cells had little esterolytic activity if grown at 35 or 55°C. The esterases of these strains were generally specific for short chain acids. Piatkiewicz (1987), who investigated the lipase and esterase activities of *Lb. casei,* found that cells had higher activities in the logarithmic than in the stationary phase of growth. Khalid *et al.* (1990) reported that *Lb. helveticus* CNRZ 32 (a strain thought to have potential in accelerated cheese ripening), *Lb. helveticus* ATCC10797, and especially *Lb. delbrueckii* ssp. *bulgaricus,* possessed esterolytic activity. Thus, several *Lactobacillus* spp, the principal nonstarter bacteria in Cheddar and Dutch-type cheeses, possess both lipolytic and esterolytic activity but none of these enzymes has been isolated and fully characterized.

Other nonstarter bacteria (e.g., *Micrococcus* and *Pediococcus*) also produce lipases. It is generally believed that lipases from *Micrococcus* spp., when present in cheese, can contribute to lipolysis during ripening (Bhowmik and Marth, 1990b). The lipase of *M. freudenreichii* was strongly inhibited by organophosphates and divalent metal ions, but less so by EDTA or pCMB (Lawrence *et al.,* 1967).

Bhowmik and Marth (1990a) studied the esterases in cell-free extracts of five strains of *Micrococcus,* all of which showed esterolytic activity; most strains hydrolyzed *p*-nitrophenyl derivatives of fatty acids faster than *o*-nitrophenyl derivatives. The esterase of *Micrococcus* spp. ATCC 8459 was studied in detail; it was optimally active at pH 8.0 and 40°C and was inhibited by organophosphates, divalent metal ions, NaCl, and redox reagents.

The lipase/esterase of *Pediococcus* spp. has received little attention. Tzanetakis and Litopoulou-Tzanetaki (1989) found only weak esterase and lipase activities in a number of strains of *P. pentosaceus* of dairy origin by means of the API-ZYM system. Bhowmik and Marth (1989) found esterase activity in six strains of *P. pentosaceus* but none in two strains of *P. acidilactici.* The lipases of *Propionibacterium shermanii* studied by Oterholm *et al.* (1970) were optimally active at pH 7.2 and 47°C on tributyrin; the enzymes showed a high preference for tripropionate and tributyrin and were inhibited by Hg^{2+} and Na_2HAsO_4 but not by pCMB or EDTA. Some esterase activity was observed but the enzyme was more active on emulsified than on soluble substrates.

Lipases and esterases of *Brevibacterium linens* were described by Sørhaug and Ordal (1974) and Foissy (1974). Sørhaug and Ordal (1974) found only intracellular lipase and esterase activities in *Br. linens.* Most of the strains studied were more active on tributyrin than on triacetin or methyl butyrate. According to Foissy (1974), 15 *Br. linens* isolates possessed intracellular esterase(s); they also possessed weak extracellular activity, which, based on zymogram patterns, may have been of intracellular origin.

Extensive lipolysis occurs in mould-ripened cheese, particularly blue varieties. In some cases, up to 25% of the total FFA may be liberated (see Gripon, 1987, 1993). However, the impact of FFA on the flavor of blue mould-ripened cheeses is less than in hard Italian varieties, possibly due to neutralization as the pH increases during ripening and to the dominant influence of methyl ketones on the flavor of blue cheese.

Lipolysis in mould-ripened varieties is due primarily to the lipases of *Penicillium roqueforti* or *P. camemberti,* which secrete potent extracellular lipases. *Penicillium* lipases are well characterized (see Kinsella and Hwang, 1976; Gripon, 1987, 1993). *P. camemberti* appears to excrete only one lipase which is optimally active at ca. pH 9.0 and at ca. 35°C. *P. roqueforti* excretes two lipases, one with a pH optimum at 7.5 to 8.0 (or perhaps 9.0 to 9.5), the other at pH 6.0 to 6.5 The acid and alkaline lipases exhibit different specificities. Lipases of *Geotrichum candidum* have been studied by Sidebottom *et al.* (1991) and Charlton *et al.* (1992). This organism produces two lipases of different substrate specificities.

Psychrotrophs, which can dominant the microflora of refrigerated milk, are a potentially important source of potent lipases in cheese. Cousins *et al.* (1977) considered that active lipase would be present in cheese if psychrotroph numbers exceed 10^7 CFU ml^{-1}. Many psychrotroph lipases are heat stable and thus may cause rancidity in cheese over the course of a long ripening period. The subject of psychrotroph enzymes in cheese was discussed by Mottar (1989). Unlike psychrotroph proteinases, which are largely water-soluble and are lost in the whey, psychrotroph lipases adsorb onto the fat globules and are therefore concentrated in the cheese.

4. Pattern and Levels of Lipolysis in Selected Cheeses

Lipolysis is considered to be undesirable in most cheese varieties. Cheddar, Gouda, and Swiss-type cheeses containing even a moderate level of free fatty acids would be considered rancid; however, certain cheese varieties are characterized by extensive lipolysis (e.g., Romano, Parmesan, and Blue cheeses). Bills and Day (1964) quantified FFA ($C_{2:0}$ to $C_{18:3}$) in 14 Cheddar cheeses with wide variations in flavor but found only small differences, qualitatively or quantitatively, between cheeses of different flavor. The

proportions of FFAs ($C_{6:0}$ to $C_{18:3}$) in cheese were similar to those in milk fat, indicating that these FFAs were released in a nonspecific manner. However, free butyric acid was found at a higher concentration than could be explained by its proportion in milk fat, suggesting that it was selectively liberated or synthesized by the cheese microflora. Lipolysis in hard Italian varieties is extensive and due primarily to the action of PGE in the rennet paste used in the manufacture of these cheeses. Lipolysis in Blue cheese varieties is extensive due to the action of lipases from *Penicillium* spp. Free fatty acid levels in a number of cheese varieties are listed in Table IV.

5. *Catabolism of Fatty Acids*

The taste and aroma of Blue cheese are dominated by saturated *n*-methyl ketones, a homologous series in which odd-numbered carbon chains from C_3 to C_{15}, as well as a number of even-numbered carbon chains (including C_4 to C_{10}), is present (Patton, 1950). Concentrations of methyl ketones in Blue

TABLE IV

TYPICAL CONCENTRATIONS OF FREE FATTY ACIDS (FFA) IN SOME CHEESE VARIETIES[a]

Variety	FFA (mg kg^{-1})
Sapsago	211
Edam	356
Mozzarella	363
Colby	550
Camembert	681
Port Salut	700
Monterey Jack	736
Cheddar	1,028
Gruyere	1,481
Gjetost	1,658
Provolone	2,118
Brick	2,150
Limburger	4,187
Goat's milk	4,558
Parmesan	4,993
Romano	6,754
Blue (U.S.)	32,230
Roquefort	32,453

[a] Adapted from Woo *et al.* (1984) and Woo and Lindsay (1984).

cheese fluctuate, presumably due to reduction to secondary alcohols; however heptan-2-one, nonan-2-one, and undecan-2-one are dominant (Dartley and Kinsella, 1971). Jolly and Kosikowski (1975a) showed that C_5, C_7, C_9, and C_{11} were dominant methyl ketones in a Blue cheese flavor concentrate.

The metabolism of fatty acids in cheese by *Penicillium* spp. involves four main steps (Fig. 4): (1) release of fatty acids by the lipolytic systems discussed above (Sections IVD2–IVD4), (2) oxidation to β-ketoacids, (3) decarboxylation to methyl ketone with one less carbon atom, and (4) reduction of methyl ketones to the corresponding secondary alcohol (Hawke, 1966); step 4 is reversible under aerobic conditions (Adda *et al.*, 1982). The concentration of methyl ketones is related to lipolysis. Methyl ketones can also be formed by the action of the mould on the ketoacids naturally present at low concentrations in milk fat (ca. 1% of total fatty acids). They could also be formed by the oxidation of monounsaturated acids, but Adda *et*

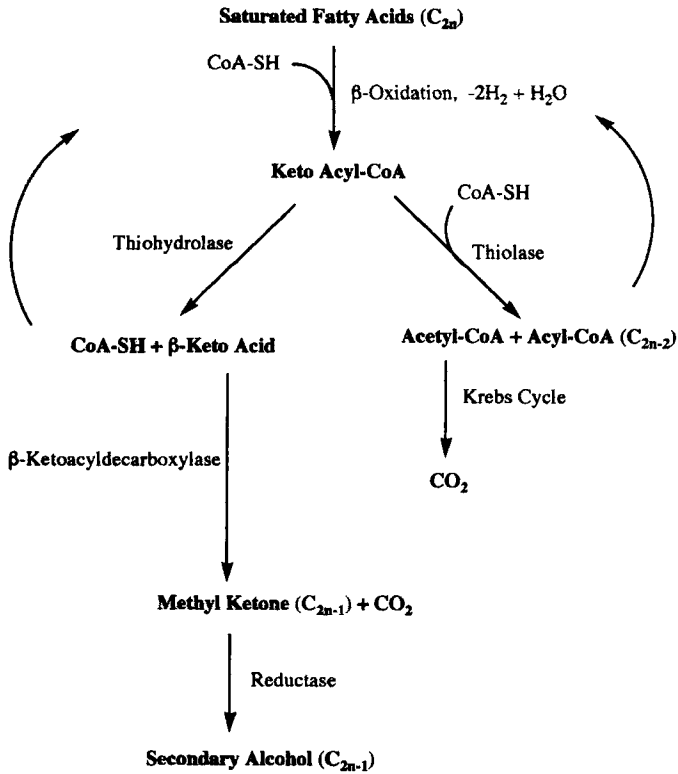


FIG. 4. Catabolism of fatty acids by *Penicillium roqueforti* (modified from Kinsella and Hwang, 1976).

al. (1982), who discussed the implications of such a pathway for methyl ketone formation in cheese, considered the evidence for such a pathway is equivocal.

A number of factors affect the rate of methyl ketone production, including temperature, pH, physiological state of the mould, and the ratio of the concentration of fatty acid to the dry weight of spores (Adda *et al.*, 1982). Fan *et al.* (1976) found that both resting spores and fungal mycelium are capable of producing methyl ketones. The rate of production of methyl ketones does not depend directly on the concentrations of FFA precursors; indeed high concentrations of FFAs are toxic to *P. roqueforti.*

Lactones are cyclic esters resulting from the intramolecular esterification of a hydroxyacid through the loss of water to form a ring structure. α- and β-Lactones are highly reactive and are used or occur as intermediates in organic synthesis; γ- and δ-lactones are stable and have been found in cheese. Lactones possess a strong aroma, which although not specifically cheese-like, may be important in the overall cheese flavor impact.

Eriksen (1975) concluded that γ- and δ-lactones in freshly secreted milk originated from the corresponding γ- and δ-hydroxyacids esterified in triglycerides. Dimick *et al.* (1969) reported a δ-oxidation system for fatty acid catabolism in the mammary gland of ruminants and thus oxidation within the mammary gland is the primary source of lactone precursors. The potential for lactone production depends on such factors as feed, season, stage of lactation, and breed (Dimick *et al.*, 1969). The formation of γ- or δ-lactones is spontaneous following release of the corresponding hydroxy acid.

In a study on Cheddar cheese, Wong *et al.* (1975) found that longer chain lactones (C_{14} to C_{16}) increased disproportionately to other lactones in rancid cheese. Two alternative methods of formation were proposed. The first involved the reduction of the corresponding keto acid, but investigations tended to disprove this hypothesis. The other possible method involved the microbial metabolism of homoricinoleic acid to shorter chain hydroxy acids and lactones.

δ-Lactones have very low flavor thresholds (Kinsella *et al.*, 1965). Jolly and Kosikowski (1975b) found that the concentration of lactones in Blue cheese was higher than that in Cheddar and concluded that the extensive lipolysis in Blue cheese influences the formation of lactones; δ-C_{14} and δ-C_{16} were the principal lactones in Blue cheese (as found also for Cheddar) (Wong *et al.*, 1973). A stronger typical Blue cheese flavor was found in cheeses containing added lipase, perhaps because lactones blend or modify harsher flavors.

O'Keefe *et al.* (1969) identified γ-C_{12}, γ-C_{14}, γ-C_{16} δ-C_{10}, δ-C_{12}, δ-C_{14}, δ-C_{15}, δ-C_{16}, and δ-C_{18} lactones in Cheddar cheese. The presence of most of these (γ-C_{12}, δ-C_{10}, δ-C_{12}, δ-C_{14}, γ-C_{16}) in Cheddar cheese was confirmed

by Wong *et al.* (1973) who showed a correlation between the number and concentration of lactones with age and flavor, suggesting that certain lactones are significant in Cheddar cheese flavor. In a further quantitative study of lactones in Cheddar cheese, Wong *et al.* (1975) failed to find a close correlation between flavor and lactone concentration. In general, the above δ-lactones were produced more quickly and to higher concentrations than γ-C_{12}. Lactone levels increased more rapidly early in the ripening period and the levels found were well above the flavor threshold; it was considered likely that they influence flavor.

E. PROTEOLYSIS

1. Introduction

Of the three primary biochemical events that occur during cheese ripening, proteolysis is the most complex and, according to many investigators, the most important. It is primarily responsible for textural changes, including changes in stretchability, meltability, adhesiveness, and emulsifying properties, and makes a major contribution to cheese flavor and the perception of flavor (through release of sapid compounds); unfortunately, some peptides produced are bitter and if present at sufficient concentrations will cause bitterness, which is probably the principal flavor defect in cheese.

Proteolysis during maturation is essential in most cheese varieties. The extent of proteolysis varies from very limited (e.g., Mozzarella) to very extensive (e.g., Blue mould varieties) and the products range in size from large polypeptides, comparable in size to intact caseins, through a range of medium and small peptides to free amino acids. Clearly, no one proteolytic agent is responsible for such a wide range of products.

2. Assessment of Proteolysis

Techniques for the assessment of proteolysis in cheese fall into two general classes: specific and nonspecific methods. The latter include determination of nitrogen soluble in, or extractable by, one of number of solvents or precipitants (e.g., water, pH 4.6 buffers, $CaCl_2$, NaCl, ethanol, trichloroacetic acid, phosphotungstic, or sulfosalicilic acids), or permeable through ultrafiltration membranes or the formation of reactive groups (e.g., $-NH_2$). Such methods are valuable for assessment of the overall extent of proteolysis and the general contribution of each proteolytic agent. Nonspecific techniques are generally simple and are valuable for the routine assessment of cheese maturity since soluble nitrogen correlates well with cheese age and to a lesser extent with quality.

Specific techniques, such as chromatography and electrophoresis, resolve individual peptides. Various forms of chromatography have been used to study cheese peptides, including paper, thin-layer, ion-exchange, gel permeation, metal chelate, and, more recently, a variety of high-performance techniques, particularly reverse-phase high-performance liquid chromatography (RP-HPLC). Electrophoresis is a very effective and popular technique for assessing primary proteolysis in cheese, especially alkaline urea-PAGE, but also SDS–PAGE and isoelectric focusing. Capillary electrophoresis has not yet been used widely but this technique will probably find widespread application for the analysis of peptides from cheese.

Techniques for the assessment of proteolysis in cheese during ripening have been the subject of a number of recent reviews, including Grappin *et al.* (1985), Rank *et al.* (1985), Fox (1989a), IDF (1991a), McSweeney and Fox (1993), and Fox *et al.* (1995).

3. *Relative Importance of Proteolytic Agents in Cheese*

Much of the information on the relative importance of individual proteinases has been obtained from studies on model cheese systems. The most comprehensive of these studies is that of Visser (1976, 1977a,b,c) and Visser and de Groot-Mostert (1977) in which the relative importance of enzymes from rennet, starter bacteria, and milk to proteolysis in Gouda cheese was assessed. The results indicated that rennet is mainly responsible for initial proteolysis and the production of most of the water- or pH 4.6-soluble nitrogen. However, the production of small peptides and free amino acids is due primarily to the action of enzymes from starter bacteria. The results of other studies on controlled-microflora cheese (Reiter *et al.*, 1969; Gripon *et al.*, 1975; Desmazeaud *et al.*, 1976; R. B. O'Keeffe *et al.*, 1976a,b; A. M. O'Keeffe *et al.*, 1978) were generally similar. Visser (1977c) found that only ~5% of the total nitrogen in a 6-month-old aseptic starter-free, rennet-free cheese was soluble at pH 4.6, with very low levels of free amino acids, indicating only a minor role for plasmin. Farkye and Fox (1991), who inhibited plasmin in Cheddar cheese by AHA, found differences between electrophoretograms of experimental and control cheeses, especially γ-caseins (produced from β-casein by plasmin) and in the level of water-soluble nitrogen, suggesting a role for plasmin in the initial hydrolysis of caseins.

Bovine milk also contains an indigenous acid proteinase which appears to be cathepsin D (see Kaminogawa *et al.*, 1980); procathepsin D has been identified in bovine milk (Larsen *et al.*, 1993). The action of cathepsin D

on the caseins is very similar to that of chymosin (Kaminogawa *et al.,* 1980; McSweeney *et al.,* 1995).

Although lactic acid bacteria, including the genera *Lactococcus* and *Lactobacillus,* are weakly proteolytic, they possess a great variety of proteolytic enzymes, especially peptidases, which contribute significantly to the later stages of proteolysis during cheese ripening, i.e., the formation of small peptides and amino acids. The proteolytic system of lactic acid bacteria is discussed in Section IV E6.

Although nonstarter lactic acid bacteria (NSLAB) can dominate the microflora of Cheddar-type cheese during much of its ripening (see Peterson and Marshall, 1990; Khalid and Marth, 1990a), their influence on proteolysis in cheese has been neglected by most authors. Visser (1977a,b,c) used aseptic cheesemaking techniques to eliminate NSLAB, as did Desmazeaud *et al.* (1976), R. B. O'Keeffe *et al.* (1976a), and A. M. O'Keeffe *et al.* (1978). A wide range of proteolytic enzymes have been identified in NSLAB (see Peterson and Marshall, 1990; Kahlid and Marth, 1990a), and therefore it is likely that they contribute to proteolysis in cheese.

The influence of adjunct starters, e.g., *Penicillium roqueforti,* on proteolysis can be great in varieties in which they are used. *P. roqueforti* degrades both α_{s1}- and β-caseins rapidly and releases large amounts of small peptides and free amino acids (Desmazeaud *et al.,* 1976).

The progress of proteolysis in most ripened cheeses can be summarized as follows: initial hydrolysis of caseins is caused primarily by residual coagulant, and to a lesser extent by plasmin and perhaps cathepsin D, resulting in the formation of large and intermediate-sized peptides which are subsequently degraded by the coagulant and enzymes from the starter and nonstarter flora of the cheese. The production of small peptides and free amino acids results from the action of bacterial proteinases and peptidases. This general outline of proteolysis can vary substantially between varieties due to differences in manufacturing practices. In Mozzarella, Swiss, and other high-cook varieties, coagulant is extensively or completely denatured and plasmin is therefore a more important contributor to primary proteolysis than in Cheddar.

4. Proteinases from the Coagulant

Chymosin (EC 3.4.23.4), the principal proteinase in traditional rennets used for cheesemaking (Rothe *et al.,* 1977), is an aspartyl proteinase of gastric origin, secreted by young mammals. The principal role of chymosin in cheesemaking is to coagulate the milk. However, about 6% of the chymosin added to cheese milk is retained in the curd for Cheddar and plays a

major role in the initial proteolysis of caseins in many cheese varieties (Fox, 1989a).

The action of chymosin on the B-chain of insulin indicates that it is specific for hydrophobic and aromatic amino acid residues (Fish, 1957). Chymosin is relatively weakly proteolytic; indeed, limited proteolysis is one of the characteristics to be considered when selecting proteinases for use as rennet substitutes (Fox, 1989a).

The primary chymosin cleavage site in the milk protein system is the Phe_{105}–Met_{106} bond in κ-casein. This bond is many times more susceptible to chymosin than any other bond in milk proteins (Vreeman *et al.*, 1986) and its hydrolysis leads to coagulation of the milk (see Section IIIA). Cleavage of κ-casein Phe_{105}–Met_{106} yields *para*-κ-casein (κ-CN f1–105) and glycomacropeptides (GMP; κ-CN f106–169). Most of the GMP is lost in the whey but the *para*-κ-casein remains attached to the casein micelles and is incorporated into the cheese. α_{s1}-, α_{s2}-, and β-caseins are not hydrolyzed during milk coagulation but may be hydrolyzed in cheese during ripening.

A number of authors (Pelissier *et al.*, 1974; Creamer, 1976a; Visser and Slangen, 1977; Carles and Ribadeau-Dumas, 1984) have investigated the action of chymosin on β-casein. In solution in 0.05 *M* Na acetate buffer, pH 5.4, chymosin cleaves β-casein at seven sites: Leu_{192}–Tyr_{193} > Ala_{189}–Phe_{190} > Leu_{165}–Ser_{166} ≥ Gln_{167}–Ser_{168} ≥ Leu_{163}–Ser_{164} > Leu_{139}–Leu_{140} ≥ Leu_{127}–Thr_{128} (Visser and Slangen, 1977). The Michaelis parameters, K_m and k_{cat}, for the action of chymosin on the bond Leu_{192}–Tyr_{193} are 0.075 m*M* and 1.54 sec^{-1}, respectively, for micellar β-casein and 0.007 m*M* and 0.56 sec^{-1} for the monomeric protein (Carles and Ribadeau-Dumas, 1984). NaCl inhibits the hydrolysis of β-casein by chymosin to an extent dependent on pH; hydrolysis is strongly inhibited by 5% NaCl and is completely inhibited by 10% (Mulvihill and Fox, 1978).

The primary site of chymosin action on α_{s1}-casein is Phe_{23}–Phe_{24} (Hill *et al.*, 1974; Carles and Ribadeau-Dumas, 1985). Cleavage of this bond is believed to be responsible for softening of cheese texture and the small peptide (α_{s1}-CN f1–23) is further hydrolyzed by starter proteinases. The specificity of chymosin on α_{s1}-casein in solution was studied by Pellissier *et al.* (1974), Mulvihill and Fox (1979), Pahkala *et al.* (1989), and McSweeney *et al.* (1993b). The hydrolysis of α_{s1}-casein by chymosin is influenced by pH and ionic strength (Mulvihill and Fox, 1977, 1979). In 0.1 *M* phosphate buffer, pH 6.5, chymosin cleaves α_{s1}-casein at Phe_{23}–Phe_{24}, Phe_{28}–Pro_{29}, Leu_{40}–Ser_{41}, Leu_{149}–Phe_{150}, Phe_{153}–Tyr_{154}, Leu_{156}–Asp_{157}, Try_{159}–Pro_{160}, and Trp_{164}–Tyr_{165} (McSweeney *et al.*, 1993b). These bonds are also hydrolyzed at pH 5.2 in the presence of 5% NaCl (i.e., conditions in cheese) and, in addition, Leu_{11}–Pro_{12}, Phe_{32}–Gly_{33}, Leu_{101}–Lys_{102}, Leu_{142}–Ala_{143}, and Phe_{179}–Ser_{180}. The rate at which many of these bonds are hydrolyzed de-

pends on the ionic strength and pH, particularly Leu_{101}–Lys_{102}, which is cleaved far faster at the lower pH. The k_{cat} and K_m for the hydrolysis of Phe_{23}–Phe_{24} bond of α_{s1}-casein by chymosin are 0.7 sec^{-1} and 0.37 m*M*, respectively (Carles and Ribadeau-Dumas, 1985).

α_{s2}-Casein appears to be relatively resistant to proteolysis by chymosin; cleavage sites are restricted to the hydrophobic regions of the molecule, i.e., residues 90–120 and 160–207: Phe_{88}–Tyr_{89}, Tyr_{95}–Leu_{96}, Gln_{97}–Tyr_{98}, Tyr_{98}–Leu_{99}, Phe_{163}–Leu_{164}, Phe_{174}–Ala_{175}, Tyr_{179}–Leu_{180} (McSweeney *et al.*, 1994b). Although *para*-κ-casein has several potential chymosin cleavage sites, it does not appear to be hydrolyzed either in solution or in cheese (Green and Foster, 1974).

Calf rennet contains about 10% bovine pepsin (EC 3.4.23.1, Rothe *et al.*, 1977). The proteolytic products produced from Na-caseinate by bovine pepsin are similar to those produced by chymosin (Fox, 1969), although as far as we are aware the specificity of bovine or porcine pepsins on bovine caseins has not been rigorously determined.

For several years, the supply of calf rennet has been insufficient to meet demand and much effort has been expended on searching for suitable rennet substitutes for cheesemaking (see Green, 1977; Phelan, 1985). A number of enzymes have been studied, including bovine, porcine, ovine, and chicken pepsins and proteinases from *Cryphonectria parasitica, Rhizomucor pusillus, R. miehei, Penicillium janthinellum, Rhizopus chinensis,* and *Aspergillus usameii.* The specificity of many of these enzymes on the oxidized B-chain of insulin were summarized by Green (1977). The specificity of the fungal proteinases on caseins differ substantially from chymosin but have not been determined rigorously.

Recombinant calf chymosins, expressed in *Aspergillus niger* var. *awamori, Kluveromyces marxianus* var. *lactis* or *E. coli.* were introduced recently and have been accepted by the regulatory authorities in many countries for use in foods; they are now used widely for cheesemaking. Cheesemaking trials, involving a number of cheese varieties, have shown only small differences between cheese made using calf rennet or recombinant chymosins (Green *et al.*, 1985; Hicks *et al.*, 1988; Bines *et al.*, 1989; van den Berg and de Koning, 1990; O'Sullivan and Fox, 1991; Nuñez *et al.*, 1992). Recombinant chymosins contain only one genetic variant of this enzyme (Harboe, 1992), while calf rennet can contain three chymosin variants (A, B, and C; Teuber, 1990) as well as bovine pepsin.

5. *Indigenous Milk Proteinases*

a. Plasmin. Plasmin (fibrinolysin, EC 3.4.21.7) has been the subject of much study (for review, see Grufferty and Fox, 1988). The physiological

role of plasmin is solubilization of fibrin clots; it is a component of a complex system consisting of the active enzyme, its zymogen, activators and inhibitors of the enzyme, and its activators, all of which are present in milk. Plasmin, plasminogen, and plasminogen activators are associated with the casein micelles in milk, while the inhibitors are in the serum phase (see Grufferty and Fox, 1988; Fox and Law, 1991).

Plasmin is a trypsin-like serine proteinase with a pH optimum at about 7.5 and a high specificity for peptide bonds involving lysyl residues. It is active on all caseins, but especially on α_{s2}- and β-caseins (Grufferty and Fox, 1988). Plasmin cleaves β-casein at three primary sites: Lys_{28}–Lys_{29}, Lys_{105}–His_{106}, and Lys_{107}–Glu_{108}, with the formation of the polypeptides, β-CN f29–209 (γ_1-CN), f106–209 (γ_2-CN), and f108–209 (γ_3-CN), β-CN f1–105 and f1–107 (proteose peptone 5), β-CN f29–105 and f29–107 (proteose peptone 8-slow) and β-CN f1–28 (proteose peptone 8-fast) (see Eigel *et al.*, 1984). Additional cleavage sites are at Lys_{113}–Tyr_{114} and Arg_{183}–Asp_{184} (Fox *et al.*, 1994).

Plasmin cleaves α_{s2}-casein in solution at eight sites: Lys_{21}–Gln_{22}, Lys_{24}–Asn_{25}, Arg_{114}–Asn_{115}, Lys_{149}–Lys_{150}, Lys_{150}–Thr_{151}, Lys_{181}–Thr_{182}, Lys_{188}–Ala_{189}, and Lys_{197}–Thr_{198} (Visser *et al.*, 1989; Le Bars and Gripon, 1989), producing about 14 peptides, 3 of which are potentially bitter (Le Bars and Gripon, 1989).

Although plasmin is less active on α_{s1}-casein than on α_{s2}- and β-caseins, the formation of λ-casein, a minor casein component, has been attributed to its action on α_{s1}-casein (Aimutis and Eigel, 1982). The specificity of plasmin on α_{s1}-casein in solution has been reported by McSweeney *et al.* (1993c) who found the principal plasmin cleavage sites to be Arg_{22}–Phe_{23}, Arg_{90}–Tyr_{91}, Lys_{102}–Lys_{103}, Lys_{103}–Tyr_{104}, Lys_{105}–Val_{106}, Lys_{124}–Glu_{125}, and Arg_{151}–Gln_{152}.

Plasmin has very low activity on κ-casein, although it contains several potential sites; Eigel (1977) found no hydrolysis of κ-casein under conditions adequate for the hydrolysis of α_{s1}-casein. However, Andrews and Alichanidis (1983) reported that 4% of the peptides produced by indigenous plasmin in pasteurized milk stored at 37°C for 7 days and detectable by PAGE originated from κ-casein. The specificity of plasmin on κ-casein has not been determined.

Cathepsin D. The indigenous acid proteinase in milk has received little attention. This activity was first recognized by Kaminogawa and Yamauchi (1972), who isolated and characterized the enzyme and considered it to be similar to the lysozomal acid proteinase, cathepsin D (EC 3.4.23.5). The presence of procathepsin D in milk has been reported (Larsen *et al.*, 1993). Cathepsin D is relatively heat labile (completely inactivated by 70°C × 10

min) and has a pH optimum of 4.0 (Kaminogawa and Yamauchi, 1972). The specificity of cathepsin D on the caseins has not been determined, although electrophoretograms of caseins incubated with milk acid proteinase (Kaminogawa and Yamauchi, 1972) or cathepsin D (McSweeney *et al.*, 1995) indicate a specificity very similar to that of chymosin; surprisingly, it coagulates milk very slowly (McSweeney *et al.*, 1995).

Other Indigenous Milk Proteinases. The presence of other minor proteolytic enzymes in milk has been reported, including thrombin and a lysine aminopeptidase (Reimerdes, 1983) and proteinases from leucocytes (Grieve and Kitchen, 1985; Verdi and Barbano, 1991), but they are considered not to be very significant (Grieve and Kitchen, 1985; Grufferty and Fox, 1988).

6. *Proteolytic Enzymes from Starter*

Although lactic acid bacteria (LAB) are weakly proteolytic they do possess a proteinase and a wide range of peptidases which are principally responsible for the formation of small peptides and amino acids in cheese. The genus most widely used as a cheese starter is *Lactococcus,* the proteolytic system of which has been studied thoroughly at the molecular, biochemical, and genetic levels. The proteolytic system of *Lactobacillus* spp. is less well characterized than that of *Lactococcus,* but the systems of both genera appear to be generally similar.

The extensive literature has been comprehensively reviewed by Monnet *et al.* (1993), Tan *et al.* (1993a), Visser (1993), and Law and Haandrikman (1996), to which the reader is referred. The principal properties of the peptidases isolated to date are summarized in Table V. The proteolytic system is capable of hydrolyzing casein completely to free amino acids; the sequential action of the peptidase system is shown schematically in Fig. 5. This complex proteolytic system is required by LAB for growth to high numbers in milk which contains a low concentration of small peptides and free amino acids.

The proteinase in LAB is anchored to the cell membrane and protrudes through the cell wall, giving it ready access to extracellular proteins. All the peptidases are intracellular although some, e.g., Pep X, appear to be oriented toward the outer surface of the cell membrane (Tan *et al.*, 1992). The oligopeptides produced by the proteinase are actively transported into the cell where they are hydrolyzed further by the battery of peptidases.

Cell wall-associated proteinases of *Lactococcus* can be classified into three groups, P_I-, P_{III}-, and mixed-type; P_I-type proteinases degrade β- but not α_{s1}-casein at a significant rate, while P_{III}-type proteinases rapidly degrade both α- and β-caseins (S. Visser *et al.*, 1986). The nucleotide sequences

TABLE V

PEPTIDASES OF LACTIC ACID BACTERIA

Organism	Principal assay substrate	MW/kDa	Optimum pH	Activity (°C)	Subunits	Class	Reference
Oligoendopeptidases (LEP, MEP, NOP, PepO)							
Lc. bv. diacetylactis CNRZ 267	Peptides	49	—	—	—	—	Desmazeaud and Zevaco (1976)
Lc. lactis ssp. *cremoris* H61	Peptides	98	7–7.5	40	1	M[a]	Yan *et al.* (1987b)
Lc. lactis ssp. *cremoris* H61	α_{s1}-CN fl-23	80	6	37	2	M	Yan *et al.* (1987a)
Lc. lactis ssp. *cremoris* Wg2	Metenkephalin	70	6–6.5	30–38	1	M	Tan *et al.* (1991)
Lc. lactis ssp. *cremoris* HP	α_{s1}-CN fl-23	180	8–9	42	>2	M	Baankreis (1992)
Lc. lactis ssp. *cremoris* C13	α_{s1}-CN fl-23	70	6–7	35	1	N	Baankreis (1992)
Lc. lactis ssp. *lactis* MG 1363	α_{s1}-CN fl-23	70	7.5	40	1	M	Stepaniak and Fox (1995)
Lc. lactis ssp. *cremoris* SK11	Bradykinin	70	6.0	—	1	M	Pritchard *et al.* (1994)
Aminopeptidases							
Aminopeptidase N (*General aminopeptidase, AMP, PepN*)							
Lc. bv. diacetylactis CNRZ 267	Lys-*p*-NA	85	6.5	35	—	M	Desmazeaud and Zevaco (1979)
Lc. lactis ssp. *cremoris* AC1	Lys-*p*-NA	36	7	40	1	M	Geis *et al.* (1985)
Lc. lactis ssp. *cremoris* Wg2	Lys-*p*-NA	95	7	40	1	M, -SH	Tan and Konings (1990)
Lb. delbrueckii ssp. *lactis* 1183	Lys-*p*-NA	78–91	6.2–7.2	47.5	1	M	Eggimann and Bachmann (1980)
Lb. acidophilus R-26	Lys-*p*-NA	38	—	—	—	M	Machuga and Ives (1984)
Lb. delbrueckii ssp. *bulgaricus* CNRZ 397	Lys-*p*-NA	95	—	—	—	M	Atlan *et al.* (1989)
Lb. helveticus CNRZ 32	Lys-*p*-NA	97	—	—	—	M	Khalid & Marth (1990c)
Lb. delbrueckii ssp. *bulgaricus* B14	Lys-*p*-NA	95	7	50	1	M	Bockelmann *et al.* (1992)
Lb. helveticus LME-511	Leu-*p*-NA	92	7	37	1	M	Miyakawa *et al.* (1992)
Lb. casei ssp. *casei* LLG	Leu-*p*-NA	87	7	39	1	M	Arora and Lee (1992)
Lb. delbrueckii ssp. *bulgaricus* ACA-DC233	Lys-*p*-NA	98	6	40	1	M	Tsakalidou *et al.* (1993)
Lb. helveticus ITGL1	Lys-*p*-NA	97	6.5	50	1	M	Blanc *et al.* (1993)

Str. thermophilus CNRZ 1199	Lys-*p*-NA	89	6.5	35	1	M	Tsakalidou *et al.* (1993)
Str. thermophilus CNRZ 302	Lys-*p*-NA	97	7.0	36	1	M	Rul *et al.* (1994)
Aminopeptidase A (Glutamyl aminopeptidase, GAP, PepA)							
Lc. lactis ssp. *cremoris* HP	Glu/Asp-*p*-NA	130	—	50–55	3	M	Exterkate and de Veer (1987)
Lc. lactis ssp. *lactis* NCDO 712	Glu-*p*-NA	245	8	65	6	M	Niven (1991)
Lc. lactis ssp. *cremoris* HP	Glu-*p*-NA	520	8	50	~10	M	Baankreis (1992)
Aminopeptidase C (Thiol amiopeptidase, PepC)							
Lc. lactis ssp. *cremoris* AM2	His-β-NA	300	7	40	6	-SH	Neviani *et al.* (1989)
Lb. delbrueckii ssp. *bulgaricus* B14	Leu–Gly–Gly	220	6.5–7	50	4	-SH	Wohlrab and Bockelmann (1993)
Pyrrolidonyl Carboxylyl Peptidase (pyroglutamyl aminopeptidase, PCP)							
Lc. lactis ssp. cremoris HP	Pyr-*p*-NA	—	—	—	—	—	Exterkate (1977)
Lc. lactis ssp. *cremoris* HP	Pyr-*p*-NA	80	8–8.5	37	2	S	Baankreis (1992)
X-Prolyldipeptidyl aminopeptidase (XPDA, PPDA, XAP, PepX)							
Lc. lactis ssp. *cremoris* P8-2-47	X-Pro-*p*-NA	180	7	45–50	2	S	Kiefer-Partch *et al.* (1989)
Lc. lactis ssp. *lactis* NCDO 763	Ala–Pro-*p*-NA	190	8.5	40–45	2	S	Zevaco *et al.* (1990)
Lc. lactis ssp. *cremoris* AM2	Gly–Pro-NH-Mec	117	6–9	—	—	S	Booth *et al.* (1990a)
Lc. lactis ssp. *lactis* H1	X-Pro-*p*-NA	150	6–9	—	—	S	Lloyd and Pritchard (1991)
Lb. delbrueckii ssp. *lactis*	X-Pro-*p*-NA	165	7	50–55	2	S	Meyer and Jordi (1987)
Lb. helveticus CNRZ 32	X-Pro-*p*-NA	72	7	40	1	S	Khalid and Marth (1990d)
Lb. delbrueckii ssp. *bulgaricus* CNRZ 397	X-Pro-*p*-NA	82	7	50	—	S	Atlan *et al.* (1990)
Lb. delbrueckii ssp. *bulgaricus* B14	Ala–Pro-*p*-NA	170–200	6.5	45	2	S	Bockelmann *et al.* (1991)
Lb. acidophilus 357	Ala–Pro-*p*-NA	170–200	6.5	45	2	S	Bockelmann *et al.* (1991)
Lb. delbrueckii ssp. *bulgaricus* LBU-147	Gly–Pro-*p*-NA	270	6.5	50	3	S	Miyakawa *et al.* (1991)
Lb. helveticus LHE-511	Gly–Pro-*p*-NA	90	6.5	50	1	S	Miyakawa *et al.* (1994)

(*continues*)

TABLE V
(*Continued*)

Organism	Principal assay substrate	MW/kDa	Optimum pH	Activity (°C)	Subunits	Class	Reference
Proline Iminopeptidase (PIP)							
Pr. freud ssp. *shermanii* 13673	—	—	—	—	—	—	Panon (1990)
Lc. lactis ssp. *cremoris* HP	Pro–Gly–Gly	100	8.5	37	2	M	Baankreis (1992)
Lb. delbrueckii ssp. *bulgaricus* CNRZ 397	Pro-*p*-NA	100	6–7	40	3	S	Gilbert *et al.* (1994)
Lb. casei ssp. *casei* LLG	Pro-AMC	46	7.5	40	1	SH	Habibi-Najafi and Lee (1995)
Dipeptidases (DIP)							
Lc. bv. diacetylactis CNRZ 267	Leu–Leu	51	7.5	—	1	M	Desmazeaud and Zevaco (1977)
Lactococcus spp.	Dipeptides	25 & 34	7	30	—	M	Law (1979)
Lc. lactis ssp. *cremoris* H61	Leu–Gly	100	8	—	—	M	Hwang *et al.* (1981)
Lc. lactis ssp. *cremoris* Wg2	Dipeptides	49	8	50	1	M	van Boven *et al.* (1988)
Lb. delbrueckii ssp. *bulgaricus* B14	Dipeptides	51	7	50	1	M	Wohlrab and Bockelmann (1992)
Prolidase (PRD)							
Lc. lactis ssp. *cremoris* H61	Leu–Pro	43	6.5–7.5	—	—	M	Kaminogawa *et al.* (1984)
Lc. lactis ssp. *cremoris* AM2	Leu–Pro	42	7.35–9	—	—	M	Booth *et al.* (1990b)
Tripeptidase (TRP)							
Lc. bv. diacetylactis CNRZ 267	Tripeptides	75	7	35	—	M	Desmazeaud and Zevaco (1979)
Lc. lactis ssp. *cremoris* Wg2	Leu–Leu–Leu	103–105	7.5	35	2	M	Bosman *et al.* (1990)
Lc. lactis ssp. *cremoris* AM2	Tripeptides	105	8.6	—	2	M	Bacon *et al.* (1993)
Lc. lactis ssp. *cremoris* IMN-C12	Leu–Leu–Leu	72	5.8	33	3	-SH	Sahlström *et al.* (1993)
Lb. delbrueckii ssp. *bulgaricus* B14	Leu–Gly–Gly	85	6.0	40	>1	M	Bockelmann *et al.* (1995)

[a] M, metallo; S, serine; SH, thiol; N, neutral; AMC, aminomethyl coumarin.

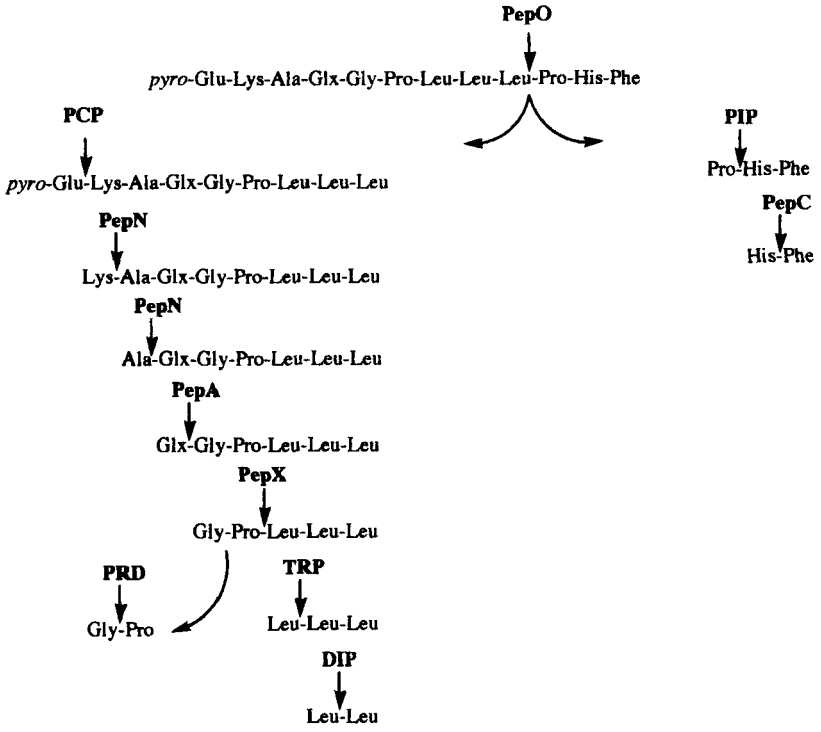


FIG. 5. Schematic representation of the hydrolysis of a hypothetical dodecapeptide by the combined action of endo- and exopeptidases of *Lactococcus* spp. Modified from Fox *et al.* (1995).

of the genes for both P_I- and P_{III}-type proteinases are known (Kok *et al.*, 1988; Vos *et al.*, 1989a,b); few differences are apparent and alteration of a few residues by site-directed mutagenesis can alter the specificity of the proteinase (Kok, 1990). However, the specificity of the lactococcal proteinases appears to be more diverse than proposed by S. Visser *et al.* (1986), e.g., Exterkate *et al.* (1993) classified the proteinases of 16 *Lactococcus* strains into seven groups based on their specificity on α_{s1}-CN f1–23.

The specificity of the CEP from several *Lactococcus* strains on α_{s1}-α_{s2}-, β- and κ-caseins and short peptide substrates has been established; these studies have been reviewed by Fox *et al.* (1994) and are summarized in Figs. 6–10. The lactococcal CEP appears to be of great importance in proteolysis in cheese (see Section IV E9).

7. *Proteolytic Enzymes of the Nonstarter Microflora*

Despite the findings by a number of authors that the NSLAB can dominate the microflora of Cheddar-type cheese during much of its ripening,

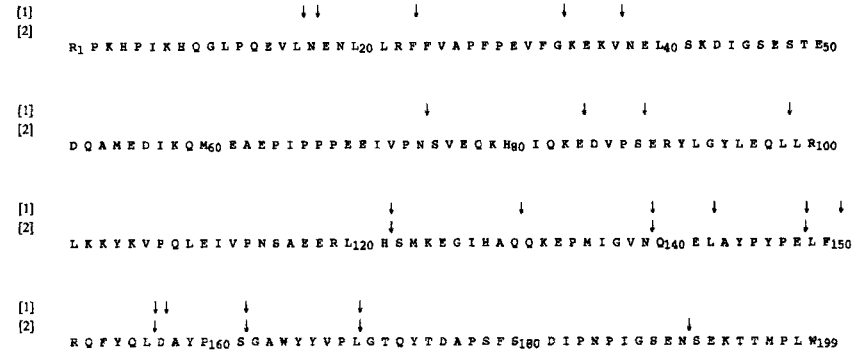


FIG. 6. Specificity of lactococcal cell envelope-associated proteinase on α_{s1}-casein. [1] *Lactococcus lactis* spp. *cremoris* SK112 (Reid *et al.*, 1991). [2] *L. lactis* ssp. *lactis* NCDO 763 (Monnet *et al.*, 1992). Modified from Fox *et al.* (1995).

the proteolytic system of NSLAB has received little attention compared with that of *Lactococcus.* The proteolytic specificity of proteinases from NSLAB on the caseins has not been determined.

The predominant NSLAB in Cheddar and Dutch-type cheeses are mesophilic *Lactobacillus,* which possess a cell wall-associated and intracellular proteinases. A range of intracellular peptidases, including dipeptidases, aminopeptidases, and endopeptidases, have been identified in *Lactobacillus* (see reviews by Khalid and Marth 1990a; Peterson and Marshall, 1990). Interestingly, carboxypeptidase activity, which has not been found in lactococci, has been reported in *Lactobacillus casei* (El Soda *et al.*, 1978).

β-Casein is preferentially degraded by a number of strains *Lactobacillus plantarum* and *L. casei,* but some strains degraded α_{s1}-casein also (Khalid and Marth, 1990b).

NSLAB also include *Micrococcus* and *Pediococcus.* All *Micrococcus* spp. appear to produce intracellular proteinases and some also produce

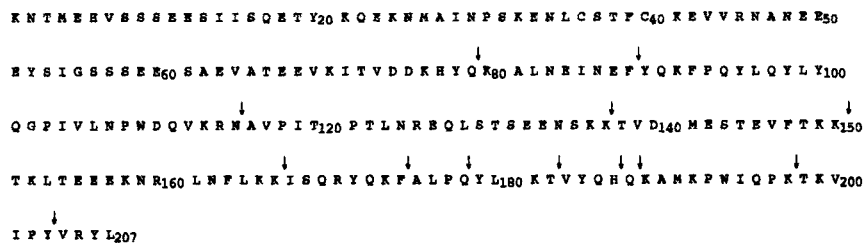


FIG. 7. Specificity of the cell envelope-associated proteinase from *Lactococcus lactis* ssp. *lactis* NCDO 763 (Monnet *et al.*, 1992) on α_{s2}-casein. Modified from Fox *et al.* (1995).

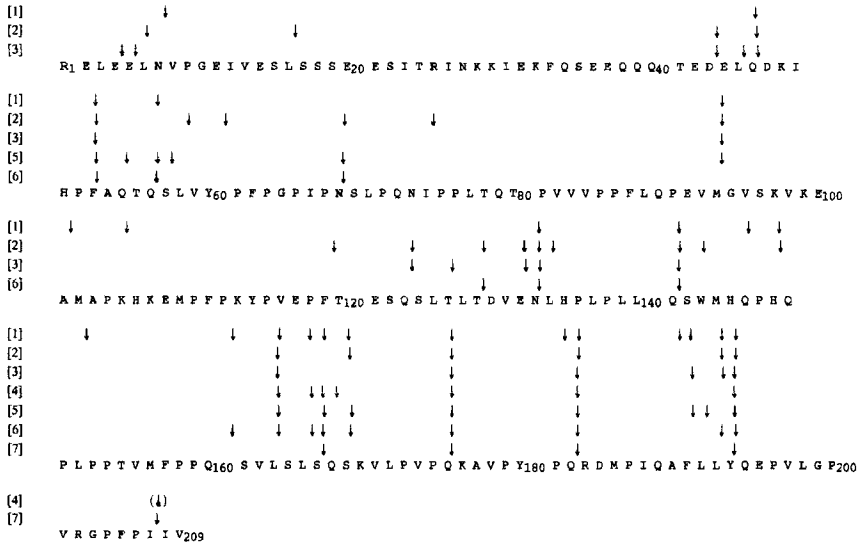


FIG. 8. Specificity of lactococcal cell envelope-associated proteinase on β-casein. [1] *L. lactis* ssp. *cremoris* H2 (Reid *et al.*, 1991b). [2] *L. lactis* ssp. *cremoris* SK112 (Reid *et al.*, 1991b). [3] *L. lactis* ssp. *cremoris* AM_1 (Visser *et al.*, 1991). [4] *L. lactis* ssp. *cremoris* JP (Visser *et al.*, 1988). [5] *L. lactis* ssp. *cremoris* AC1 (Monnet *et al.*, 1989). [6] *L. lactis* ssp. *lactis* NCDO 763 (Monnet *et al.*, 1989). [7] *L. lactis* ssp. *lactis* NCDO 763 (Monnet *et al.*, 1986). Modified from Fox *et al.* (1995).

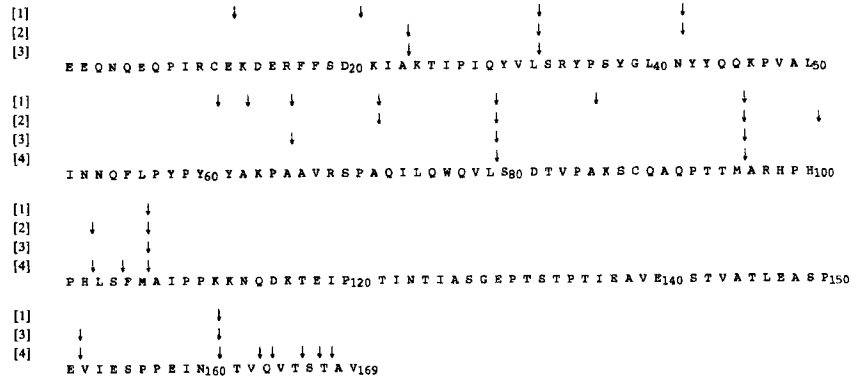
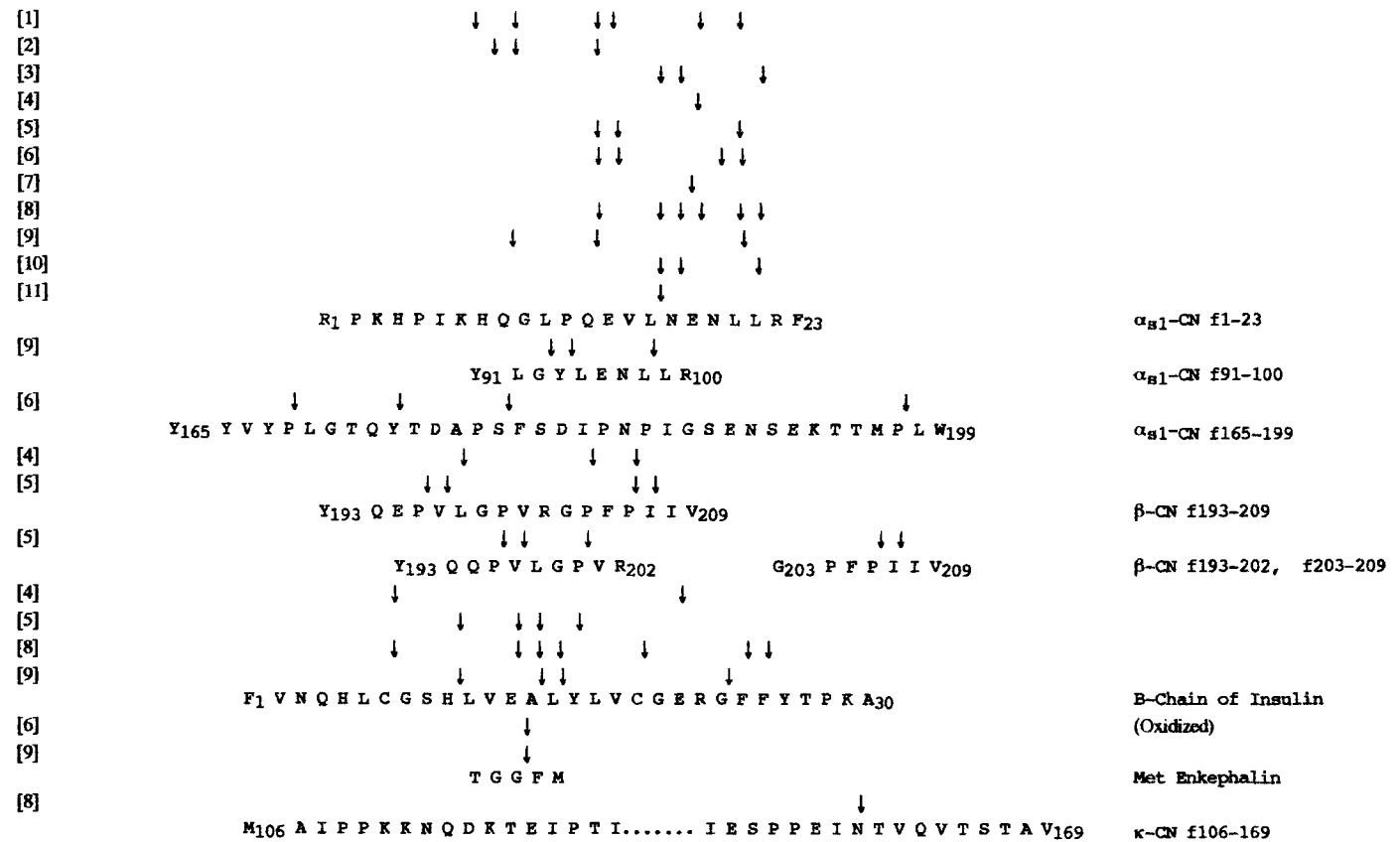


FIG. 9. Specificity of lactococcal cell envelope-associated proteinase on κ-casein. [1] *Lactococcus lactis* ssp. *lactis* NCDO 763 (Monnet *et al.*, 1992). [2] *L. lactis* ssp. *cremoris* H2 (Reid *et al.*, 1994). [3] *L. lactis* ssp. *cremoris* SK11 (Reid *et al.*, 1994). [4] *L. lactis* ssp. *cremoris* AM_1 (Visser *et al.*, 1994). Modified from Fox *et al.* (1995).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αs1-CN f1-23
[9]
Y91 L G Y L E N L L R100
αs1-CN f91-100
[6]
Y165 Y V Y P L G T Q Y T D A P S F S D I P N P I G S E N S E K T T M P L W199
αs1-CN f165-199
[4]
[5]
Y193 Q E P V L G P V R G P F P I I V209
β-CN f193-209
[5]
Y193 Q Q P V L G P V R202
G203 P F P I I V209
β-CN f193-202, f203-209
[4]
[5]
[8]
[9]
F1 V N Q H L C G S H L V E A L Y L V C G E R G F F Y T P K A30
B-Chain of Insulin
[6]
(Oxidized)
[9]
T G G F M
Met Enkephalin
[8]
M106 A I P P K K N Q D K T E I P T I..... I E S P P E I N T V Q V T S T A V169
κ-CN f106-169

extracellular proteinases. All strains studied by Nath and Ledford (1972) and Bhowmik and Marth (1988) possessed intracellular proteoloytic activity. Baribo and Foster (1952) studied some of the characteristics of the intracellular proteolytic activity of *M. freudenreichii* 325. *Micrococcus* spp. also produce extracellular proteinases with alkaline pH optima. Desmazeaud and Hermier (1968a,b) studied an extracellular neutral metalloproteinase from *M. caseolyticus* and Desmazeaud and Hermier (1971) determined its specificity on glucagon; this enzyme, which did not possess exopeptidase activity, rapidly cleaved at bonds containing Phe, Leu, or Ala. Nath and Ledford (1972) found that 3 of the 18 strains of *Micrococcus* studied produced extracellular proteinases which preferentially hydrolyzed α_{s1}-casein while intracellular proteinases preferetially hydrolyzed β-casein. The extracellular metalloproteinase (28.9 kDa) of *Micrococcus* MCC-315 was isolated by Prasad *et al.* (1986) who found this enzyme to be optimally active at pH 10.6 and at temperatures between 50 and 60°C (whole casein) or 37 to 40°C (β-casein). García de Fernando and Fox (1991) purified two extracellular metalloproteinases (23.5 and 42.5 kDa) from *Micrococcus* GF, a strain which had been isolated from farmhouse Blue cheese. The enzymes were optimally active at ~45°C and pH 8.5 to 11. One proteinase preferentially hydrolyzed β-casein while the other hydrolyzed both α_{s1}- and β-caseins at approximately the same rate. Membrane-associated and intracellular proteinases have also been found (see review by Bhowmik and Marth, 1990b).

There are few reports on the proteolytic activity of *Pediococcus;* Tzanetakis and Litopoulou-Tzanetaki (1989) found Leu and Val aminopeptidase activities in *P. pentosaceus* and El-Soda *et al.* (1991) reported aminopeptidase and dipeptidase activity in *Pediococcus* sp. LR.

8. Proteinases from Secondary Starter

Proteinases and peptidases from the secondary (adjunct) starter can play an important role in proteolysis in cheese varieties where such adjuncts

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FIG. 10. Specificity of lactococcal cell envelope-associated proteinase or endopeptidase (Pep O) on various peptides. [1] *L. lactis* ssp. *cremoris* H61 (Kaminogawa *et al.*, 1986). [2] *L. lactis* ssp. *cremoris* HP (Exterkate *et al.*, 1991). [3] *L. lactis* ssp. *cremoris* AM_1 (Exterkate *et al.*, 1991). [4] *L. lactis* ssp. *cremoris* (Baankreis, 1992). [5] *L. lactis* ssp. *cremoris* C13 (Baankreis, 1992). [6] *L. lactis* ssp. *lactis* MG 1363 (Stepaniak and Fox, 1995). [7] *L. lactis* ssp. *cremoris* H61 (Yan *et al.*, 1987b). [8] *L. lactis* ssp. *lactis* NCDO 763 (Monnet *et al.*, 1992). [9] *L. lactis* ssp. *cremoris* H61 (Yan *et al.*, 1987a). [10] *L. lactis* ssp. *cremoris* AM_1, pH 6.5 (Exterkate and Alting, 1993). [11] *L. lactis* ssp. *cremoris* AM_1, pH 5.2 + NaCl (Exterkate and Alting, 1993). Modified from Fox *et al.* (1995).

are used. A number of authors (e.g., Broome *et al.*, 1990, 1991) have used lactobacilli as adjuncts in the manufacture of Cheddar; their proteolytic system is presumably the same as that of starter and nonstarter lactobacilli, as was discussed in Section IV E6 and IV E7. In this section, enzymes from traditional adjuncts, i.e., *Penicillium* spp. (mould-ripened varieties), *Brevibacterium linens* (smear-ripened varieties), and *Propionibacterium* spp. (Swiss varieties) will be discussed.

Blue-veined cheeses are characterized by the growth of *Penicillium roqueforti* throughout the cheese and Camembert and Brie by the growth of *P. camemberti* on the surface. These moulds produce aspartyl and metalloproteinases which have generally similar specificities on α_{s1}- and β-caseins (Trieu-Cuot and Gripon, 1981). The aspartyl proteinases from both species cleave β-casein at positions Lys_{29}–Ile_{30}, Lys_{97}–Val_{98}, and Lys_{99}–Glu_{100}, releasing five peptides: β-CN f98–209, f100–209, f1–97/99, f30–209, and f1–29 (Trieu-Cuot and Gripon, 1982). The aspartyl proteinase of *Penicillium* spp. also cleaves α_{s1}-casein at a number of sites; according to Trieu-Cuot and Gripon (1982), the primary site is at or near the primary site of chymosin action, i.e., Phe_{23}–Phe_{24}.

The metalloproteinases of *P. roqueforti* and *P. camemberti* are generally similar; the primary cleavage sites for the *P. camemberti* enzyme on β-casein are Lys_{28}–Lys_{29}, Pro_{90}–Glu_{91}, and Glu_{100}–Ala_{101} (Trieu-Cuot *et al.*, 1982).

Intracellular acid proteinase(s) and exopeptidases (amino and carboxy) have been found in *P. roqueforti* and *P. camemberti* but have not been well studied (see Gripon *et al.*, 1991; Gripon, 1993). *P. roqueforti* excretes a carboxypeptidase with an acid pH optimum and an alkaline metallo aminopeptidase (see Gripon *et al.*, 1991; Gripon, 1993).

Brevibacterium linens, a characteristic component of the surface microflora of smear-ripened varieties, has a strong proteolytic activity (Gripon *et al.*, 1991). The extracellular proteinases secreted by a number of *Br. linens* strains have been partially purified (see Rattray *et al.*, 1995). The proteinase secreted by *Br. linens* ATCC 9174 was purified to homogeneity by Rattray *et al.* (1995) and found to be a serine proteinase with optimum activity at pH 8.5 and 50°C. It appears to be a trimeric enzyme with a monomeric mass of 56 kDa. The sequence of the 20 N-terminal amino acids showed no homology with the published sequences of other bacterial proteinases (Rattray 1995). Foissy (1974) reported on both intra- and extracellular proteinases but they were not studied further; an extracellular aminopeptidase was purified by Foissy (1978). Sørhaug (1981) described a number of peptidase activities in cell-free extracts of *Br. linens.*

There are few studies on the proteinase and peptidase activities of *Propionibacterium shermanii,* the characteristic microorganism in Swiss cheese

varieties. Langsrud *et al.* (1977, 1978) studied the release of Pro by *Propionibacterium* in growth media. The location of peptidases from *P. shermanii* α and ATCC 9614 was studied by Sahlström *et al.* (1989) who found a number of peptidases (assayed on a range of di- and tripeptides) associated with the wall, membrane, and intracellular fractions. Perez Chaia *et al.* (1990) studied the aminopeptidase and proline iminopeptidase activities of cell-free extracts of *P. freudenreichii* ssp. *shermanii* ATCC 1367 and $E_2$2, which were optimal at 37–45°C and pH 6.4–7.2. Panon (1990) isolated a proline iminopeptidase from *P. shermanii* 13673; this serine enzyme (61 kDa) was optimally active at pH 8 and 40°C. *P. shermanii* (NZ) was one of the bacteria studied by El-Soda *et al.* (1991) who found that its partially purified aminopeptidase was optimally active at pH 7.5 and 50°C. El-Soda *et al.* (1992) studied the intracellular peptide hydrolase system of *P. freudenreichii, P. acidipropionicci,* and *P. jensenii;* aminopeptidase and dipeptidase activities were found in all species studied but no carboxypeptidase or endopeptidase activities were detected. The majority of the strains studied were able to degrade casein. The cell wall-associated peptide hydrolase activities of a number of cheese-related microorganisms, including *P. acidipropionici,* were studied by Ezzat *et al.* (1993).

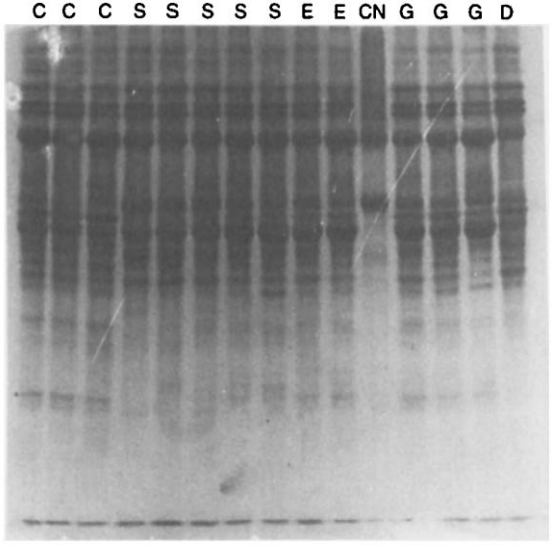

FIG. 11. Urea-polyacrylamide gel electrophoretograms of casein (CN) and water-insoluble fractions from a number of Cheddar (C), Swiss (S), Edam (E), Gouda (G), or Blarney (D) cheeses (E. Olthoff and R. Schmidt, unpublished).

9. *Characterization of Proteolysis in Cheese*

As mentioned in Section IV E1, the extent of proteolysis varies from very limited, e.g., Mozzarella, to very extensive, e.g., blue-mould varieties. The use of PAGE showed that the proteolytic pattern, as well as its extent, exhibit marked intervarietal differences (Ledford *et al.*, 1966; Marcos *et al.*, 1979). The PAGE patterns of both the water-insoluble and water-soluble fractions are, in fact, quite characteristic of the variety, as shown in Figs. 11 and 12 for a number of Cheddar, Dutch, and Swiss-type cheeses. RP-HPLC of the water-soluble fraction or subfractions thereof also shows varietal characteristics (Fig. 13). Both the PAGE and HPLC patterns vary and become more complex as the cheese matures and are in fact very useful indices of cheese maturity and to a lesser extent of its quality (O'Shea, 1993). Therefore, they have potential in the objective assessment of cheese quality.

Complete characterization of proteolysis in cheese requires isolation and identification of the individual peptides. Various fractionation techniques were compared to Kuchroo and Fox (1983), some of which have been developed further by O'Sullivan and Fox (1990), Singh *et al.* (1994), and Fox *et al.* (1994). Using these techniques, many of the water-insoluble and water-soluble peptides in Cheddar cheese have been isolated and identified

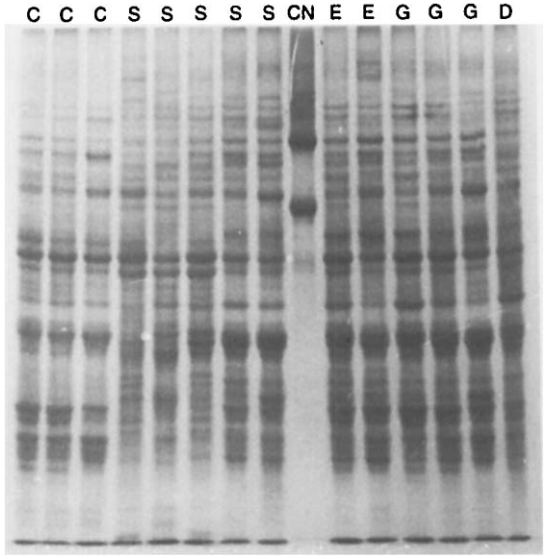

FIG. 12. Urea-polyacrylamide gel electrophoretograms of casein (CN) and water-soluble fractions prepared from a number of Cheddar (C), Swiss (S), Edam (E), Gouda (G), or Blarney (D) cheeses. (E. Olthoff, R. Schmidt, and P. F. Fox, unpublished).

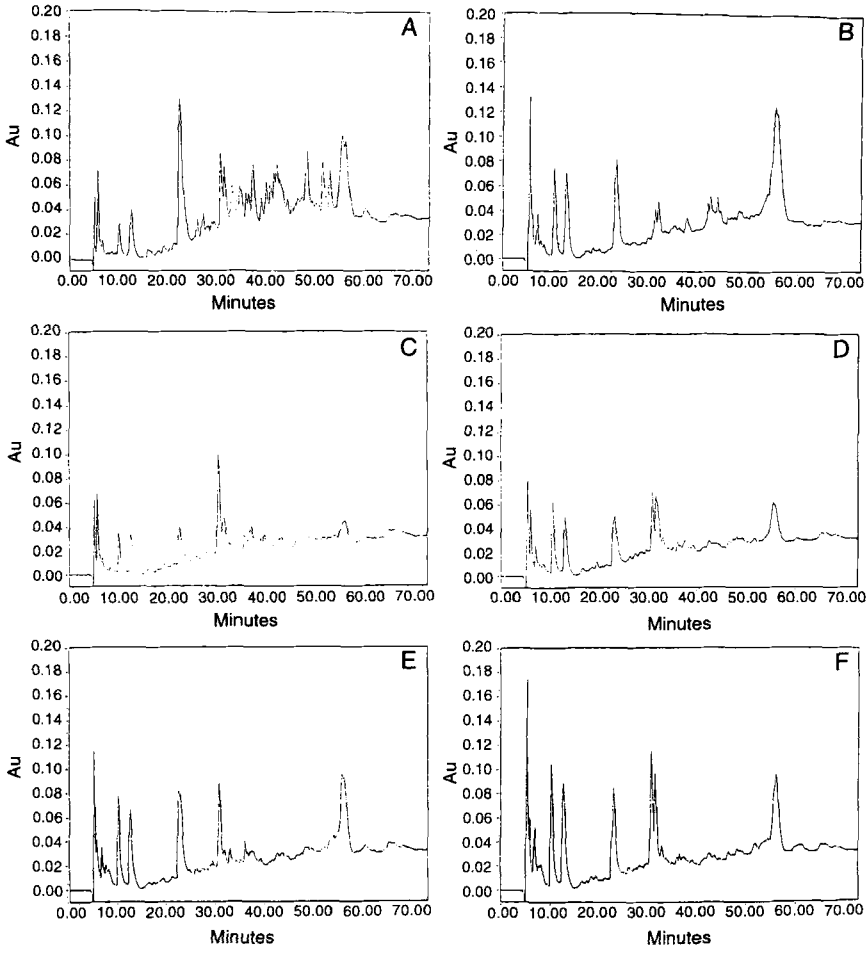

FIG. 13. Typical RP-HPLC (C_8 column, acetonitrile/water gradient, TFA as ion pair reagent, detection at 214 nm) chromatograms of the water-soluble fraction of Cheddar (A), Emmental (B), Edam (C), Gouda (D), Leerdamer (E), and Jarlsberg (F).

by amino acid sequencing and mass spectrometry. The results of these studies have been reported by Singh *et al.* (1994, 1995), Fox *et al.* (1994), and McSweeney *et al.* (1994c) and are summarized in Fig. 14. All the principal water-insoluble peptides are produced either from α_{s1}-casein by chymosin or from β-casein by plasmin (McSweeney *et al.*, 1994c). In mature Cheddar (>6 months old), all the α_{s1}-casein is hydrolyzed by chymosin at Phe_{23}–Phe_{24}. The peptide α_{s1}-CN f1–23 does not accumulate but is rapidly hydrolyzed at the bonds Gln_9–Gly_{10} and Gln_{13}–Glu_{14} by the lactococcal

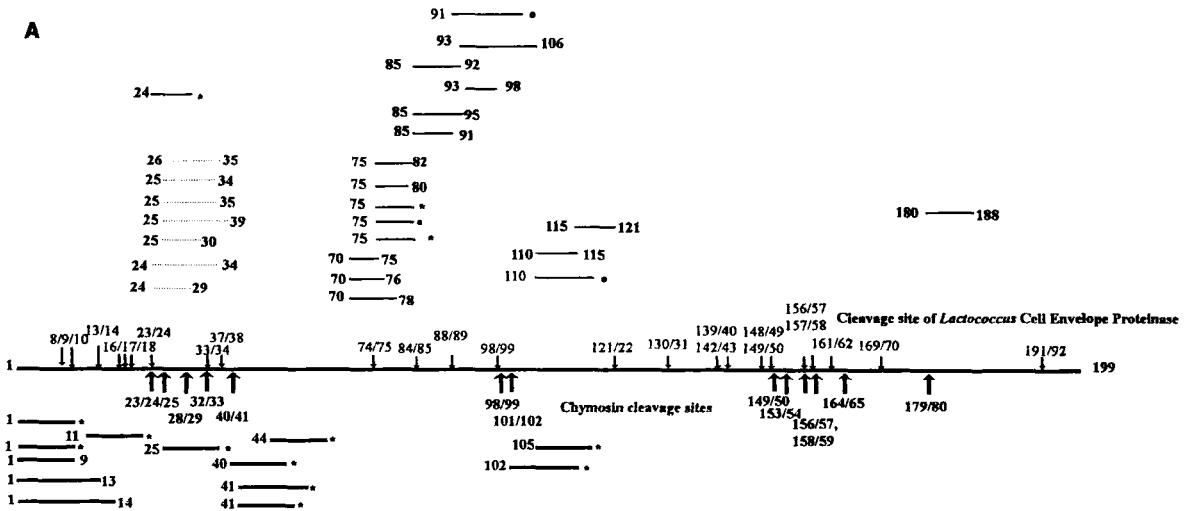

FIG. 14. Identity of peptides isolated from the water-soluble fraction of Cheddar cheese. Peptides derived from α_{s1}-casein are shown in A, those from β-casein in B. The principal chymosin cleavage sites in α_{s1}-casein, the principal plasmin cleavage sites in β-casein, and the principal cleavage sites of lactococcal cell envelope proteinase on α_{s1}- and β-casein are shown by arrows (from Fox *et al.*, 1994, 1995, unpublished).

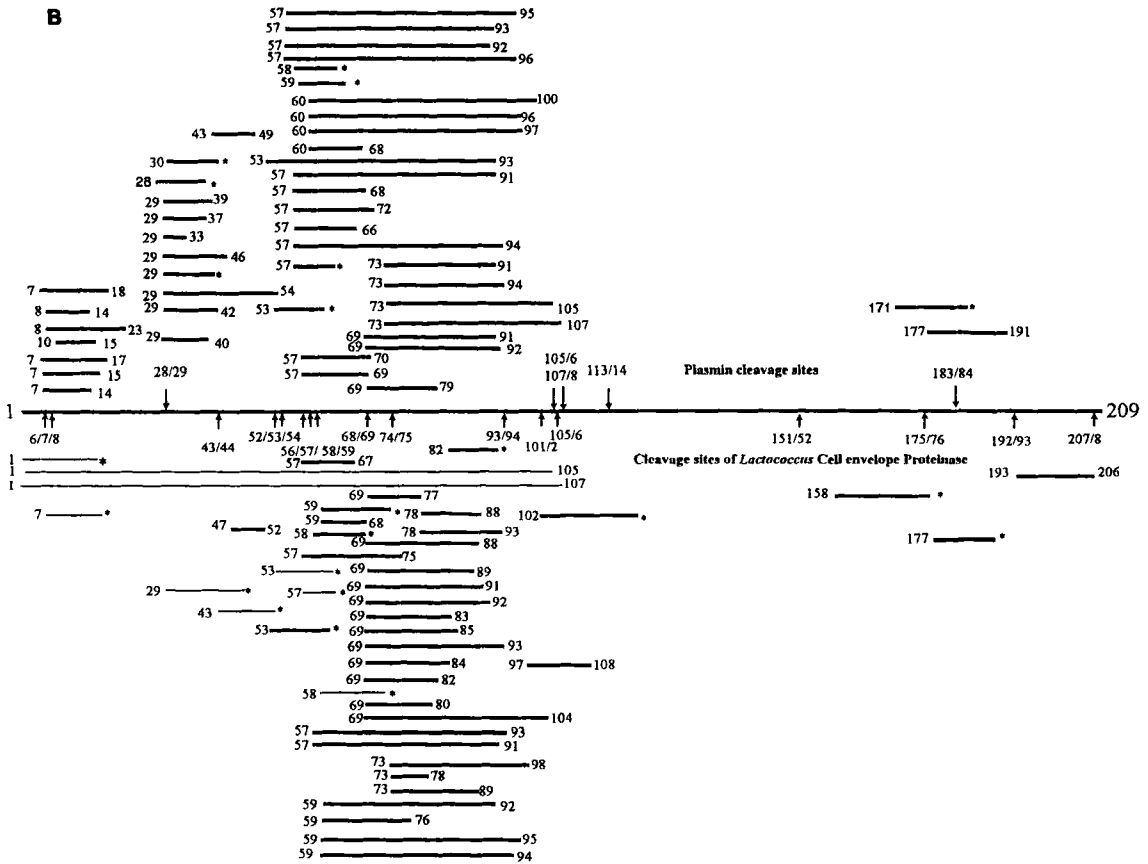

FIG. 14. (*Continued*).

cell wall proteinase. A significant amount of the larger peptide α_{s1}-CN f24–199 is hydrolyzed at Leu_{101}–Lys_{102}. In mature Cheddar, ~50% of the β-casein is hydrolyzed, mainly by plasmin, to γ-caseins (β-CN f29–209, f106–209, and f108–209) and proteose peptones (β-CN f1–28, f1–105, f1–107, f29–105, f29–107). These polypeptides do not appear to be hydrolyzed by chymosin or lactococcal proteinases. Although α_{s2}-casein gradually disappears from PAGE patterns (McSweeney *et al.*, 1993a), the polypeptides produced from it, if any, have not been identified. *para*-κ-Casein appears to be resistant to proteolysis and no peptides produced from it have been identified.

Most of the peptides in the UF retentate of the water-soluble fraction are derived from β-casein, especially from the region residues 53 to 91 (Fig. 14). In contrast, most of the peptides in the UF permeate are produced from α_{s1}-CN (Fig. 14). The N terminus of some of these peptides corresponds to a chymosin (α_{s1}-CN) or plasmin (β-CN) cleavage site but many appear to be produced by the lactococcal cell wall proteinase. However, the N terminus and especially the C terminus of many peptides does not correspond precisely to the known cleavage sites of chymosin, plasmin, or lactococcal proteinase. This strongly suggests the action of bacterial aminopeptidases. Carboxypeptidase activity would explain why the C terminus of many peptides does not correspond to known proteinase cleavage sites but *Lactococcus* spp. have not been reported to possess a carboxypeptidase and there is only one report (El-Soda *et al.*, 1978) of carboxypeptidase activity in *Lactobacillus*. It must be presumed that other proteinase, e.g., from NSLAB, or starter or NSLAB endopeptidases (Pep O), are involved or perhaps other cleavage sites for lactococcal cell wall proteinase remain to be identified.

The N-terminal sequence of α_{s1}-CN f1–9 and f1–13 is RPKHPIK; therefore, it should be susceptible to Pep X. The accumulation of these peptides in Cheddar and the apparent absence of peptides with a sequence commencing at Lys_3 of α_{s1}-CN suggest that Pep X is not active in cheese; isolated Pep X is in fact inactive on α_{s1}-CN f1–23 (W. Bockelmann and P. F. Fox, unpublished).

The very small peptides in the UF permeate have not yet been identified. A number of authors (Aston and Creamer, 1986; Cliffe *et al.*, 1993; Engels and Visser, 1994) have shown that the very small peptides (<500 Da) make a significant contribution to Cheddar flavor; therefore, identification of the small peptides should prove interesting.

Fractionation and identification of the small water-soluble peptides in cheese varieties is much less advanced than for Cheddar. Although not fractionated systematically, a large number of 12% TCA-soluble and -insoluble peptides were identified in water extracts of Parmesan by Addeo

et al. (1992, 1994) using fast atom bombardment mass spectrometry. Parmesan is quite an exceptional cheese; while it undergoes extensive proteolysis and has a very high concentration of free amino acids, it contains low concentrations of medium-sized peptides (Resmini *et al.*, 1988).

Although very extensive proteolysis occurs in Blue cheese and some of the larger peptides detectable by PAGE have been partially identified (see Gripon, 1993), very little work has been done on the small (pH 4.6-soluble) peptides. The only study we are aware of is the partial identification of four PTA-soluble peptides from Gamonedo Blue cheese by Gonzalez de Llano *et al.* (1991). Tsuda *et al.* (1993) identified the dipeptide Leu–Leu in Camembert using capillary isotacophoresis. Some of the peptides resulting from the cleavage of α_{s1}-CN f1–23 (produced by chymosin) by lactococcal cell envelope-associated proteinase have been identified in Gouda (Kaminogawa *et al.*, 1986; Exterkate and Alting, 1995). Proteolysis in Swiss-type cheeses has been studied using PAGE and RP-HPLC (Steffen *et al.*, 1993; Bican and Spahni, 1993) but as far as we are aware, small peptides have not been isolated and characterized.

Significant concentrations of amino acids, the final products of proteolysis, occur in all cheeses that have been investigated (see McSweeney and Fox, 1993). Relative to the level of water-soluble nitrogen, Cheddar contains a low concentration of amino acids (see Fig. 19, Section VC2). The principal amino acids in Cheddar are Glu, Leu, Arg, Lys, Phe, and Ser (Wilkinson, 1992) (Fig. 15). Parmesan contains a very high concentration of amino

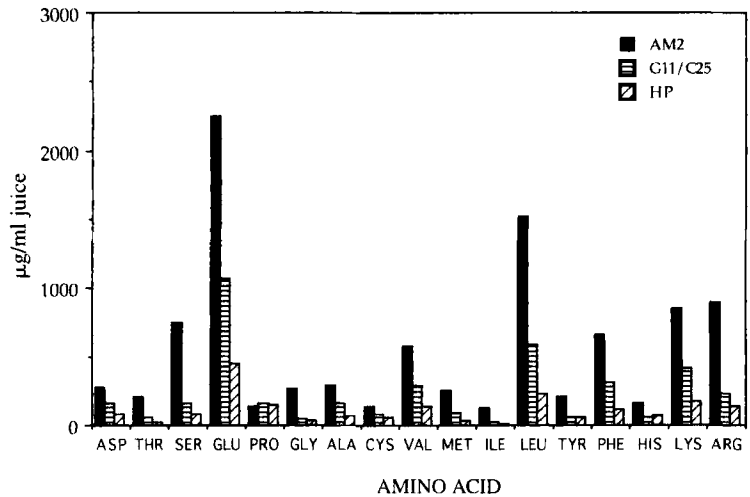

FIG. 15. Free amino acids in Cheddar cheese made using different starters and ripened at 10°C for 42 days (Wilkinson, 1992).

acids which appear to make a major contribution to the characteristic flavor of this cheese (Resmini *et al.*, 1988). The presence of amino acids in cheeses clearly indicates aminopeptidase activity; since these enzymes are intracellular, their action indicates lactococcal cell lysis. On the presumption that amino acids contribute to cheese flavor, interest is now being focussed on a search for fast-lysing lactococcal strains, e.g., heat-induced (Feirtag and McKay, 1987), phage-induced (Crow *et al.*, 1996), or bacteriosin-induced (Morgan *et al.*, 1995). Amino acids have characteristic flavors (Table VI); although none has a cheese-like flavor, it is believed that they contribute to the savory taste of mature cheese.

10. Catabolism of Amino Acids and Related Events

Catabolism of free amino acids probably plays some role in all cheese varieties but is particularly significant in mould- and smear-ripened varieties. The first stage in amino acid catabolism involves decarboxylation, deamination, transamination, desulfuration, or perhaps hydrolysis of the amino acid side chains. The second stage involves conversion of the resulting compounds (amines and α-ketoacids), as well as amino acids themselves, to aldehydes, primarily by the action of deaminases on amines. The final level of amino acid catabolism is the reduction of the aldehydes to alcohols or their oxidation to acids (Hemme *et al.*, 1982). Sulfur-containing amino acids can undergo extensive conversion, leading to the formation of a number of compounds, including methanethiol and other sulfur derivatives. General pathways of amino acid catabolism are summarized in Fig. 16. The catabolism of amino acids has been reviewed by Hemme *et al.* (1982) and Law (1984, 1987).

Decarboxylation involves the conversion of an amino acid to the corresponding amine, with the loss of CO_2. The presence of primary amines (e.g., tyramine) in cheese can be explained in terms of simple decarboxylation, although the presence of secondary and tertiary amines is more difficult to explain. The principal amine in cheese is tyramine (Law, 1987). The decarboxylase activities of microorganisms of significance in cheese ripening were discussed by Hemme *et al.* (1982). Deamination results in the formation of NH_3 and α-ketoacids. Ammonia is an important constituent of a number of cheeses, such as Camembert, Gruyere, and Comte (Hemme *et al.*, 1982). Ammonia can also be formed by the oxidative deamination of amines (yielding aldehydes), and products of complex reactions involving amino acid side chains can also be deaminated. Transamination results in the formation of other amino acids by the action of transaminases. Aldehydes formed by the above processes can then be oxidized to acids or reduced to the corresponding alcohols.

TABLE VI

TASTE DESCRIPTOR AND THRESHOLD VALUES OF AMINO ACIDS (ADAPTED FROM O'CALLAGHAN, 1994)

Amino acid	Q (cal mol^{-1})	Taste threshold (mg 100 ml^{-1})	Concentration[a] in Cheddar ($\mu g\ g^{-1}$)	Concentration[a] in Cheddar (mg 100 ml^{-1} extract)[b]	Perception[c]	Taste[d] Sweet	Salt	Sour	Bitter	Umami
Gly	0	130	370	15.6	−	***				
Ser	300	150	1210	51.1	−	***			*	
Thr	400	260	650	27.4	−	***		*		
His	500	20	440	18.6	±		***			
Asp	0	3	610	25.7	+		**		*	
Glu	0	5	5080	214.4	+		***		**	
Arg	750	50	1740	73.4	+			***		
Ala	500	60	340	14.3	−	***				
Met	1300	30	870	36.7	+			***		
Lys	1500	50	2330	98.3	+	**		**		
Val	1500	40	2020	85.2	+			***		
Leu	1800	190	4610	194.5	±			***		
Pro	2600	300	390	16.5	−	***		***		
Phe	2500	90	2400	101.3	+			***		
Tyr	2300	—	610	25.7				***		
Ile	2950	90	470	19.8	−			***		
Trp	3400	90	—	—	−				***	

[a] Wilkinson (1992); total concentration 24.1 mg g^{-1} cheese.

[b] 50 g cheese containing 37% moisture to 100 ml water.

[c] Amino acids in Cheddar cheese are deemed to be perceived if their concentration in the water extract is greater than their threshold concentration.

[d] Asterisks indicate degree of taste sensation.

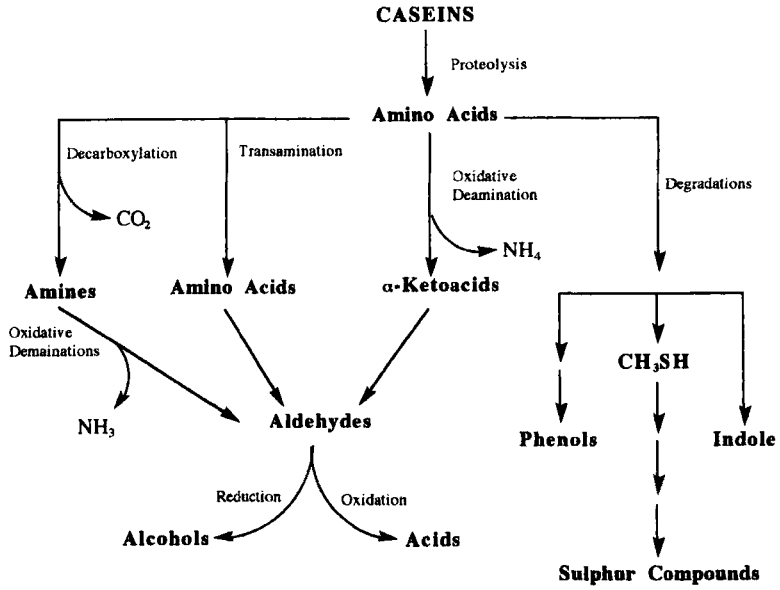

FIG. 16. Catabolism of amino acids in cheese (adapted from Fox *et al.*, 1995).

Amino acid side chains can also be modified in cheese. Hydrolases can release ammonia from Asn or Gln or by the partial hydrolysis of the guanidino group of Arg, forming citrulline or its degradation to ornithine (Hemme *et al.*, 1982). Phenol and indole, in addition to pyruvate and ammonia, can be produced by the action of C–C lyases on Tyr and Trp.

Volatile sulfur compounds are found in most cheeses and can be important flavor constituents. The origin of sulfur-containing compounds is generally thought to be the sulfur-containing amino acids methionine and cysteine (Law, 1987). As Cys is rare in the caseins (occurring at low levels only in α_{s2}- and κ-caseins, which are not extensively hydrolyzed in cheese), the origin of sulfur compounds must be primarily Met. Sulfur compounds formed from Met include H_2S, dimethylsulfide, and methanethiol. The importance of methanethiol and related compounds in cheese aroma is discussed by Law (1987).

A number of amines produced in cheese are biologically active, including tyramine, histamine, tryptamine, putrescine, cadaverine, and phenylethylamine. These biogenic amines can have imporant physiological effects for susceptible individuals, including migraine headaches and hypertension (see Section IXJ).

V. CHEESE FLAVOR

A. INTRODUCTION

The flavor and texture of cheeses are their most important attributes. Generally, the former is the more important but there are exceptions, e.g., Mozzarella, which has very little flavor and is judged mainly by its textural properties, especially meltability and stretchability. Color and overall physical appearance are of some importance in all varieties; in fact, poor appearance and discoloration may be the most important attributes since they are the attributes by which the consumer initially assesses cheese quality or acceptability.

Cheese flavor has been the subject of scientific investigation since the beginning of this century. Initially, it was believed that a single compound might be responsible for cheese flavor but according to the "Component Balance Theory" (Mulder, 1952; Kosikowski and Mocquot, 1958), cheese flavor results from the correct balance and concentration of numerous sapid and aromatic compounds. During the intervening 40 years, there has been extensive research on the flavor of several cheese varieties, but complete information is not yet available on the flavor chemistry of any specific variety. The extensive literature on cheese flavor has been reviewed by Reiter *et al.* (1966), Kristoffersen (1973, 1985), McGugan (1975), Aston and Dulley (1982), Adda *et al.* (1982), Lawrence *et al.* (1983), Olson (1990), Urbach (1993), Fox (1994), and Fox *et al.* (1995).

Although it is not possible to describe the flavor of cheese in precise chemical terms, very considerable progress has been made on the identification of flavor compounds in cheese and elucidation of the biochemical pathways by which these compounds are produced. It is generally recognized that the aroma of cheese is primarily in the volatile fraction while taste is largely in the aqueous phase; until recently, most researchers focussed on the volatile fraction. Intervarietal comparisons should be a valuable approach toward identifying key flavor compounds. Although several such studies on the volatile compounds have been reported, there have been relatively few comparative studies on the aqueous phase.

One of the major problems encountered in research on cheese flavor is defining what the typical flavor should be. Within any variety, a fairly wide range of flavor and textural characteristics is acceptable; this is particularly so for Cheddar which makes it especially difficult to chemically define its flavor. In cheese factories, wholesale or retail outlets and research laboratories, somebody decides what constitutes desirable and undesirable flavor, which may not be typical. Only recently have systematic attempts been

made to objectively describe the sensoric attributes of cheese, e.g., McEwan *et al.* (1989), Muir and Hunter (1992), and Hirst *et al.* (1994). An international study in the EU FLAIR-SENS programme (FLAIR Concerted Action No. 2, Cost 902, Relating Instrumental, Sensory and Consumer Data) had a similar objective, especially for cheese varieties with Appelation d'Origine Contrôlée status. An agreed vocabulary is essential if the results of instrumental studies are to be related to the sensoric attributes and quality of cheese.

Perhaps not surprisingly, chemical definition of off-flavors has been more successful than the definition of desirable flavors because off-flavors usually have a fairly well-defined cause, e.g., bitterness (peptides), rancidity (fatty acids), fruitiness (esters), and the specific compound(s) responsible can, usually, be identified.

Since cheese texture has a major impact on flavor perception, these attributes should, ideally, be considered together. For example, it has been suggested (McGugan *et al.*, 1979) that the main contribution of proteolysis to cheese flavor is due to its effect on cheese texture which affects the release of sapid compounds on mastication of the cheese. However, these two aspects of cheese quality are rarely part of the same investigation and cheese texture is even less well understood at the molecular level than cheese flavor.

This section will deal with three aspects of cheese flavor: (1) analytical techniques, (2) intervarietal comparisons, and (3) effect of composition on cheese quality. The biochemical pathways through which the principal flavor compounds are generated were described in Section VI.

B. ANALYTICAL METHODS

1. Nonvolatile Compounds

Although studies on cheese flavor date from the beginning of this century, the techniques available prior to the development of gas chromatography (GC) in the 1950s were inadequate to permit significant progress. Early investigators recognized the important contribution of proteolysis and lipolysis to cheese ripening. Studies on proteolysis relied on changes in protein/peptide solubility, e.g., in water, pH 4.6 buffers, TCA, ethanol, etc. Such techniques, which have been reviewed by Fox (1989a), IDF (1991a), and McSweeney and Fox (1993), are still widely used as useful indices of cheese maturity but are less effective indices of cheese quality (Aston *et al.*, 1983). Since the products of proteolysis are nonvolatile, they contribute to cheese taste but not to aroma.

More specific studies on proteolysis commenced with the development of paper and ion-exchange chromatography in the 1950s. Large water-soluble and insoluble peptides are best characterized by electrophoresis, especially PAGE, which was first applied to cheese by Ledford *et al.* (1966) and is now used widely for this purpose (for reviews, see Shalabi and Fox, 1987; Creamer, 1991; Strange *et al.*, 1992). Isoelectric focusing has had limited application in studies on cheese ripening and is not particularly effective. Recently developed capillary electrophoresis is a very powerful technique but has had very little application to date in cheese research.

The small peptides in cheese can be fractionated by various forms of chromatography, e.g., gel permeation, ion-exchange, and especially RP-HPLC. Using these techniques, more than 200 peptides have been demonstrated in Cheddar cheese, many of which have been isolated and identified (see Section IVE). Free amino acids are usually quantified by ion-exchange HPLC with post-column derivitization using ninhydrin or by separation of fluorescent amino acid derivatives by RP-HPLC.

The total concentration of free fatty acids is usually determined by extraction/titration methods or spectrophotometrically as Cu soaps. Early attempts to quantify the concentration of individual short-chain fatty acids involved steam distillation and adsorption chromatography. Complete separation and quantitation of free fatty acids can be achieved by GC, usually as their methyl esters, for which several preparative techniques have been published. Free fatty acids are major contributors to the flavor of some varieties, e.g., Romano, Feta, and Blue; in the latter, up to 25% of the total fatty acids may be in the free form. Short chain fatty acids are important contributors to cheese aroma, while longer chain acids contribute to taste. Excessive concentrations of either cause off-flavors (rancidity) and the critical concentration is quite low in many varieties, e.g., Cheddar and Gouda.

Several other organic acids, especially lactic, are present in cheese, and are routinely analyzed by HPLC. Enzymatic methods are available for several acids, e.g., D and L lactic (the easiest method available to distinguish between the isomers), acetic, pyruvic, and succinic (Boehringer-Mannheim, 1986).

2. *Volatile Compounds*

Compounds responsible for cheese aroma are volatile. While some preliminary work on the volatile constituents of cheese was done before 1960, e.g., short chain fatty acids and amines, significant progress was not possible until the development of GC in the 1950s. GC was first applied to the study of Cheddar cheese volatiles by Scarpellino and Kosikowski (1962) and McGugan and Howsam (1962), who used vacuum distillation and cold

trapping to recover volatiles and GC with packed columns and thermal conductivity detectors to resolve and identify them. The introduction of flame ionization detectors, capillary columns, and interfacing GC with mass spectrometry (MS) markedly increased sensitivity and greatly extended the number of compounds detected, e.g., 200 for Cheddar (Aishima and Nakai, 1987). Vacuum distillation at ca. 70°C may generate artefacts and was replaced by head-space analysis. To increase sensitivity, head-space volatiles may be trapped, e.g., Tenax traps which can be inserted directly into the port of the GC (see Bossett and Gauch, 1993).

Based on a survey of the published literature, Maarse and Vischer (1989) listed 213 volatile compounds that had been identified in 50 studies on Cheddar; these included 33 hydrocarbons, 24 alcohols, 13 aldehydes, 17 ketones, 42 acids, 30 esters, 12 lactones, 18 amines, 7 sulfur compounds, 5 halogens, 6 nitriles and amides, 4 phenols, 1 ether, and 1 pyran. The concentrations of many of these compounds were reported. The principal volatile compounds identified in Cheddar are listed in Table VII.

Thus, a great diversity of potentially sapid and/or aromatic compounds have been identified in one or more cheese varieties—these include small

TABLE VII

SIXTY-ONE VOLATILE COMPOUNDS WHICH HAVE BEEN IDENTIFIED IN CHEDDAR CHEESE[a]

Acetaldehyde	Dimethyl sulfide	Methyl acetate
Acetoin	Dimethyl disulfide	2-Methylbutanol
Acetone	Dimethyl trisulfide	3-Methylbutanol
Acetophenone	δ-Dodecalactone	3-Methyl-2-butanone
β-Angelicalatone	Ethanol	3-Methylbutyric acid
1, 2-Butanediol	Ethyl acetate	2-Nonanone
n-Butanol	2-Ethyl butanol	δ-Octalactone
2-Butanol	Ethyl butyrate	*n*-Octanoic acid
Butanone	Ethyl hexanoate	2-Octanol
n-Butyl acetate	2-Heptanone	2,4-Pentanediol
2-Butyl acetate	*n*-Hexanal	*n*-Pentanoic acid
n-Butyl butyrate	*n*-Hexanoic acid	2-Pentanol
n-Butyric acid	*n*-Hexanol	Pentan-2-one
Carbon dioxide	2-Hexanone	*n*-Propanol
ρ-Cresol	Hexanethiol	Propanal
γ-Decalactone	2-Hexenal	Propenal
δ-Decalactone	Isobutanol	*n*-Propyl butyrate
n-Decanoic acid	Isohexanal	Tetrahydrofuran
Diacetyl	Methanethiol	Thiophen-2-aldehyde
Diethyl ether	Methional	2-Tridecanone
		2-Undecanone

[a] Adapted from Urbach (1993).

peptides (200 or more), amino acids and more than 200 volatile compounds (fatty acids, other acids, carbonyls, amines, sulfur compounds, and hydrocarbons). All these classes of compounds occur in most or all cheeses—what appears to matter most is the absolute and relative concentrations of these compounds, not any major compound, or even class of compounds.

3. *Off-flavors in Cheese*

Specific flavor defects are frequently encountered in cheese. While the desirable flavor of cheese has been difficult to define precisely in chemical terms, the specific cause(s) of many defects has been established more or less definitively. The principal flavor defects in cheese are described below.

a. Bitterness. Bitterness is probably the principal taste defect in cheese. Although amino acids, amines, amides, substituted amides, long-chain ketones, some monoglycerides, *N*-acyl amino acids, and diketopiperazines may contribute to bitterness (Ney, 1979a; Roudot-Algaron *et al.,* 1993), this defect in cheese usually results from the accumulation of hydrophobic peptides (Fox *et al.,* 1995).

Ney (1979b) suggested that the mean hydrophobicity ($Q = \Sigma\Delta f_i/n$, where Δf_i is side chain hydrophobicity and n is the number of residues) of a peptide, rather than any particular amino acid sequence, is of importance in bitterness. Although further work suggested that the nature of the terminal amino acids and certain steric parameters influence the perception of bitterness (see Lemieux and Simard, 1991, 1992), the mean hydrophobicity of a peptide remains the single most important factor determining its bitterness. Peptides of mol wt 0.1 to 10 kDa and $Q < 1300$ cal residue^{-1} are not bitter while those with $Q > 1400$ cal residue^{-1} and mol wt from 0.1 to 6 kDa are bitter (Ney, 1979b); above 6 kDa, even peptides with a Q value greater than 1400 cal residue^{-1} are not bitter. Hydrolysates of proteins with a high mean hydrophobicity are likely to contain bitter peptides although the distribution of hydrophobic residues along the polypeptide and the specificity of the proteinase used to prepare the hydrolysate also influence the development of bitterness (Adler-Nissen, 1986). Since the caseins, especially β-casein, are quite hydrophobic and the hydrophobic residues are clustered, casein hydrolysates have a high propensity to bitterness.

As discussed in Section IVE, cheese contains a great diversity of proteinases and peptidases with different and complementary specificities. Although detailed kinetic studies are lacking, at least some of the peptides in cheese are transient and hence bitterness may be transient as bitter peptides are formed and hydrolyzed, or masked by other sapid compounds. It is very likely that all cheeses contain bitter peptides, which probably

contribute positively to the overall desirable flavor; bitterness becomes a problem only when bitter peptides accumulate to excessive, unbalanced levels. Although bitter peptides can originate from either α_{s1}- or β-casein, the primary action of chymosin and/or lactococcal cell envelope-associated proteinase (CEP) on the very hydrophobic C-terminal region of β-casein may result in the early production of bitter peptides, while the peptides produced initially from α_{s1}-casein are generally not hydrophobic. The production of bitter peptides also depends on the specificity of the lactococcal CEP; e.g., P_{III}-type CEP (*Lc. lactis* ssp. *cremoris* AM_1) produced less bitter casein hydrolysates than P_I-type CEP (*Lc. lactis* ssp. *cremoris* HP) (Visser *et al.*, 1983), perhaps due to the initial release of more peptides from the hydrophobic C-terminal region of β-casein by the latter. The concentration of bitter peptides depends on the rate at which they are degraded by lactococcal peptidases (S. Visser, 1993) and perhaps, in the case of larger bitter peptides, by the CEP. The debittering effect of aminopeptidase N on a tryptic digest of β-casein was demonstrated by Tan *et al.* (1993b).

Certain starters have a propensity to cause bitterness (Lowrie *et al.*, 1972; Lawrence *et al.*, 1972; Stadhouders, 1974). Nonbitter cheese can be made using these strains provided they are used in combination with "nonbitter" strains. Since chymosin may cause the release of bitter peptides, factors that affect its retention in cheese curd (type and quantity used in cheesemaking, drain pH, and cook temperature) will influence the development of bitterness. Certain rennet substitutes produce bitter cheese, owing to excessively high activity and/or incorrect specificity. The pH of cheese also influences the activity of residual coagulant and other enzymes. Cheese with a low salt concentration is very prone to bitterness (Stadhouders *et al.*, 1983; Visser *et al.*, 1983), perhaps because the susceptibility of β-casein to hydrolysis by chymosin, with the production of the bitter peptide, β-CN f193–209, is strongly affected by the NaCl concentration in cheese (Kelly, 1993). Salt also inhibits lactococcal CEP (Exterkate, 1990) and may promote the aggregation of large, nonbitter hydrophobic peptides which would otherwise be degraded to bitter peptides (Visser, 1993). Bitterness can be particularly problematic in low-fat cheeses, presumably as a result of reduced partitioning of hydrophobic peptides into the fat phase.

Bitter peptides that have been isolated from cheese are summarized in Table VIII. As expected, bitter peptides originate principally from hydrophobic regions of the caseins, e.g., sequences 14 to 34, 91 to 101, and 143 to 151 of α_{s1}-casein, and 46 to 90 or 190 to 209 of β-casein. As discussed by McSweeney *et al.* (1996) the majority of these peptides show evidence of some degradation by lactococcal proteinases and/or peptidases.

b. Astringency. Astringency is a taste-related phenomenon perceived as a dry feeling in the mouth and a puckering of the oral tissue (Lindsay,

TABLE VIII

BITTER PEPTIDES ISOLATED FROM CHEESE[a]

Cheese	Origin	Amino acid sequence	Hydrophobicity (Q, cal residue^{-1})
Cheddar	α_{s1}-CN f14–17	E.V.L.N.	1162.5
	α_{s1}-CN f17–21	N.E.N.L.L	1074.0
	α_{s1}-CN f26–32	A.P.F.P.E.V.F	1930.0
	α_{s1}-CN f26–33	A.P.F.P.E.V.F.G	1688.8
	β-CN f46–67	Q.D.K.I.H.P.F.A.Q.T.Q.S.L.V.Y.P. F.P.G.P.I.P	1580.5
	β-CN f46–84	Q.D.K.I.H.P.F.A.Q.T.Q.S.L.V.Y.P. F.P.G.P.I.P.N.S.L.P.Q.N.I.P.P.L. T.Q.T.P.V.V.V	1508.5
	β-CN f193–209	Y.Q.Q.P.V.L.G.P.V.R.G.P.F.P.I.I.V	1762.4
Gouda	β-CN f84–89	V.P.P.F.L.Q	1983.3
	β-CN f193–207	Y.Q.Q.P.V.L.G.P.V.R.G.P.F.P.I	1686.7
	β-CN f193–208	Y.Q.Q.P.V.L.G.P.V.R.G.P.F.P.I.I	1766.9
	β-CN f193–209	Y.Q.Q.P.V.L.G.P.V.R.G.P.F.P.I.I.V	1762.4
Alpkäse	α_{s1}-CN f198–199	L.W	2710.0
Butterkäse	β-CN f61–69	P.F.P.G.P.I.P.N.S	1792.2

[a] Adapted from Lemieux and Simard (1992).

1985). Astringency usually involves the formation of aggregates or precipitates between tannins (polyphenols) and proteins in the siliva (Lindsay, 1985). Harwalkar and Elliott (1971) isolated an astringent fraction from Cheddar cheese by extraction with chloroform–methanol but did not characterize it. Since these authors monitored the chromatographic separation of astringent and bitter compounds by absorbance at 280 nm, it appears that they may be peptides. The aqueous fraction of Comté contains *N*-propionyl methionine, which is slightly bitter, astringent, and pungent, and also fractions which had umami or pungent tastes (Roudot-Algaron *et al.*, 1993).

c. Fruitiness. The principal compounds responsible for fruitiness in Cheddar are ethyl butyrate and ethyl hexanoate, formed by esterification of free fatty acids (FFAs) with ethanol; production of ethanol appears to be the limiting factor as FFAs are present in cheese at relatively high concentrations. Ethyl esters are present in low concentrations in nonfruity cheeses and thus the fruity defect occurs as a result of excessive production of ethanol or its precursors.

d. "Unclean" Off-Flavors. The origin of "unclean" and related flavors in Cheddar was investigated by Dunn and Lindsay (1985) who quantified

a number of Strecker-type compounds, including phenylacetaldehyde, phenethanol, 3-methyl butanol, 2-methyl propanol, phenol, and *p*-cresol. Phenylacetaldehyde concentrations were elevated in cheeses with an "unclean-rosy" off-flavor and the addition of this compound to clean-flavored mild Cheddar reproduced this defect. At higher concentrations (> 500 μg/kg), phenylacetaldehyde imparted astringent, bitter, and stinging flavors to cheese. Concentrations of phenethanol were similar in most of the cheeses studied (ca. 100 μg/kg). *p*-Cresol imparted a "utensil"-type flavor when present at high concentrations. Dunn and Lindsay (1985) also discussed the potential of short-chain fatty acids to potentiate the flavor impact of *p*-cresol. Branched chain Strecker-type aldehydes (3-methyl butanal, 2-methyl butanal, and 2-methyl propanal) did not cause flavor defects when added at concentrations below 200 μg/kg to clean-flavored cheese. Phenol contributed to the unclean flavor of cheese and indeed enhanced the sharpness of Cheddar flavor.

C. INTER- AND INTRAVARIETAL COMPARISON OF CHEESE RIPENING

As discussed in Section VB, most ripened cheeses contain essentially the same sapid and aromatic compounds but at different concentrations and proportions. Therefore, it appears reasonable to presume that inter- and intravarietal comparison, especially of closely related varieties, might help to identify compounds most likely to contribute to characteristic cheese flavors. However, although both the water-soluble and volatile fractions of several cheese varieties have been analyzed, there are relatively few intervarietal comparisons, especially of the water-soluble fraction. In this section, the results of some such studies will be discussed.

1. Gel Electrophoresis

Gel electrophoresis, especially alkaline (pH 9.0) urea–PAGE, is the best method for characterizing the large water-insoluble (WISF) peptides and is also useful for characterizing the larger water-soluble (WSF) peptides. Ledford *et al.* (1966) and Marcos *et al.* (1979) showed differences between the electrophoretograms of 7 and 34 cheese varieties, respectively, by urea–PAGE; both studies involved only one sample, of unknown age, history, or quality, for each variety and hence may not have been typical.

We have made several comparative studies on Cheddar cheese in relation to starter type, manufacturer, ripening temperature, age, and quality. In one such study, urea–PAGE of the WSF and WISF from 22 6-month-old cheeses made using single strain starters showed very little difference

between the cheeses, which differed substantially in quality; perhaps, this is not surprising since starter proteinases contribute little to primary proteolysis in Cheddar, as detected by PAGE. In another study (McSweeney *et al.*, 1993a), PAGE failed to show substantial differences between cheeses made from pasteurized, ultrafiltered, or raw milk, although the flavor of the raw milk cheese was very much more intense than that of the others. O'Shea (1993) characterized proteolysis in 60 commercial Cheddar cheeses ranging in age from 3 to 33 months and varying in quality. Urea–PAGE of the WISF generally reflected the age of the cheese and was a useful index of its textural quality (if the age was known), but not of its flavor. Since an elevated ripening temperature accelerates proteolysis (Folkertsma *et al.*, 1996), the history of the cheese must be known if PAGE is to be a useful index of the age of cheese. Electrophoretograms are fairly characteristic of the variety, as shown in Figs. 11 and 12 for a selection of commercial samples of Cheddar, Swiss, and Dutch varieties. Studies of more samples and a wider range of varieties are warranted.

The peptides detected by PAGE are probably too large to have a direct impact on cheese flavor but probably do reflect the overall ripening process and hence might be useful as an indirect index of quality, including maturity.

2. *High-Performance Liquid Chromatography*

HPLC is more effective than PAGE, which does not detect peptides less than ~3000 Da, for analysis of the WSF. HPLC profiles of the UF permeate and retentate of a mild, mature, and extramature Cheddar are shown in Fig. 17; clearly, the complexity of the chromatograms, especially of the retentate (medium-sized peptides), increased as the cheese matured. Unfortunately, it is not possible at present to relate cheese flavor or texture to HPLC chromatograms. HPLC of water- or TCA-soluble peptides shows clear varietal characteristics (Fig. 13). Engels and Visser (1994) concluded that very small peptides and free amino acids contributed significantly to the flavor of Cheddar, Edam, Gouda, Gruyère, Maasdam, Parmesan, and Proosdij. Glu, Leu, and Phe were the principal free amino acids, in agreement with other studies, e.g., Wilkinson (1992). The concentration of free amino acids (measured by reaction with Cd-ninhydrin) in Cheddar is highly correlated with age (Fig. 18) and hence with the intensity of cheese flavor. The ratio of free amino acids to water-soluble nitrogen (WSN) appears to be characteristic of the variety (Fig. 19); Cheddar appears to contain a very low level of free amino acids relative to small peptides. Parmesan contains a particularly high concentration of amino acids (Engels and Visser, 1994) which have a major effect of its flavor (Resmini *et al.*, 1988).

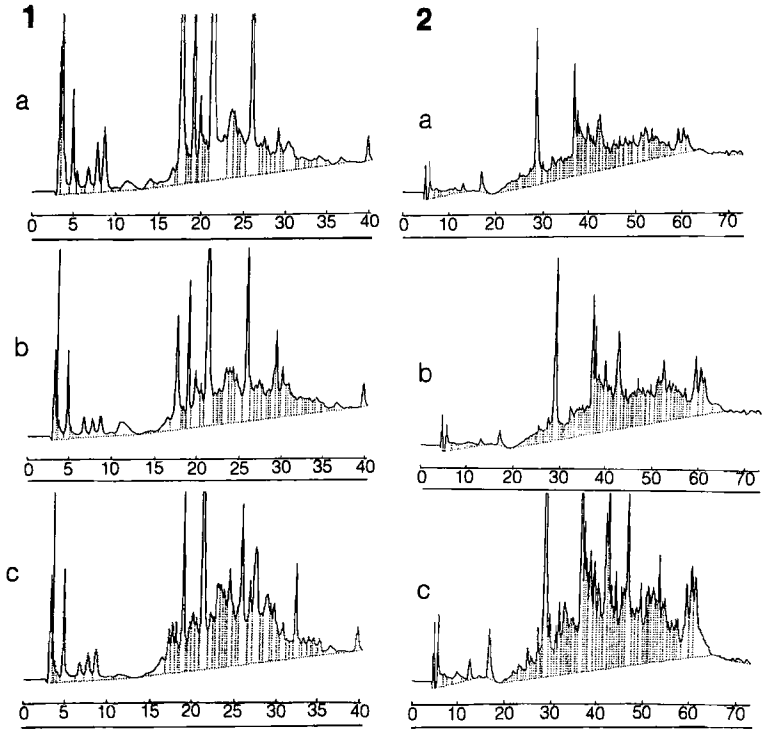

FIG. 17. Reverse-phase HPLC (C_8 column, acetonitrile/water gradient, TFA as ion pair reagent, detection at 214 nm) profiles of (1) permeates and (2) renetates from (a) mild, (b) mature, and (c) extra mature Cheddar cheese (from O'Shea, 1993).

The WSF also contains short chain fatty acids ($< C_{9:0}$) which impart a "cheesy" aroma; there are substantial intervarietal differences with respect to short chain fatty acids (Engels and Visser, 1994). This is the only study we are aware of on the free fatty acids in the WSF of cheese, on which further work appears to be warranted.

3. *Cheese Volatiles*

A number of intra- and intervarietal comparisons of cheese volatiles have been published. An early example is that of Manning and Moore (1979) who analyzed head-space volatiles of nine fairly closely related varieties; considerable intervarietal differences were evident but the four samples of "Cheddar" also differed markedly. The intensity of cheese flavor was reported to be related to the concentration of sulfur compounds (peaks 1 and 2); 2-pentanone was also considered to be important for Cheddar cheese flavor.

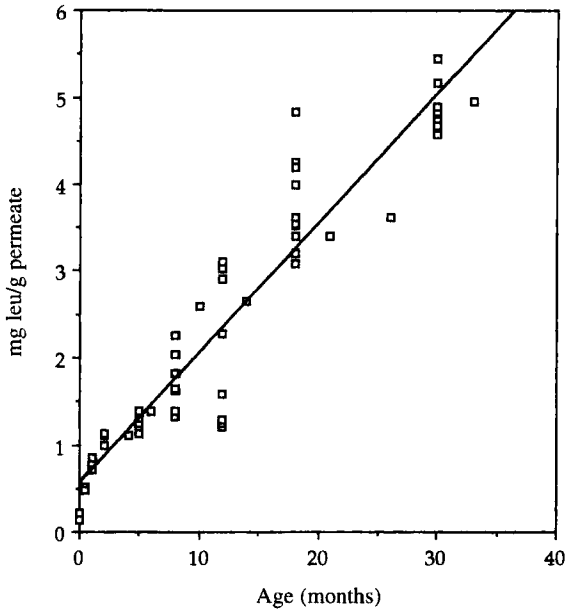

FIG. 18. Concentration of total free amino acids (Cd-ninhydrin assay) in Cheddar cheese as a function of age (from O'Shea, 1993).

A more comprehensive study on Cheddar, Gouda, Edam, Swiss, and Parmesan (total of 82 samples) was reported by Aishima and Nakai (1987). The volatiles were extracted by CH_2Cl_2 and analyzed by GC. More than 200 peaks were resolved in every chromatogram, 118 of which were selected as variables for discriminative analysis. Expression of the area of each of the 118 peaks as a percentage of total chromatogram area clearly permitted classification of the five varieties. The compounds likely to be responsible for the characteristic flavor of each variety were not discussed.

Bosset and Gauch (1993) concentrated the head-space volatiles from six cheese varieties by a "purge and trap" method for analysis by GC-MS; a total of 81 compounds were isolated and identified (Fig. 20), 20 of which were found in all six varieties and a further nine in five of the six varieties. The authors concluded that "practically all types of cheese analyzed contain more or less the same constituents, but at varying concentrations" and that the flavor of these cheeses seems to depend not on any particular key compound, but rather on a "critical balance" or a "weighted concentration ratio of all components present."

Thus, the component balance theory still applies and we appear to have advanced relatively little during the last 40 years. While analytical techniques have improved greatly during that period and data are available on

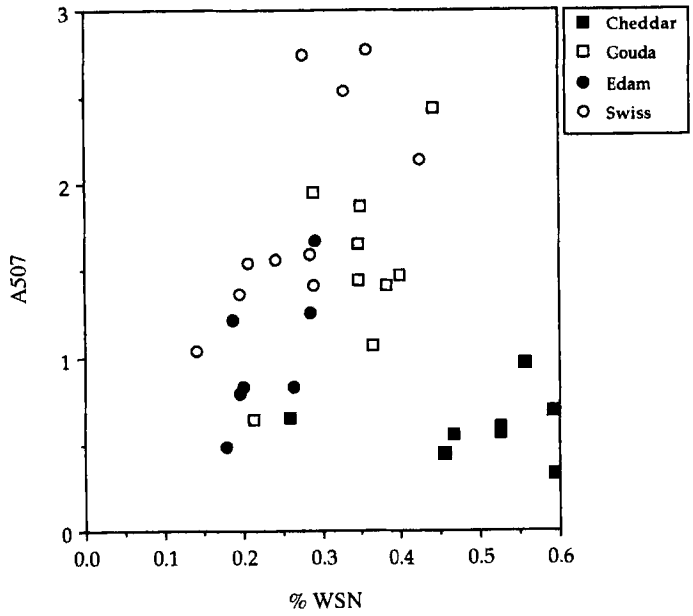

FIG. 19. Relation between the concentration of total free amino acids by the Cd-ninhydrin assay, A_{507}, and water-soluble nitrogen for some cheese varieties (E. Olthoff, R. Schmidt, and P. F. Fox, unpublished).

the concentrations of many flavor compounds present in cheese, we still do not know what the critical compounds are, if any. Further intervarietal comparisons may be useful if quantitative data are provided, although usually they are not. Perhaps it would be fruitful to reinvestigate cheeses with controlled microflora. There have been no studies on such models since the 1970s and these were concerned mainly or totally with proteolysis. It would seem to be particularly useful to combine studies on controlled microflora cheese with intervarietal comparisons but perhaps such an undertaking is beyond the capabilities of a single laboratory.

D. FACTORS THAT AFFECT CHEESE QUALITY

As discussed in Section IV, the ripening of cheese, and hence its quality, is due to the activity of microorganisms and enzymes from four or five sources. Therefore, it might reasonably be expected that it should be possible to produce premium quality cheese consistently by controlling these agents; however, in spite of considerable research and quality control efforts, it is not yet possible to do so.

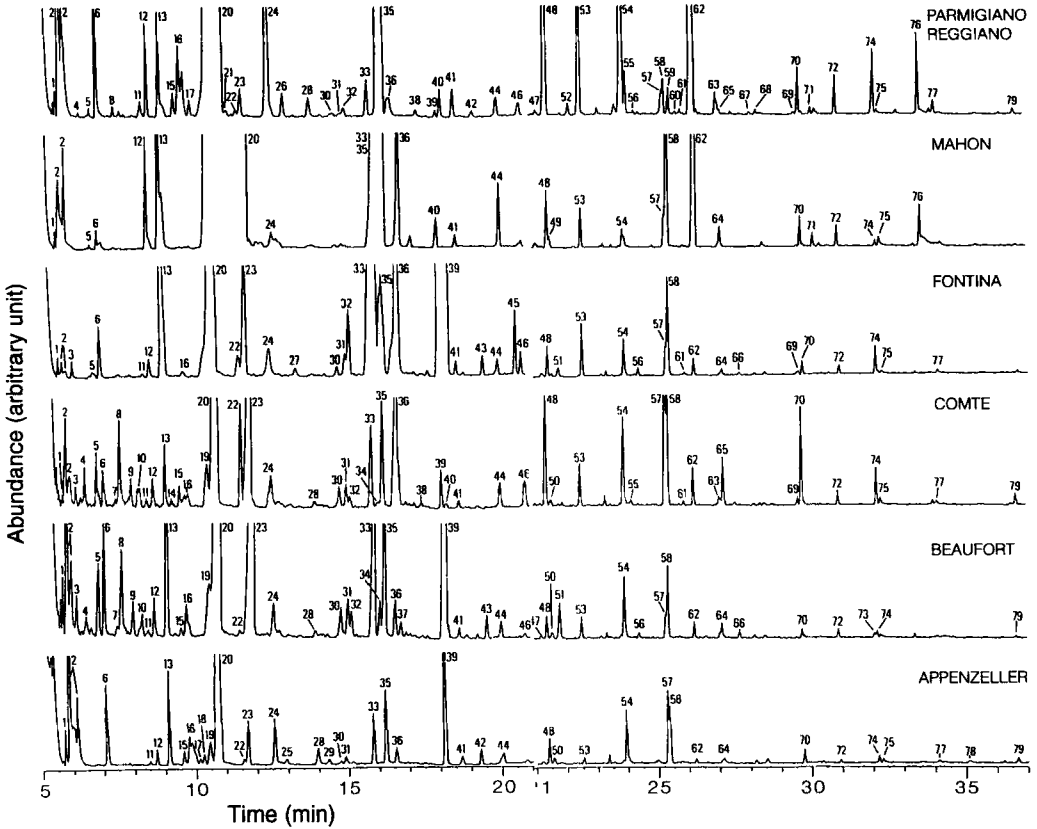

FIG. 20. GC-MS chromatograms of the head-space volatiles for six cheese varieties (from Bosset and Gauch, 1993, with permission).

Certain factors/agents can be manipulated easily and precisely, while others are more difficult to control. In this section, possible factors responsible for the variability of cheese quality and how they might be controlled will be considered. Interactions (Fig. 21) between these factors were reviewed by Lawrence and Gilles (1980).

1. Milk Supply

It is well recognized that the quality of the milk supply has a major impact on the quality of the resultant cheese. Three aspects of quality must be considered: microbiological, enzymatic, and chemical.

a. Microbiological. In developed dairying countries, the quality of the milk supply has improved markedly during the past 30 years—total bacterial count (TBC) is now usually <20,000 CFU/ml exfarm. The TBC probably increases during transport and storage at the factory, but growth can be minimized by thermization (65°C × 15 sec) of the milk supply, as is standard practice in some countries.

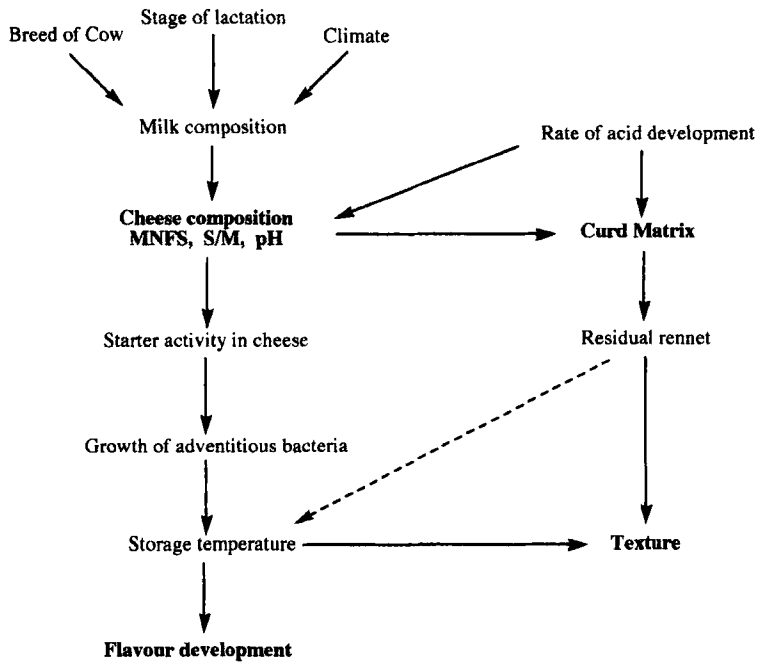

FIG. 21. Principal factors that affect the quality of Cheddar cheese.

Although many cheeses are made from raw milk, in quantitative terms, most cheese is made from milk pasteurized at or close to 72°C × 15 sec. If produced from good quality raw milk and if subsequently handled under hygienic conditions, pasteurized milk should have very low TBCs and therefore represents a very uniform product from a microbiological viewpoint.

b. Indigenous Enzymes. Milk contains as many as 60 indigenous enzymes, the significance of which to cheese quality has not yet been researched adequately. Several of these enzymes have the potential to affect cheese quality, especially lipase, proteinase, acid phosphatase, and perhaps xanthine oxidase, sulfydryl oxidase, lactoperoxidase, and γ-glutamyl transpeptidase. Most of these survive HTST pasteurization to a greater or lesser extent and at least some, e.g., proteinase (plasmin), are active during cheese ripening.

Although precise information is lacking, it is our opinion that indigenous milk enzymes are not major causes of variability in cheese quality, although some contribute to cheese ripening and may contribute to the superior quality of raw milk cheese, a possibility that warrants investigation.

c. Chemical Composition. The chemical composition of milk, especially the concentrations of casein, fat, calcium, and pH, has a major influence on several aspects of cheese manufacture, especially rennet coagulation, gel strength, curd syneresis, and hence cheese composition. When seasonal milk production is practiced, as in New Zealand, Ireland, and parts of Australia, milk composition varies widely but there is some variability even with random calving patterns. It is possible to reduce, but not eliminate, the variability in the principal milk constituents by standardizing with respect to fat and casein content, not just the ratio (protein content can be standardized by adding UF retentate), the pH (using gluconic acid δ-lactone), and the calcium content (by adding $CaCl_2$).

2. *Coagulant (Rennet)*

It is generally accepted that calf chymosin produces the best quality cheese. An adequate supply of chymosin from genetically engineered microorganisms is now available and therefore rennet quality should not be a factor in cheese quality.

As discussed in Section IVE4, the proportion of added rennet retained in cheese curd varies with rennet type, cook temperature, and drain pH; these variables should be standardized if cheese of consistent quality is to be produced. Johnston *et al.* (1994) found that increased retention of the coagulant in the curd resulted in greater initial hydrolysis of α_{s1}-casein

although this did not appear to be reflected in sensory assessment of cheese texture. It has been suggested (Exterkate and Alting, 1995) that the activity of chymosin in cheese curd is the limiting factor in cheese ripening; however, excessive rennet activity leads to bitterness. There have been relatively few studies on the significance of chymosin activity to cheese quality, an aspect which appears to warrant further research.

3. *Starter*

Since the starter plays a key role in cheese manufacture and ripening, it might reasonably be expected that differences between the enzyme profile of starter strains affect cheese quality. Modern single-strain starters produce acid very reproducibly and if properly managed, show good phage resistance. *Lactococcus* strains have been selected mainly on the basis of acid-producing ability, phage resistance, and compatibility. Empirical studies have indicated strains that produce unsatisfactory, especially bitter, cheese but systematic studies on strains with positive cheesemaking attributes are lacking. This probably reflects the lack of information on precisely what attributes of a starter are desirable from a flavor-generating viewpoint. Studies on genetically engineered strains that superproduce proteinase and/or the principal aminopeptidase (Pep N) showed that cheese quality was not improved although proteolysis was accelerated (McGarry *et al.*, 1994). Since all lactococcal enzymes, except the cell wall-associated proteinase, are intracellular or membrane associated, the cells must lyse before these enzymes can participate in ripening; therefore, the lytic rate of *Lactococcus* strains is being studied, with the objective of selecting strains with improved cheesemaking properties (see Section VIIC).

Sulfur compounds have long been considered as contributors to Cheddar cheese flavor. Some strains of *Lc. lactis* ssp. *cremoris,* but not *Lc. lactis* ssp. *lactis,* can absorb glutathione (γ-Glu–Cys–Gly; GSH) from the growth medium (Fernandes and Steele, 1993). Release of GSH into the cheese on cell lysis may affect the redox potential (E_h) of cheese, and hence the concentration of thiol compounds. Cheesemaking studies using starter strains that accumulate glutathione or those that do not are warranted.

It is very likely that the desirable cheesemaking properties of starters are due to a balance between certain, perhaps secondary, enzymatic activities, which have not yet been identified.

4. *Nonstarter Lactic Acid Bacteria*

The significance of lactobacilli for Cheddar cheese quality is controversial (see Sections IVD3 and IVE7). Many researchers consider their contribu-

tion to be negative (in the Netherlands, a maximum of 2×10^6 NSLAB/g is specified for Gouda). Although there are several studies on controlled microflora cheeses, we are not aware of studies in which cheese free of NSLAB was compared with "control" cheeses containing "wild" NSLAB. McSweeney *et al.* (1994a) reported comparative studies on cheese made under aseptic conditions using *Lactococcus* starter alone or with selected *Lactobacillus* adjuncts; preliminary results suggested that inoculation of cheese milk with selected strains of *Lactobacillus* improves cheese flavor and possibly accelerates ripening. These findings confirm those of other researchers; *Lactobacillus* adjuncts are used commercially in Canada and are being investigated by starter supply companies.

Since the numbers and strains of lactobacilli in cheese are uncontrolled, it is likely that they contribute to variability in cheese quality. Since it is impossible to eliminate NSLAB completely, even under experimental conditions, it appears worthwhile to determine what factors affect their growth. The number of NSLAB in Cheddar is strongly influenced by the rate at which the curd in cooled and subsequently ripened (Folkertsma *et al.*, 1996). The growth of NSLAB does not appear to be influenced by the concentration of NaCl in the cheese (Turner and Thomas, 1980). It is likely that the moisture content of cheese affects the growth of NSLAB but we are not aware of studies in which this effect has been studied.

NSLAB grow mainly after the lactose has been metabolized by residual starter activity. Although the growth substrates in cheese for *Lactobacillus* are not known, it is likely that they are limited (NSLAB normally plateau at $\sim 10^7$ CFU/g) and hence it might be possible to outcompete wild NSLAB by adding selected strains of *Lactobacillus,* thereby offering better control.

5. *Cheese Composition*

The quality of cheese is influenced by its composition, especially moisture content, NaCl concentration (preferably expressed as S/M), pH, moisture in nonfat substances (MNFS; essentially ratio of protein : moisture) and % fat in dry matter (FDM). At least five studies (O'Connor, 1971; Gilles and Lawrence, 1973; Fox, 1975; Pearce and Gilles, 1979; Lelievre and Gilles, 1982) have attempted to relate the quality of Cheddar cheese to its composition. While these authors agree that moisture content, %S/M and pH are the key determinants of cheese quality, they disagree as to the relative importance of these parameters.

O'Connor (1971) found that flavor and aroma, texture, and total score were not correlated with moisture content but were significantly correlated with %NaCl and particularly with pH. Salt content and pH were themselves strongly correlated, as were salt and moisture.

Based on the results of a study on experimental and commercial cheeses in New Zealand, Gilles and Lawrence (1973) proposed a grading scheme which has since been applied commercially in New Zealand for young (14 day) Cheddar cheese. The standards prescribed for Premium grade were: pH, 4.95–5.10; %S/M, 4.0–6.02%; MNFS, 52–56%; FDM, 52–55%. The corresponding values for First grade cheeses were: 4.85–5.20%, 2.5–6%, 50–57%, and 50–56%; young cheeses with a composition outside these ranges were considered unlikely to yield good quality mature cheese. Quite wide ranges of FDM are permitted; Lawrence and Gilles (1980) suggested that since relatively little lipolysis occurs in Cheddar cheese, fat content plays a minor role in determining cheese quality but if FDM fell below about 48%, the cheese was noticeably more firm and less attractive in flavor. Pearce and Gilles (1979) found that the grade of young (14-day-old) cheeses produced at the New Zealand Dairy Research Institute was most highly correlated with moisture content; the optimum compositional ranges were: MNFS, 52–54%; %S/M, 4.2–5.2%; pH, 4.95–5.15%.

Fox (1975) reported a poor correlation between grade and moisture, salt, and pH for Irish Cheddar cheeses but a high percentage of cheeses with compositional extremes were downgraded, especially those with low salt (<1.4%), high moisture (>38%), or high pH (>pH 5.4). Salt concentration seemed to exercise the strongest influence on cheese quality and the lowest percentage of downgraded cheeses can be expected in the salt range 1.6–1.8% (S/M, 4.0–4.9%); apart from the upper extremes, pH and moisture appeared to exercise little influence on quality. High salt levels tended to cause curdy textures, probably due to insufficient proteolysis; pasty body, often accompanied by off-flavors, was associated with low salt and high moisture levels. In the same study, the composition of extramature cheeses was found to vary less and the mean moisture content was 1% lower than that of regular cheeses.

A very extensive study of the relationship between the composition and quality of nearly 10,000 cheeses produced in five commercial New Zealand factories was reported by Lelievre and Gilles (1982). As in previous studies, considerable compositional variation was evident but was less for some factories than others. While the precise relationship between quality and composition varied between plants, certain generalizations emerged: (1) within the compositional range suggested by Gilles and Lawrence (1973) for premium quality cheese, composition does not have a decisive influence on grade, which decreases outside this range; (2) composition alone does not provide a basis for grading as currently acceptable in New Zealand; (3) MNFS was again found to be the principal factor affecting quality; (4) within the recommended compositional bands, grades declined marginally as MNFS increased from 51 to 55%, grades increased slightly as %S/M

decreased from 6 to 4%, pH had no consistent effect within the range 4.9–5.2, and FDM had no influence in the range 50–57%. The authors stress that since specific interplant relationships exist between grade and composition, each plant should determine the optimum compositional parameters pertinent to that plant.

One could summarize the results of the foregoing investigations as indicating that high or low values for moisture, salt, and pH lead to flavor and textural defects. The desired ranges suggested by Gilles and Lawrence (1973) appear to be reasonable, at least for New Zealand conditions, but within the prescribed zones, composition is not a good predictor of cheese quality. Presumably, several other factors, e.g., microflora, activity of indigenous milk enzymes, relatively small variations in milk composition, and probably other unknown factors, influence cheese quality but become dominant only under conditions where the principal determinants, moisture, salt, and pH, are within appropriate limits.

Although the role of calcium concentration in cheese quality has received occasional mention, its significance was largely overlooked until the work of Lawrence and Gilles (1980) who pointed out that the calcium level in cheese curd determines the cheese matrix and, together with pH, indicates whether proper procedures were used to manufacture a specific cheese variety. As the pH decreases during cheese manufacture, colloidal calcium phosphate dissolves and is removed in the whey. The whey removed at running constitutes 90–95% of the total whey lost during cheesemaking and this whey contains, under normal conditions, ~85% of the calcium and ~90% of the phosphorus lost. Thus, the calcium content of cheese reflects the pH of the curd at whey drainage. Lawrence and Gilles (1982) showed strong correlations between the calcium content of cheese and the pH at 1 day, pH at 14 days, and the amount of starter used. Since the pH of cheese increases during ripening, the pH of mature cheese may be a poor index of the pH of the young cheese. Therefore, calcium concentration is probably a better record of the history of a cheese with respect to the rate of acidification than the final pH. Reduction in calcium phosphate concentration by excessively rapid acid development also reduces the buffering capacity of cheese and hence the pH of the cheese will fall to a lower value for any particular level of acid development. Unfortunately, no recent work on the levels and significance of calcium in Cheddar cheese appears to be available.

6. *Ripening Temperature*

The final factor known to influence the rate of ripening and cheese quality is ripening temperature. Ripening at elevated temperatures is normally

considered with the objective of accelerating ripening but it also affects cheese quality. The literature on the accelerated ripening of cheese is discussed briefly in Section VII.

VI. CHEESE TEXTURE

The rheological properties of cheese, commonly referred to as body and texture or consistency, are important aspects of cheese quality as perceived by the consumer. In many cases, texture is as important as a parameter of quality as taste and aroma. In addition to its significance to the eating quality of cheese, the texture of cheese is also very important with respect to the domestic and catering use of cheese as an ingredient, e.g., ease of cutting, grating, and melting and handling properties, e.g., whether the cheese holds its shape, whether grated cheese particles remain discrete or stick together (adhesiveness), and eye formation.

The texture is characteristic for the variety and ranges from crumbly, e.g., Parmesan and Cheshire, to elastic, e.g., Emmental or Gouda, from close, e.g., Cheddar, to open, e.g., Blue, from very firm, e.g., Gruyere, to fluid, e.g., Camembert.

Texture is determined by composition, manufacturing conditions, the duration and extent of ripening, and assay temperature. For example, Young's modulus, E, decreases with increasing temperature, increasing moisture content, increasing fat content (with a concomitant decrease in protein content), and increasing pH but increases with increasing salt content and age (partly due to loss of moisture and partly to proteolysis which reduces the amount of free moisture). The concentration of calcium in cheese (which is influenced mainly by the pH of the curd/whey at draining) and its association with casein (which is influenced by the final pH of the cheese) have major effects on the elasticity of cheese, e.g., cheeses with a high calcium/high drainage pH, e.g., Emmental, are elastic while those with a low calcium content (low draining pH), e.g., Cheshire, are crumbly. Proteolysis softens the texture of cheese, putatively owing to breakdown of the α_{s1}-casein matrix which is commonly regarded as forming the continuous solid network in young cheese (although this is debatable). However, extensive proteolysis in low-moisture cheeses causes an increase in crumbliness due to increased water binding by the liberated carboxyl and amino groups.

Cheese is an inhomogeneous system due to fissures/cracks of various types, eyes or mechanical openings, or differences in composition, e.g., between the rind and center of a cheese. Therefore, precise studies on cheese rheology are difficult. However, as the amount and depth of knowledge on the biochemistry, chemistry, and physical chemistry of cheese

has increased, researchers are showing increased interest in the study of cheese rheology.

The subject is largely outside the scope of the present review and the interested reader is referred to a number of recent reviews/monographs on various aspects of cheese rheology and texture (Chen *et al.*, 1979; Creamer and Olson, 1982; Lawrence *et al.*, 1983; Masi and Addeo, 1986; Prentice, 1987; Walstra *et al.*, 1987; Luyten, 1988; Tunick *et al.*, 1990; IDF, 1991b; Pagliarini *et al.*, 1991; Luyten *et al.*, 1991a,b; Jack and Peterson, 1992; Konstance and Holsinger, 1992; Prentice *et al.*, 1993).

Traditionally, cheese texture has been assessed subjectively by trained or untrained graders. Such procedures are expensive and not very reproducible and there is a major problem in describing and defining textural characteristics as determined by a grader. A useful attempt to standardize the evaluation and description of cheese texture is presented by Lavanchy *et al.* (1994). To overcome at least some of these limitations, objective instrumental methods for textural analysis of foods in general, and cheese in particular, have been developed. However, it is very difficult to evaluate and assess the sensory qualities of food by instrumental methods, largely because it is very difficult to replicate the process of food mastication. While various rheological properties of foods can be quantified readily by instrumental methods, the problem lies in relating these rheological parameters to sensory characteristics. These problems are discussed in some of the references cited above.

VII. ACCELERATED CHEESE RIPENING

Cheese ripening is a slow, and hence an expensive, process, e.g., Parmesan and extramature Cheddar are ripened for at least 18 months. Ripening is still not controllable precisely, i.e., the quality and intensity of flavor cannot be predicted precisely. Therefore, there is an economic incentive for the development of methods for the acceleration of cheese ripening, provided that the flavor and texture can be maintained and characteristic of the variety.

Of the three primary events in cheese ripening, i.e., glycolysis, lipolysis, and proteolysis, proteolysis is usually the rate-limiting one. Glycolysis is normally very rapid and is complete in most varieties within 24 hr; therefore, acceleration of glycolysis is not of interest. The modification and catabolism of lactate is either of little or no consequence (e.g., Cheddar or Dutch varieties) or is quite rapid—2–3 weeks (e.g., Swiss types, Camembert)—and consequently its acceleration is not important. Lipolysis is limited in most cheese varieties, exceptions being some Italian varieties, e.g., Romano and

Provolone, and blue varieties. In the Italian and blue varieties, lipolysis does not appear to be rate-limiting and there appears to be little interest in accelerating it. As discussed in Section IVD, it is claimed that the intensity of the flavor of several varieties, including Cheddar, can be increased by adding exogenous lipases, but as far as we are aware, such practices are not used commercially.

Therefore, studies on the acceleration of cheese ripening have focussed on proteolysis, especially in hard, low-moisture varieties, in particular Cheddar. Low-fat cheeses have attracted much attention recently; such cheeses have poor texture and flavor and the techniques being considered to accelerate the ripening of normal cheeses are being applied to low-fat cheeses also. The third area of interest is the production of cheese-like products, e.g., enzyme modified cheeses, for use in the preparation of food products, e.g., processed cheeses, cheese sauces, cheese dips, etc.

Literature on the acceleration of cheese ripening and related topics has been reviewed extensively (e.g., Law, 1984, 1987; Fox, 1988–1989; El-Soda and Pandian, 1991; El-Soda, 1993; Wilkinson, 1993). Therefore, it is not intended to exhaustively review the literature again but rather to provide a summary and suggest possible developments.

Ripening can be accelerated by:

1. Increasing the ripening temperature.
2. Using exogenous enzymes.
3. Using modified starters.
4. Using cheese slurries.

Each of these methods has advantages and limitations (see Fox, 1988–1989).

A. ELEVATED TEMPERATURES

Traditionally, different cheese varieties are ripened at a characteristic temperature which is frequently chosen to suit the secondary microflora, e.g., *Propionibacterium* or moulds. Temperatures of 12–15°C are common, an exception being Swiss-type cheeses which are exposed at ~20°C for a period of 3–4 weeks to induce the growth of *Propionibacterium* responsible for eye formation and typical flavor. Traditionally, Cheddar was ripened at ~15°C but during the last 40 years or so it has become widespread commercial practice to ripen at 6–8°C. Ripening at lower temperatures reduces the risk of off-flavor development, but obviously retards ripening and probably the intensity of the final flavor attained. The use of low ripening temperatures is often augmented by rapidly cooling the curd, perhaps to as low as 10°C immediately after manufacture. The principal objective of rapid cooling is to retard the growth of NSLAB, which are

considered to have a negative effect on cheese quality. However, this conclusion depends on ones definition of Cheddar cheese flavor; almost certainly, high numbers of NSLAB lead to more strongly flavored cheese.

The microbiological quality of cheesemilk today is vastly superior to that available even 20 years ago, total counts <20,000 CFU/ml are now common. Thermization of milk on receipt at the factory is common in some countries and it is normal commercial practice in most countries to pasteurize cheesemilk. When good hygienic practices are followed at farm and factory, milk is almost sterile as it enters the cheese vats; mechanized curd handling systems and enclosed cheddaring and salting systems (in the case of Cheddar) minimize contamination and consequently modern Cheddar, and probably Dutch varieties, are essentially free of NSLAB at the end of manufacture—in our experience, NSLAB counts of <100 CFU/g are normal for 1-day-old Cheddar (although these grow to $>10^7$ during a 6-month ripening period). Starters have been greatly refined; it is now common practice to use a starter containing only one or two carefully selected strains of *Lc. cremoris*. While this practice leads to better control over cheesemaking, it further limits the diversity of ripening agents in cheese.

The overall effect of these various practices, i.e., improved milk quality, pasteurization, defined starters, enclosed cheesemaking equipment, rapid cooling, and low ripening temperature, is the production of very mild cheese, free of off-flavors. While the latter is, obviously, a desirable development, not all consumers are happy with the very mild flavor of modern Cheddar.

Several studies, especially in Australia, have shown that provided cheese of good composition and with a low count of NSLAB is used, the ripening of Cheddar cheese can be accelerated and its flavor intensified by using higher than normal ripening temperatures. Optimum results have been reported at 13–15°C at which the ripening time required for the production of mature cheese can be reduced by 50% (Folkertsma *et al.*, 1996). Such practices are in fact reverting to traditional methods.

B. EXOGENOUS ENZYMES

On the assumption that proteolysis is the rate-limiting event in cheese ripening, there has been interest for several years in adding exogenous proteinases to cheese curd. The first problem encountered is the method of enzyme addition. Direct addition of the proteinase to the cheesemilk ensures its uniform distribution throughout the curd but since most proteinases are water-soluble, most of the added enzyme is lost in the whey, which is economically undesirable, and significant proteolysis may occur prior to coagulation with consequent loss of peptides in the whey and a reduction in cheese yield.

The enzyme may be added to the curd but this method can be practiced only with Cheddar-type cheeses, the curd for which are milled prior to moulding; to facilitate uniform dispersion, the enzyme is usually diluted with salt but nonuniform distribution of enzyme may still occur, resulting in uneven ripening.

Microencapsulation appears very attractive as a technique for preparing enzymes for addition to cheese and has attracted considerable attention. The microcapsules are added to the cheesemilk and are occluded in the curd, thus ensuring uniform distribution in the curd and minimizing losses of enzyme in the whey. The microcapsules disintegrate during cooking or ripening, releasing the entrapped enzyme into the cheese matrix. Liposomes are the preferred form of microcapsule and appear to give satisfactory results with respect to the efficiency of encapsulation and retention in the coagulum. However, microencapsulated enzymes have not been commercialized, probably owing to cost. The use of microencapsulation in cheese technology was reviewed by Skeie (1994).

Early studies on the use of exogenous enzymes concentrated on individual proteinases, of which Neutrase (a neutral proteinase from *B. subtilis*) gave best results. However, gross proteolysis is not rate-limiting and current trends are to combine proteinases and peptidases. Such preparations appear to accelerate ripening (Wilkinson *et al.*, 1992) but perhaps equally good results can be obtained using elevated ripening temperatures, with less inconvenience and risk and at no cost. Exogenous enzymes are not yet used commercially to accelerate the ripening of natural cheeses.

C. MODIFIED STARTERS

Since current evidence indicates that the starter cells and their enzymes are responsible for the final stages of proteolysis, i.e., production of small peptides and free amino acids, probably the modification of amino acids and probably other important changes, it would appear that increasing starter cell numbers should accelerate ripening. Much of the recent work on acceleration of cheese ripening has been based on the above assumption and several approaches have been adopted.

Increasing starter cell numbers is not satisfactory owing to the consequent increase in rate of acid production which has undesirable consequences (see Section IIIA5). High starter numbers may also cause bitterness. To overcome these problems, either lactase-negative (Lac^-) or attenuated starters have been investigated and reported to accelerate ripening. The inclusion of a proportion of Lac^- cells in cheese starters has also been recommended to reduce the incidence of bitterness and apparently is widely used for this reason in the Netherlands. Attenuated starters are prepared by heat-shocking or freeze-shocking *Lactococcus* or *Lactobacillus* cells such

that their acid-producing ability is destroyed but much of their proteolytic and especially their peptidolytic activity is retained. Attenuated starters can be regarded as microencapsulated enzymes. Several reports indicate that their use accelerates ripening but they are probably too expensive in most circumstances and to our knowledge they are not used commercially.

The alternative approach is to develop starters that superproduce key enzymes. An engineered starter producing three times as much cell wall-associated proteinase as the parent did not accelerate ripening (Law *et al.*, 1993), which supports the results of earlier studies using Prt^- starters which indicated that the cell wall-associated proteinase is not rate-limiting in cheese ripening. McGarry *et al.* (1994) used an engineered strain that produced high levels of aminopeptidase (Pep N) in Cheddar cheese but found no beneficial effect. Thus, it is not clear at present which starter enzymes might be limiting.

A further approach is to select or develop starter strains that lyse and release their intracellular enzymes quickly. This is based on the premise that if intracellular lactococcal enzymes are important, then the more rapidly they are released from the cells the better. *Lactococcus* strains die and lyse at considerably different rates and fast-lysing strains have been reported to give faster rates of ripening in Cheddar (Wilkinson *et al.*, 1994) and Saint Paulin (Chapot-Chartier *et al.*, 1994). Further work in this area appears warranted.

D. CHEESE SLURRIES

Flavor has been reported to develop very rapidly (1 week) in slurries containing ~40% solids. Such systems have been used to screen exogenous enzymes. Fast-ripening slurries could be useful in the preparation of cheese sauces, cheese flavoring, processed cheeses, etc. Enzyme-modified cheeses, which can be regarded as being based on the slurry principle, are used commercially as ingredients in processed cheese and cheese products.

Thus, there is undoubtedly a commercial economic incentive to develop techniques to accelerate the ripening of cheese. However, in spite of a considerable amount of published research, and presumably unpublished work, the number of viable options appears to be rather limited—at present the best method appears to be a higher ripening temperature.

VIII. PROCESSED CHEESE PRODUCTS

A. INTRODUCTION

Pasteurized processed cheese products are produced by comminuting, melting, and emulsifying, into a smooth, homogeneous molten blend, one

or more natural cheeses and optional ingredients using heat, mechanical shear, and (usually) emulsifying salts. Optional ingredients permitted are determined by product type, i.e., whether processed cheese, processed cheese food, or processed cheese spread and include dairy ingredients, vegetables, meats, stabilizers, emulsifying salts, flavors, colors, preservatives, and water (Tables IX and X).

Although a product of recent origin compared to natural cheese, processed cheese products (PCPs) show a parallel increase in growth rate of ~3% p.a. Documented world production amounts to 8–10% of total cheese manufactured (MMB, 1991). Factors contributing to the continued growth of these products are:

(i) Their versatility as foods which offer almost unlimited variety in flavor, consistency, functionality, and consumer appeal as a result of differ-

TABLE IX

PERMITTED INGREDIENTS IN PASTEURIZED PROCESS CHEESE PRODUCTS

Product	Ingredients
Pasteurized blended cheese	Cheese; cream, anhydrous milk fat, dehydrated cream [in quantities such that the fat derived from them is less than 5% (w/w) in finished product]; water; salt; food-grade colors, spices, and flavors; mould inhibitors (sorbic acid, potassium/sodium sorbate, and/or sodium/calcium propionates), at levels $\not>$ 0.2% (w/w) of finished product.
Pasteurized process cheese	As for pasteurized blended cheese, but with the following extra optional ingredients: emulsifying salts [sodium phosphates, sodium citrates; 3% (w/w) of finished product], food-grade organic acids (e.g., lactic, acetic, or citric) at levels such that pH of finished product is $\not<$5.3.
Pasteurized process cheese foods	As for pasteurized process cheese, but with the following extra optional ingredients: dairy ingredients (milk, skim milk, buttermilk, cheese whey, whey proteins—in wet or dehydrated forms).
Pasteurized process cheese spread	As for pasteurized process cheese food but with the following extra optional ingredients: food-grade hydrocolloids (e.g., carob bean gum, guar gum, xanthan gums, gelatin, carboxymethylcellulose, and/or carageenan) at levels < 0.8% (w/w) of finished products; food-grade sweetening agents (e.g., sugar, dextrose, corn syrup, glucose syrup, hydrolyzed lactose).

TABLE X

COMPOSITIONAL SPECIFICATIONS FOR PASTEURIZED PROCESS CHEESE PRODUCTS[a,b]

Product category	Moisture (%, w/w)	Fat (%, w/w)	Fat in dry matter (%, w/w)
Pasteurized blended cheese	$\geq$ 43	—	$<$ 47
Pasteurized process cheese	$\geq$ 43	—	$<$ 47
Pasteurized process cheese food	$\geq$ 44	$<$ 23	—
Pasteurized process cheese spread	40–60	$<$ 20	—

[a] Minimum temperatures and times specified for processing are 65.5°C for 30 sec.

[b] The compositional specifications for pasteurized process cheese may differ from those given, depending on the type of product; for more detail, see the Code of Federal Regulations (CFR) (1988).

ences in formulation, condiment addition, processing conditions, and packaging in various shapes and sizes.

(ii) Lower cost relative to natural cheese due to incorporation of low-grade natural cheese and cheaper noncheese dairy ingredients.

(iii) Adaptability to the fast food trade, the most notable examples being the use of cheese slices in burgers and dried processed cheeses as snack and popcorn coatings.

(iv) Relatively long shelf-life and no waste.

(v) Development of companies specializing in the manufacture of equipment, emulsifying salts, and other ingredients tailor-made to the industry's needs in fulfilling consumer needs.

B. CLASSIFICATION OF PROCESSED CHEESE PRODUCTS

Four main categories of PCPs are identified [Code of Federal Regulations (CFR), 1988], namely, processed cheese, processed cheese foods, processed cheese spreads, and texturized blended cheeses (Table IX). The criteria for classification include permitted ingredients and compositional parameters. Processed cheese is usually sold in the form of sliceable blocks (e.g., processed Cheddar) or slices; cheese spreads and foods may be in the form of blocks, slices, spreads, dips, sauces, or pastes (e.g., in tubes). Texturized blended cheese, which is the least common category, is usually sold in forms giving a natural cheese image. Processed cheese analogues/substitutes, although similar to PCPs in make procedure and product characteristics, are not PCPs per se. Analogue production is based on vegetable fat–caseinate/rennet casein blends and usually does not incorporate natural cheese, except where it is added in small quantities in the form of enzyme-modified cheeses,

very mature cheese, or cheese powders to impart a more natural cheese flavor.

Other processed cheese-derived products include cheese powders (obtained by drying pasteurized PCPs) and cheese sauce preparations (prepared by cooking blends of cheese powders, starches, skimmed milk solids, flavors, and water).

C. MANUFACTURING PROTOCOL

Manufacture involves the following steps: (i) formulation of blend, which involves selection of the correct type and quantity of natural cheeses, emulsifying salts, water, and optional ingredients, (ii) shredding/comminuting of cheese and blending with optional ingredients, (iii) processing of the blend, (iv) homogenization of the hot molten blend; this step is optional and implementation depends on the fat content of the blend, type of cooker used, smoothness, and body characteristics required in the end product, and (v) packaging and cooling.

Processing refers to the heat treatment of the blend, with direct or indirect steam, with constant agitation; a partial vacuum may be used to regulate moisture, when using indirect steam injection, and to remove air from the product. In batch processing, the temperature–time combination varies (i.e., 70–95°C for 4–15 min), depending on the formulation, extent of agitation, the desired product texture, body, and shelf-life characteristics. At a given temperature, the processing time generally decreases with agitation rate which may vary, depending on the kettle (cooker) type, from 50 to 3000 rpm. In continuous cookers, mainly used for dips and sauces, the blend is mixed and heated to 80–90°C in a vacuum mixer from where it is pumped through a battery of tubular heat exchangers and heated to 130–145°C for a few seconds and then flash-cooled to 90°C. Cooked product is then pumped to a surge tank which feeds the packaging machine.

In the case of slice production, the hot molten cheese is pumped through a manifold with 8–12 nozzles which extrude ribbons of cheese onto the first two or three counter-rotating chill rolls over which the cheese ribbons pass and are thereby cooled from 70–80 to 30°C. The ribbons are automatically cut into slices, which are stacked and packed.

D. PRINCIPLES OF MANUFACTURE OF PROCESSED CHEESE

Natural cheese may be viewed as a three-dimensional particulate network, resembling a loose semirigid sponge, in the pores of which naturally emulsified fat globules and moisture are entrapped (Brooker, 1979; Kalab, 1979). The integrity of the network, which consists of overlapped and

crosslinked chains of partially fused aggregates (consisting of fused para-casein micelles) is maintained by various intra- and interaggregate bonds, including hydrophobic and electrostatic (e.g., calcium cross links via casein phosphoserine and ionized carboxyl residues) attractions (Knoop, 1977; Walstra and van Vliet, 1986).

Application of heat (70 to 90°C) and mechanical shear to natural cheese, as in processing, in the absence of stabilizers, generally results in a heterogeneous, gummy pudding-like mass with extensive oiling-off and moisture exudation, particularly on cooling. These defects arise from shearing of fat globule membranes and aggregation and partial dehydration of the protein phase (especially in low-pH cheeses); consequently, free moisture and de-emulsified liquified fat seep through the more porous, modified structure.

The addition of emulsifying salts (1–3%) during processing promotes emulsification of free fat and rehydration of protein and thus contributes greatly to the formation of a smooth, homogeneous, stable product. However, it is also possible to manufacture stable pasteurized cheese products (i.e., pasteurized blended cheese) without the addition of emulsifying salts or hydrocolloids when certain changes in formulation and processing conditions are implemented (McAuliffe and O'Mullane, 1991; Guinee, 1991); such products are now commercially available in Ireland and elsewhere in the form of slices and spreads. In practice, the development of body, creaminess, and sheen during processing is commonly termed creaming of the product, especially when making spreadable, high-moisture PCPs. It is generally observed as an increase in viscosity on holding at elevated temperatures (75–90°C), especially when shearing continously.

The emulsifying salts most commonly used for the manufacture of pasteurized processed cheese are sodium citrates, sodium orthophosphates, sodium pyrophosphates, sodium tripolyphosphates, sodium polyphosphates (e.g., Calgon), basic sodium aluminium phosphates (e.g., Kasal), and phosphate blends (e.g., Joha, Solva blends). These salts generally contain a monovalent cation (i.e., sodium) and a polyvalent anion (e.g., phosphate). While they are not emulsifiers per se, they cause, with the aid of heat and shear, a series of concerted physicochemical changes within the cheese blend which convert the aggregated, inactive calcium *para*-casein gel (network) into an active emulsifying and water-binding agent. These changes include calcium sequestration, pH displacement and stabilization, dispersal and hydration of *para*-casein, emulsification, and structure formation (Fig. 22) and are discussed briefly below.

1. Calcium Sequestration

This involves the exchange of the divalent Ca^{2+} (attached to casein via carboxyl and phosphoseryl residues) of the calcium *para*-caseinate net-

A. Overall Reaction

Raw cheese (calcium paracasein network with entrapped moisture and globular fat) + water + melting salts and energy

↓

Processed cheese

B. Process Responsible for Transition

Process	Main Causative Agent
Ion exchange (Ca sequestration)	Emulsifying salt
pH buffering	Emulsifying salt
Protein dispersion	Emulsifying salt Thermal and mechanical energy
Protein hydration	Processing salt Thermal energy
Emulsification	Dispersed hydrated protein Mechanical energy
Structure formation	Protein-protein interactions
	Emulsified fat Globules (pseudo-protein particles)

FIG. 22. Summary of principles operative in process cheese manufacture (from Guinee, 1990).

work for the monovalent Na^+ of the emulsifying salt (Nakajima *et al.*, 1975; Lee and Alais, 1980). The removal (sequestration) of Ca^{2+} results in: (i) disintegration of the inter- and intrastrand linkages keeping the *para*-caseinate network intact and hence a reduction in matrix continuity and gel-like structure, (ii) conversion of the *para*-caseinate gel network into sodium- and/or sodium phosphate *para*-caseinate dispersion (sol) (Tatsumi *et al.*, 1975; Ito *et al.*, 1976) to a greater or lesser degree, depending on the processing conditions and type of salt (calcium chelating strength, pH, and buffering capacity). The formation of sodium phosphate *para*-caseinate, as affected by the probable surface adsorption of the polyvalent emulsifying salt anions to the *para*-casein (van Wazer, 1971; Melnychyn and Wolcott, 1971; Shimp, 1985), is supported by the higher concentration of protein-bound phosphorus in processed cheese compared to natural cheese (Nakajima *et al.*, 1975).

2. *pH Displacement and Stabilization*

The use of the correct blend of emulsifying salts usually shifts the cheese pH from ~5.0–5.5 in the natural cheese to 5.5–5.9 in PCPs and stabilizes it by virtue of their high-buffering capacity (Meyer, 1973; Tatsumi *et al.,* 1975; Gupta *et al.,* 1984; Caric and Kalab, 1987). This change contributes to successful processing by increasing the calcium-sequestering ability of the emulsifying salts per se (Irani and Callis, 1962; Lee *et al.,* 1986) and the negative charge on the *para*-caseinate, which in turn promotes further disintegration of the calcium *para*-caseinate network and a more open, reactive caseinate conformation with superior water-binding and emulsification properties.

Thus, the extent of pH displacement is a critical factor controlling the textural attributes of PCPs (Swiatek, 1964; Rayan *et al.,* 1980; Gupta *et al.,* 1984).

3. *Para-Casein Dispersion/Water Binding*

Dispersion of *para*-casein, also called peptization, refers to the disintegration of the cheese network and conversion of the calcium *para*-caseinate into a charged, hydrated sodium (phosphate) *para*-caseinate as effected by the above-mentioned emulsifying salt-induced changes in combination with the mechanical and thermal energy inputs of processing (Lee *et al.,* 1979, 1986; Kirchmeier *et al.,* 1978; Buchheim and Thomasow, 1984). The conversion of calcium *para*-caseinate to sodium (phosphate) *para*-caseinate during processing is the major factor affecting protein hydration. This is supported by the inverse relationship found between casein-bound calcium and casein solvation (Sood *et al.,* 1979). Protein hydration is reflected in the large increases in nonsedimentable protein and bound water (Templeton and Sommer, 1936; Nakajima *et al.,* 1975; Ito *et al.,* 1976; M. A. Thomas *et al.,* 1980; Csok, 1982; Lamure *et al.,* 1988); the level of hydration varies with formulation and processing conditions. However, prolonged holding of the molten processed cheese at a high temperature results in some reaggregation of the protein, a decrease in protein-bound water, and an increase in the level of sedimentable protein (Csok, 1982; Tatsumi *et al.,* 1991).

4. *Emulsification*

Under the conditions of cheese processing, the dispersed, hydrated *para*-caseinate contributes to: (i) emulsification, via coating of dispersed free

fat droplets, resulting in the formation of fat globule membranes, and (ii) emulsion stability by immobilization of large amounts of free water.

E. STRUCTURE FORMATION ON COOLING

During the cooling of PCPs, the homogeneous, molten, viscous mass sets to form a characteristic body, which, depending on blend formulation, processing conditions, and cooling rate, may vary from a firm sliceable product to a semisoft spreadable consistency. While little or no information is available on the physicochemical mechanisms responsible for structure formation (setting) of PCPs on cooling, factors which probably contribute include: fat crystallization, protein–protein interactions, and incorporation of recombined fat globules, which may be considered as increasing the effective protein concentration, into the new structural matrix.

Electron microscopical studies on processed cheese (Kimura *et al.*, 1979; Taneya *et al.*, 1979, 1980; Rayan *et al.*, 1980; Heertje *et al.*, 1981; Lee *et al.*, 1981; Kalab *et al.*, 1987; Savello *et al.*, 1989; Tamime *et al.*, 1990) indicate that the structure consists of emulsified fat globules dispersed in a protein network. The fat globules are evenly distributed (unlike natural cheese) and generally range from 0.3 to 5 μm in diameter. Fat globule size, as affected by the degree of emulsification, varies with formulation (i.e., type and quantity of emulsifying salt and other ingredients, age of cheese) and processing conditions (shear rate, temperature, and time). Rayan *et al.* (1980) found that the diameter of fat globules in processed Cheddar decreased progressively with processing time and at any given time the mean fat globule diameter was smallest on using tetrasodium pyrophosphate, largest with basic sodium aluminium phosphate (SALP), and intermediate with trisodium citrate or disodium phosphate. Indeed, after processing for 10 min with SALP, many of the fat globules had diameters greater than 10 μm (Rayan *et al.*, 1980); hence, in practice, SALP is generally claimed to give processed cheeses with good melt and stretch properties. Lee *et al.* (1981) found that increasing the concentration of emulsifying salt (1–4%) and processing temperature (80–140°C) resulted in a progressive decrease in mean fat globule diameter and a parallel increase in firmness.

The protein fraction of processed cheeses exists as relatively short strands which are connected to varying degrees, resulting in a matrix with different degrees of continuity, depending on product type. The matrix strands are much finer than those of natural cheese and appear to be composed of *para*-caseinate particles (20–30 mm diameter) which undergo limited aggregation or touching; it is suggested that these particles may correspond to casein submicelles (Kimura *et al.*, 1979; Taneya *et al.*, 1980; Heertje *et al.*, 1981) released from the cheese *para*-caseinate network as a result of calcium

chelation by the emulsifying salts. The *para*-caseinate membranes of emulsified fat globules, distributed uniformly throughout the protein phase, appear to attach to the matrix strands; the ensuing anchoring of the relatively short strands by the recombined fat globules probably contributes to the continuity and elasticity of the matrix in the cooled product. The positive correlation between the degree of emulsification (surface area of the fat phase) and firmness and the inverse relationship between the degree of emulsification and melt (Rayan *et al.*, 1980; Savello *et al.*, 1989) lend support to this suggestion.

The following differences exist between the protein matrices in hard and soft processed cheeses (Kimura *et al.*, 1979; Taneya *et al.*, 1980):

(i) The network in hard processed cheeses consists of interconnecting strands (up to 100 μm in length) made up of *para*-casein aggregates (20 μm diameter) strung together in a necklace-like structure.
(ii) In soft products, the matrix-building *para*-casein aggregates are more dispersed and there are fewer interaggregate connections.

The increase in the length of matrix strands with processing time and temperature (Heertje *et al.*, 1981) probably reflects the decrease and increase in protein hydration and aggregation, respectively. The occurrence of more numerous and longer strands in hard processed cheeses ensure more interstrand connections and hence a more continuous and elastic protein matrix. Hence, Rayan *et al.* (1980) found that increasing the processing time, while scarcely changing the dimensions of the emulsified fat particles (i.e., when using sodium aluminum phosphate as emulsifying salt), resulted in processed Cheddar which was firmer, more elastic, and less meltable.

F. PROPERTIES OF EMULSIFYING SALTS

The emulsifying salts most commonly used are citrates, phosphates, polyphosphates, and sodium aluminium phosphates (Caric and Kalab, 1987) (Table XI). Other potential emulsifying agents include gluconates, lactates, malates, ammonium salts, glucono lactones, and tartarates (Price and Bush, 1974). Today, salts are generally supplied as blends of phosphates (e.g., Joha C special) or phosphates and citrates (e.g., Solva NZ 10), tailor-made to the processor's requirements.

Citrates are usually used as sodium salts, although potassium salts have also been used (Gupta *et al.*, 1984). The trisodium citrate is used most commonly; the mono- and disodium salts ($NaH_2C_6H_5O_7$ and $Na_2HC_6H_5O_7$), when used alone, generally give overacid PCPs which are mealy, acid, and crumbly and show a tendency toward oiling-off due to poor emulsification

TABLE XI

PROPERTIES OF EMULSIFYING SALTS FOR PROCESSED CHEESE PRODUCTS[a]

Group	Emulsifying salt	Formula	Solubility at 20°C (%)	pH value (1% solution)
Citrates	Trisodium citrate	$2Na_3C_6H_5O_7.1H_2O$	High	6.23–6.26
Orthophosphates	Monosodium phosphate	$NaH_2PO_4.2H_2O$	40	4.0–4.2
	Disodium phosphate	$Na_3HPO_4.12H_2O$	18	8.9–9.1
Pyrophosphates	Disodium pyrophosphate	$Na_2H_2P_2O_7$	10.7	4.0–4.5
	Trisodium pyrophosphate	$Na_3HP_2O_7.9H_2O$	32.0	6.7–7.5
	Tetrasodium pyrophosphate	$Na_4P_2O_7.10H_2O$	10–12	10.2–10.4
Polyphosphates	Pentasodium tripolyphosphate	$Na_5P_3O_{10}$	14–15	9.3–9.5
	Sodium tetrapolyphosphate	$Na_6P_4O_{13}$	14–15	9.0–9.5
	Sodium hexametaphosphate (Graham's salt)	$Na_{n+2}P_nO_{3n+1}$ (n = 10–25)	Infinite	6.0–7.5
Aluminium Phosphates	Sodium aluminium phosphate	$NaH_{14}Al_3(PO_4)_8.4H_2O$	—	8.0

[a] From van Wazer (1971) and Caric and Kalab (1987).

(Gupta *et al.*, 1984). The dissociation constants (pK_a's) of citric acid at the ionic strength of milk are 3.0, 4.5, and 4.9 (Walstra and Jenness, 1984). Owing to their acidic properties, mono- and disodium citrates may be used to correct the pH of a cheese blend, e.g., when a high proportion of very mature cheese is used.

The phosphates used in cheese processing include sodium monophosphates (orthophosphates; $n = 1$) and linear condensed phosphates such as pyrophosphates ($n = 2$) and polyphosphates ($n = 3$–25) (van Wazer, 1971; Wissmeier, 1972). Of the orthophosphates, disodium orthophosphate (Na_2HPO_4) is the form normally used; the mono- and trisodium forms when used alone tend to give overacid and underacid products, respectively (Templeton and Sommer, 1936; Scharf, 1971; Gupta *et al.*, 1984). Comparative studies (Gupta *et al.*, 1984; Scharf, 1971) have shown that the potassium orthophosphates, pyrophosphates, and citrates give processed cheeses with textual properties similar to those made with the equivalent sodium salts at similar concentrations. Therefore, Gupta *et al.* (1984) suggested that potassium emulsifying salts may have potential in the preparation of reduced-sodium formulations. Sodium aluminium phosphate ($NaH_{14}Al_3(PO_4)_8 \cdot 4H_2O$; van Wazer, 1971) is used only on a limited scale.

The effectiveness of the different salts in promoting the various physicochemical changes during processing, summarized in Table XII, are discussed below.

TABLE XII

GENERAL PROPERTIES OF EMULSIFYING SALTS IN RELATION TO CHEESE PROCESSING[a]

Property	Citrates	Orthophosphates	Pyrophosphates	Polyphosphates	Aluminium phosphate
Ion exchange (calcium sequestration)	Low	Low	Moderate	High–very high	Low
Buffering action in the pH range 5.3–6.0	High	High	Moderate	Low–very low	
para-Caseinate dispersion (peptization)	Low	Low	High	Very high	—
Emulsification	Low	Low	Very high	Very high ($n = 3$–10) –Low	Very low
Bacteriostatic effects	Nil	Low	High	High–very high	

[a] Templeton and Sommer (1936), Glandorf (1964), Roesler (1966), Scharf (1971), van Wazer (1971), Tanaka *et al.* (1979, 1986), Rayan *et al.* (1980), Kosikowski (1982), Caric and Kalab (1987), and Marcy *et al.* (1988).

1. Calcium Sequestration

Ion exchange is best accomplished by salts containing a monovalent cation and a polyvalent anion and effectiveness generally increases with the valency of the anion. The general ranking of the calcium sequestration ability of the common emulsifying salts in cheese is polyphosphates > pyrophosphates > orthophosphates > sodium aluminium phosphate = citrates (Wagner and Wagner-Hering, 1981; Nakajima *et al.*, 1975; Lee *et al.*, 1986). However, the sequestering ability, especially of the shorter chain phosphates, is strongly influenced by pH. This may be attributed to more complete dissociation, which gives a higher valency anion, at the higher pH values (van Wazer, 1971). Thus, for the shorter chain phosphates, calcium binding increases in the following order: NaH_2PO_4, Na_2HPO_4, $Na_2H_2P_2O_7$, $Na_3HP_2O_7$, $Na_4P_2O_7$, $Na_5P_3O_{10}$ (Caric and Kalab, 1987).

2. pH Changes and Buffering

The buffering capacity of sodium phosphates, in the pH range normally encountered in PCPs (i.e., 5.3–6.0), decreases with increasing chain length and is effectively zero for the longer chain phosphates ($n > 10$). This reduction in buffering capacity with chain length is due to the corresponding reduction in the number of acid groups per molecule which occur singly at each end of the polyphosphate chain (van Wazer, 1971). The ortho- and pyrophosphates possess high buffering capacities in the pH ranges 2–3, 5.5–7.5, and 10–12 (van Wazer, 1971); thus, in cheese processing they are very suitable not only as buffering agents but also for pH correction. Within the citrate group, only the trisodium salt has buffering capacity in the pH region 5.3–6.0; the more acidic mono- and disodium citrates cause overacid, crumbly cheese with a propensity to oiling-off (Gupta *et al.*, 1984).

3. Casein Hydration and Dispersion

The ability of the different groups of emulsifying salts to promote protein hydration and dispersion during cheese processing is in the following general order: polyphosphates > pyrophosphates = monophosphates = citrates (Lee *et al.*, 1986; Tatsumi *et al.*, 1975; Wagner and Wagner-Hering, 1981). The greater swelling effect of polyphosphates, which increases with chain length, over mono- and diphosphates can be explained in terms of the greater calcium sequestrating ability of the former.

4. Ability to Promote Emulsification

The effectiveness of different emulsifying salts to promote emulsification, as determined from electron microscopical and oiling-off studies, in pro-

cessed cheese is in the following general order: pyrophosphates > polyphosphates (p = 3–15) > citrates ~ orthophosphates (slightly) > basic sodium aluminium phosphates (Templeton and Sommer, 1936; Roesler, 1966; Scharf, 1971; Thomas *et al.*, 1980; Shimp, 1985). This ability generally parallels their effectiveness in promoting hydration and dispersion.

5. *Hydrolysis (Stability)*

During processing and storage of PCPs, linear condensed phosphates undergo hydrolytic degradation which finally leads to complete conversion to orthophosphates (Glandorf, 1964; Roesler, 1966; Scharf, 1971; van Wazer, 1971; Meyer, 1973). The extent of degradation increases with increased duration of processing time and temperature, product storage time and temperature, and phosphate chain length (Glandorf, 1964). Other influencing factors include the type of cheese, quantity of emulsifying salt, and type of product being produced. In experiments with processed Emmentaler (Roesler, 1966), the level of polyphosphate (n > 4) breakdown during melting at 85°C varied from 7% for block cheese (processed for 4 min) to 45% for spreadable cheese (processed for 10 min). While the breakdown of condensed phosphates, through the tri- and di-forms, to monophosphates was complete in the spreadable cheese after 7 weeks, low levels were detectable in block processed cheese even after 12 weeks. The greater extent of polyphosphate degradation in the spreadable processed cheeses is also expected due to their higher pH and moisture content (Scharf, 1971; van Wazer, 1971).

The practical consequences of hydrolysis include variations in the functionality of the emulsifying salt blend with processing conditions, an increased propensity to precipitation of dodecahydrate disodium orthophosphate ($Na_2HPO_4 \cdot 12H_2O$) on product storage (Scharf and Kichline, 1969) and labeling difficulties in relation to declaration of emulsifying salts used.

6. *Bacteriocidal Effects*

Cheese processing normally involves temperatures (70–95°C) which are lower than those used for sterilization. Thus, PCPs may contain viable spores, especially *Clostridium* spp., which originate in the raw materials (Briozzo *et al.*, 1983; Sinha and Sinha, 1986, 1988; Caric and Kalab, 1987). Germination of spores during storage often leads to blowing of cans, putrefaction, and off-flavors (Meyer, 1973; Price and Bush, 1974). While bacterial spoilage is minimized through the addition of preservatives, some of the emulsifying salts also possess bacteriocidal properties. Polyphosphates inhibit many microorganisms, including *Staphylococcus aureus, Bacillus subtilis, Clostridium sporogenes,* and various *Salmonella* species (van Wazer,

1971). Citrates, on the other hand, possess no bacteriostatic effects and may even themselves be subject to microbial degradation, thus reducing product keeping quality (Caric and Kalab, 1987). Orthophosphates have been found to inhibit the growth of *Cl. botulinum* in processed cheese (van Wazer, 1971; Tanaka *et al.*, 1979; Marcy *et al.*, 1988). The inhibitory effect of sodium orthophosphates on *Cl. botulinum*, which has been found to be superior to that of sodium citrates in pasteurized process cheese spreads with moisture levels in the range of 52–58% (Tanaka *et al.*, 1979), depends on the moisture and sodium chloride levels and pH of the processed cheese product (Tanaka *et al.*, 1986). The general bacteriocidal effect of phosphates, which increases with chain length (Meyer, 1973; Kosikowski, 1982), may be attributed to their interactions with bacterial proteins (van Wazer, 1971) and chelation of calcium, which generally serves as an important cellular cation and cofactor for some microbial enzymes (Stanier *et al.*, 1981).

7. *Flavor Effects*

While the effect of emulsifying salts *per se* on the flavor of processed cheese is difficult to quantify because of the influence of the many processing conditions thereon, it is generally recognized that sodium citrates impart a "clean" flavor while phosphates promote off flavors such as soapiness (in the case of orthophosphates) and bitterness (Templeton and Sommer, 1936; Scharf, 1971; Meyer, 1973; Price and Bush, 1974). Potassium citrates also tend to cause bitterness (Templeton and Sommer, 1936).

G. INFLUENCE OF VARIOUS PARAMETERS ON THE TEXTURAL PROPERTIES OF PROCESSED CHEESE PRODUCTS

Numerous investigations have been carried out during the last decade on the development of cheese extenders and the functionality of ingredients for PCPs with the view to reducing formulation costs while retaining or improving textural attributes (Mann, 1986, 1987, 1990). Comparative studies on the effects on textural characteristics have frequently led to different conclusions; discrepancies may be attributed, at least partly, to differences in processing conditions and formulation and hence in the types of interactions between the component ingredients. The influence of various blend ingredients and processing conditions on product quality are discussed below.

1. *Blend Ingredients*

Cheese is the major blend constituent in PCPs, ranging from a minimum of 51% in spreads to 95% in processed cheeses (CFR, 1988). Hence, both

the type and maturity of the cheese used have a major influence on the rheological properties of the product (Meyer, 1973; Harvey *et al.,* 1982; Caric and Kalab, 1987). This is reflected by formulation practices at the commercial level; block processed cheese with good sliceability and elasticity requires predominantly young cheese (75–90% intact casein) whereas predominantly medium-ripe cheese (60–75% intact casein) is required for spreads.

In model experiments with processed Gouda, Ito *et al.* (1976) found an inverse relationship between the age (and hence the degree of proteolysis) of the natural cheese and its emulsifying capacity. Hence, it is not surprising that the melt and firmness of PCPs increase and decrease, respectively, with maturity of the cheese blend (Thomas, 1970; Lazardis *et al.,* 1981; Harvey *et al.,* 1982; Mahoney *et al.,* 1982; Tamime *et al.,* 1990) since there is a positive relationship between the degree of emulsification and firmness and an inverse relationship between the degree of emulsification and firmness/hardness (Rayan *et al.,* 1980).

Working with chemically acidified curd systems, Lazaridis *et al.* (1981) treated curd with *Aspergillus oryzae* proteinase to induce variations in the level of proteolysis prior to processing. A strong positive relationship (r = 0.96) was found between meltability and the extent of proteolysis. Excessive proteolysis, however, was associated with textural defects, including excessive shortness. The same group (Mahoney *et al.,* 1982) found that the optimum meltability of the processed acidified curd was obtained when the molecular weight of the proteolysis products was in the range 10–25 kDa; smaller peptides (<10,000) resulted in an excessively meltable soft cheese.

Because of intercheese variation in structure and level of proteolysis, different types of cheese give processed products of different textural characteristics. Hence, it is generally recognized that mature semihard cheese varieties, such as hard Italian-types, Cheddar, and Emmentaler, give firmer, longer-bodied processed products that mould-ripened cheeses of the same age.

"Rework" refers to creamed (having attained the desired increase in viscosity) processed cheese which is not packaged for sale; it is obtained from "leftovers" in the cooking/filling machines, damaged packs, and batches which have overthickened (overcreamed) and are too viscous to pump. Added at a maximum level of ~20% (w/w), rework promotes the rate of firming (i.e., enhances the creaming effect) during processing, especially in blends with a high moisture content (e.g., cheese spreads) and/or a high proportion of overripe cheese. Such cheese (overripe) tends to give poor emulsification due to very low levels of intact casein or to a high hydrophilicity; emulsification requires protein (emulsifier) with the correct hydrophile–lipophile balance. Addition of rework generally results in a

higher apparent viscosity in the processed, hot, molten blend and in cooled products with higher resistance to deformation and reduced meltability, especially if the rework has overcreamed and is cooled slowly after processing (Kalab *et al.*, 1987). The thickening effect of rework during processing becomes more pronounced when it is cooled slowly and stored for some time prior to reuse. In the above context, it is interesting to note that Tamime *et al.* (1990) found that the firmness of processed cheese increased during storage over 3 months with the effect becoming more pronounced as the storage temperature was increased from 10 to 30°C.

Cheese base (CB) is being used increasingly as a cheese substitute in processed cheese manufacture, the main advantages being its lower cost and more consistent quality (i.e., intact casein content). Production generally involves ultrafiltration and diafiltration of milk, inoculation of the retentate with lactic culture, incubation to a set pH (5.2–5.8), pasteurization, and scraped-surface evaporation to 60% dry matter (Ernström, 1985; Mortensen, 1985); similar products are fermented, high dry matter, UF retentates. Increasing the level of substitution with CB normally results in "longer-bodied," firmer, and less meltable products (Tamime *et al.*, 1990; Younis *et al.*, 1991; Collinge and Ernstrom, 1988). However, the effects vary depending on the method of CB preparation and the subsequent heat treatment during processing: (i) decreasing pH, in the range 6.6 to 5.2, of milk prior to UF resulted in CB with lower calcium levels and processed products with improved meltability (Anis and Ernstrom, 1984), (ii) rennet treatment of the UF retentate results in poorer meltability (Anis and Ernström, 1984), an effect which may be attributed to the higher degree of interaction between β-lactoglobulin and *para*-κ-casein (than with native casein) during subsequent processing (Doi *et al.*, 1983), (iii) treatment of retentate with other proteinases (i.e., Savorase-A, *Aspergillus oryzae,* and *Candida cylindracea*), which increase the level of proteolysis in the CB, yields products which are softer and more meltable than those containing untreated CB (Sood and Kosikowski, 1979; Tamime *et al.*, 1990), (iv) increasing the processing temperature in the range 66 to 82°C results in processed products with reduced meltability, an effect attributed to the gelation of whey proteins at the higher temperatures, especially when rennet-treated CB is used (Collinge and Ernstrom, 1988). In this context, it is noteworthy that processed cheese, resistant to melting on cooking, may be prepared by adding a heat-coaguable protein (3–7%, w/w, lactalbumin, egg albumen, etc.), at a temperature <70°C, to the cheese blend on completion of processing (Schulz, 1976).

Noncheese dairy ingredients may account for a maximum of ~15% of the blend in pasteurized processed cheese spreads. Addition of skim-milk powder at levels of 3–5% of the blend results in softer, more spreadable

PCPs but increases the propensity to nonenzymatic browning on storage; higher levels (>7%) promote textural defects such as crumbliness (Thomas, 1969, 1970; Kairyukshtene and Zakharova, 1982). However, high levels (7–10%) may be added if the skim-milk powder is first reconstituted and then precipitated by proteinases or citric acid and the curd then added to the blend (Thomas, 1977).

Added lactose, in the range of 0 to 5%, is reported to result in lower spreadability, lower water activity, and increased propensity to nonenzymatic browning during processing (especially at high temperatures) and storage (Kombila-Moundounga and Lacroix, 1991; Piergiovanni *et al.*, 1989). Excess lactose may also increase the propensity to form mixed crystals containing various species, e.g., Ca, P, Mg, Na, tyrosine, and/or citrate, in PCPs during storage. Owing to the relatively high level of bound water in processed cheese products (a maximum of 1.6 g/g solids nonfat; Csok, 1982), the effective lactose concentration, in the free moisture phase, may easily exceed its solubility limit (~15 g/100 g H_2O at 21°C) (Uhlmann *et al.*, 1983); the lactose crystals may then act as nuclei for crystallization of mineral species which are supersaturated. It is generally recognized that added whey proteins gel on heating and thereby result in firmer process cheese products with lower meltability (Schulz, 1976; Savello *et al.*, 1989); the magnitude of the effect, however, appears to be related to the method of preparation and form in which the whey protein is added (Hill and Smith, 1992).

The addition of milk protein coprecipitates (produced by high heat treatment of milk followed by acidification and calcium addition), at levels up to 5% of the blend, to processed Cheddar yielded products with increased firmness and sliceability and lower meltability and a reduced propensity to nonenzymatic browning (Thomas, 1970). However, the level at which meltability became noticeably impaired varied from 0.25 to 3.0% and varied with the source of the coprecipitate.

Caseinates and caseins (acid and rennet) are used widely in processed cheese and cheese analogues, the main attractions being lower cost (relative to cheese protein), a consistent level of intact casein, good emulsifying capacity (of caseinates), and stretching properties of rennet casein, making it ideal for analogue pizza cheese. Caseinates (especially sodium) find most application in processed cheese spreads where their high water-binding capacity and good emulsification properties promote creaming. Gouda *et al.* (1985) found that partial replacement of cheese solids nonfat by calcium caseinate (5–7%) improved the meltability of cheese spreads. While caseinates may be used in spreadable cheese analogues (Marschall, 1990; Hokes *et al.*, 1989), rennet casein is generally preferred in the manufacture of analogue pizza cheese, which is the major imitation cheese product (McCar-

thy, 1990). With rennet casein, the colloidal calcium to casein ratio, and hence degree of intercasein aggregate crosslinking, may be controlled by the calcium-chelating strength of the emulsifying salts during processing, to give the desired degree of casein hydration/aggregation and fat emulsification which in turn give the desired degree of meltability and stretchability on cooking the finished product. In this application, caseinates appear to overhydrate, resulting in a degree of casein aggregation which yields good meltability but which is too low to achieve satisfactory stretchability on cooking.

Hydrocolloids, including carob bean gum, guar gum, carageenan, sodium alginate, gum karaya, pectins, and carboxymethylcellulose, are allowed in pasteurized processed cheese spreads at a maximum level of 0.8%. Owing to their water-binding and/or gelation properties (Phillips *et al.*, 1986), they impart viscosity, especially in instances of high water content or low creaming action (thin consistency) as affected by, for example, the use of overripe cheese. These materials, along with polysaccharides/polysaccharide derivatives (e.g., inulin) are finding increasing application, as fillers and texturizers, in the manufacture of lowfat products, including PCPs (Brummel and Lee, 1990; Smith *et al.*, 1992; Anonymous, 1993).

2. *Composition*

Although the rheological attributes of PCPs with the same moisture level can differ significantly due to variations in blend composition and processing conditions, increasing moisture content yields products which are softer, less elastic and viscous, sticky, and spreadable (Kairyukshtene and Zakharova, 1982). Product pH has a major effect on the texture (Scharf, 1971; Gupta *et al.*, 1984; Shimp, 1985). Low pH (4.8–5.2), e.g., due to the use of monosodium citrate, monosodium phosphate, or sodium hexametaphosphate alone, give short, dry, crumbly cheeses which show a high propensity to oiling-off (Gupta *et al.*, 1984). High pH values (>6.0) lead to very soft, overmeltable products (Gupta *et al.*, 1984).

3. *Processing Conditions*

Increases in agitation speed, temperature in the range 70–100°C, and duration of processing are generally accompanied by an increase in the creaming reaction with a concomitant increase in firmness and loss of spreadability and meltability (Ryan *et al.*, 1980; Lee *et al.*, 1981; Harvey *et al.*, 1982; Kalab *et al.*, 1987; Tatsumi *et al.*, 1991). The changes may be attributed to the increased degree of protein emulsification. Hence, high moisture formulations, as in processed cheese spreads, are generally sub-

jected to conditions (higher temperature and more vigorous agitation) which promote a stronger creaming action (firming effect) than block-processed cheese.

IX. NUTRITIONAL AND SAFETY ASPECTS OF CHEESE

A. INTRODUCTION

Cheese is a nutritious, versatile food. A wide variety of cheese types are available to meet specific consumer requirements and allow convenience of use. The increasing popularity of cheese is apparent from consumption studies worldwide, Table II (IDF, 1984, 1992; National Dairy Council, 1989).

Cheese contains a high concentration of essential nutrients relative to its energy content. Its precise nutrient content is influenced by the type of milk used (species, stage of lactation, whole fat, lowfat, skim), method of manufacture, and to a lesser extent the degree of ripening. As outlined in detail elsewhere in this review, water-insoluble nutrients of milk (casein, colloidal minerals, fat, and fat-soluble vitamins) are retained in the cheese curd whereas the water-soluble constituents (whey proteins, lactose, water-soluble vitamins, and minerals) partition into the whey. However, loss of water-soluble B vitamins in whey may be compensated to a certain extent by microbial synthesis during ripening (Renner, 1987).

B. PROTEIN

The protein content of cheese ranges from ca. 3 to 40%, Table XIII (Holland *et al.*, 1989). Protein content tends to vary inversely with fat content for any one type of cheese. During traditional cheese manufacture, most of the whey proteins are lost in the whey and represent only 2–3% of the total protein, the remainder being casein which is slightly deficient in sulfur-containing amino acids. Thus, the biological value of cheese protein is slightly less than that of total milk protein. If the essential amino acid index of total milk protein is given a value of 100, the corresponding value for the proteins in most cheese varieties ranges from 91 to 97 (Renner, 1987).

When ultrafiltration is exploited in cheese manufacture (see Section IIIC), whey proteins are incorporated into the cheese, resulting in a biological value similar to milk protein. Cheese protein is almost 100% digestible as the ripening phase of cheese manufacture involves a progressive breakdown of casein to water-soluble peptides and free amino acids (see Section IVE). Hence, a significant degree of digestion of cheese protein has occurred before it is consumed.

TABLE XIII

COMPOSITION OF SELECTED CHEESES, PER 100 G[a]

Cheese type	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Cholesterol (mg)	Energy kcal	Energy kJ
Brie	48.6	19.3	26.9	Tr[b]	100	319	1323
Caerphilly	41.8	23.2	31.3	0.1	90	375	1554
Camembert	50.7	20.9	23.7	Tr[b]	75	297	1232
Cheddar (normal)	36.0	25.5	34.4	0.1	100	412	1708
Cheddar (reduced fat)	47.1	31.5	15.0	Tr[b]	43	261	1091
Cheshire	40.6	24.0	31.4	0.1	90	379	1571
Cottage cheese	79.1	13.8	3.9	2.1	13	98	413
Cream cheese	45.5	3.1	47.4	Tr[b]	95	439	1807
Danish blue	45.3	20.1	29.6	Tr[b]	75	347	1437
Edam	43.8	26.0	25.4	Tr[b]	80	333	1382
Emmental	35.7	28.7	29.7	Tr[b]	90	382	1587
Feta	56.5	15.6	20.2	1.5	70	250	1037
Fromage frais	77.9	6.8	7.1	5.7	25	113	469
Gouda	40.1	24.0	31.0	Tr[b]	100	375	1555
Gruyere	35.0	27.2	33.3	Tr[b]	100	409	1695
Mozzarella	49.8	25.1	21.0	Tr[b]	65	289	1204
Parmesan	18.4	39.4	32.7	Tr[b]	100	452	1880
Processed cheese[c]	45.7	20.8	27.0	0.9	85	330	1367
Ricotta	72.1	9.4	11.0	2.0	50	144	599
Roquefort	41.3	19.7	32.9	Tr[b]	90	375	1552
Stilton	38.6	22.7	35.5	0.1	105	411	1701

[a] Adapted from Holland *et al.* (1989).
[b] Tr, trace.
[c] Variety not specified.

C. CARBOHYDRATE

Most of the lactose in milk is lost in the whey during cheese manufacture and hence most cheese contain only trace amounts of carbohydrate (Table XIII). Furthermore, the residual lactose in cheese curd is usually fermented to lactic acid by starter bacteria. Thus, cheeses are suitable dairy foods for lactose-malabsorbing individuals who are deficient in the intestinal enzyme, lactase.

D. FAT AND CHOLESTEROL

The fat content of cheese varies considerably, depending on the milk used and the method of manufacture (Table XIII). Fat plays several roles

in cheese. It affects its firmness, adhesiveness, mouthfeel, and flavor. Its influence on cheese flavor depends on the variety; in some varieties, free fatty acids and their metabolites are important flavor constituents (see Section IVE).

From a nutritional point of view, the digestibility of the fat of different varieties of cheese is in the range 88–94% (Renner, 1987). Most cheeses are potentially significant sources of dietary fat. For example, a 50-g serving of Cheddar provides 17 g fat (Table XIII), which is a significant amount when compared with typical intake of fat in affluent Western societies, e.g., a typical Western diet providing 2000 kcal (8400 J) with 40% energy from fat contains approximately 88 g fat. Cheese fat generally contains ~66% saturated fatty acids, 30% monosaturated and 4% polyunsaturated. Thus, cheese represents a significant dietary source of both total fat and saturated fatty acids. Recent work on primates (Hayes *et al.,* 1991) shows that of the many saturated fatty acids in milk, only $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$ have the property of raising blood cholesterol and palmitic acid ($C_{16:0}$) is relatively ineffective.

Many sets of dietary guidelines issued by expert panels worldwide have recommended reductions in both total and saturated fat intakes in Western societies. Recently, the National Research Council (1989) recommended that total fat intake be restricted to not more than 30% and saturated fatty acids to not more than 10% of energy, respectively. In large measure, these recommendations are based on evidence that increased intakes of some saturated fatty acids elevate both total and low density lipoprotein cholesterol in blood which is associated with an increased risk of coronary heart disease. While some nutritionists dispute this hypothesis, the overwhelming body of medical opinion worldwide supports the concept of dietary guidelines. Market forces and consumers have responded to these guidelines and the market for food products low in fat, cholesterol, and sodium has expanded dramatically during the past decade. The cheese industry has responded by developing "light" cheese products with a reduced fat content (see Olson and Johnson, 1990).

The cholesterol content of cheese is a function of its fat content (Table XIII) and ranges from ca. 10 to 100 mg/100 g, depending on the variety. Despite considerable consumer confusion and the widespread prevalence of misinformation, dietary cholesterol has much less influence on blood cholesterol levels than dietary saturated fat (Keys, 1984). Thus, the cholesterol content of cheese is of lesser importance than its saturated fat content. The majority of individuals show little or no response in blood cholesterol levels to increased dietary cholesterol intake in the range 250–800 mg/day. However, a minority (ca. 20%) of adults do exhibit increased levels of blood cholesterol in response to increased dietary intake (McNamara, 1987). Some dietary guidelines recommend restricting dietary cholesterol intake to not

greater than 300 mg/day (National Research Council, 1989) while others make no specific recommendation regarding cholesterol [Committee on Medical Aspects of Food Policy (COMA), 1984].

In recent years, a considerable body of research has been carried out on the effects of oxidation products of cholesterol on the etiology of atherosclerosis (Hubbard *et al.,* 1989). However, cholesterol oxides are formed to a negligible extent in cheese under normal conditions of manufacture, ripening, and storage (Sunder *et al.,* 1988).

E. VITAMINS

The concentration of fat-soluble vitamins in cheese is influenced by the same factors that affect its fat content. Most of the vitamin A (80–85%) in milk fat is retained in the cheese fat. The concentration of water-soluble vitamins in cheese is generally lower than that in milk due to losses in the whey. The loss of some of the B vitamins is offset, to a certain extent, by microbial synthesis during cheese ripening. In particular, propionic acid bacteria synthesise significant levels of vitamin B_{12} in Swiss-type cheeses (Renner, 1987). In general, most cheeses are good sources of vitamin A, riboflavin, vitamin B_{12}, and to a lesser extent, folate. The vitamin content of a range of cheeses is shown in Table XIV (Holland *et al.,* 1989). Cheese contains negligible amounts of vitamin C.

F. MINERALS

Cheese is an important dietary source of several minerals, in particular calcium, phosphorus, and magnesium (Table XV). A 100-g serving of hard cheese provides ca. 800 mg Ca, which represents the Recommended Daily Allowance for most adults (Food and Nutrition Board, 1980). However, acid-coagulated cheeses, e.g., Cottage, contain considerably lower levels of calcium than rennet-coagulated varieties (Renner, 1987).

Calcium bioavailability from cheese is equivalent to that from milk. Recker *et al.* (1988) reported that 22.9, 26.7, and 25.4% of total calcium was absorbed from cream cheese, whole milk, and yogurt, respectively.

While the etiology of osteoporosis is very complex, there is widespread consensus that adequate calcium intake during childhood and in the teenage years is important in assuring the development of optimum peak bone mass. Maximizing bone mass early in life is considered to be a major preventative factor against development of osteoporosis in later years (Heaney, 1991). The National Research Council (1989) recommended an increase in calcium intake by teenage and adult females. Cheese has a potential role in supplying extra, highly bioavailable, calcium.

TABLE XIV

VITAMIN CONTENT OF SELECTED CHEESES, PER 100 G[a] (HOLLAND *ET AL.*, 1989)

Cheese type	Retinol (μg)	Carotene (μg)	Vitamin D (μg)	Vitamin E (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (μg)	Folate (μg)	Pantothenate (mg)	Biotin (μg)
Brie	285	210	0.20	0.84	0.04	0.43	0.43	0.15	1.2	58	0.35	5.6
Caerphilly	315	210	0.24	0.78	0.03	0.47	0.11	0.11	1.1	50	0.29	3.5
Camembert	230	315	0.18	0.65	0.05	0.52	0.96	0.22	1.1	102	0.36	7.6
Cheddar (normal)	325	225	0.26	0.53	0.03	0.40	0.07	0.10	1.1	33	0.36	3.0
Cheddar (reduced fat)	165	100	0.11	0.39	0.03	0.53	0.09	0.13	1.3	56	0.51	3.8
Cheshire	350	220	0.24	0.70	0.03	0.48	0.11	0.09	0.9	40	0.31	4.0
Cottage cheese	44	10	0.03	0.08	0.03	0.26	0.13	0.08	0.7	27	0.40	3.0
Cream cheese	385	220	0.27	1.0	0.03	0.13	0.06	0.04	0.3	11	0.27	1.6
Danish blue	280	250	0.23	0.76	0.03	0.41	0.48	0.12	1.0	50	0.53	2.7
Edam	175	150	0.19	0.48	0.03	0.35	0.07	0.09	2.1	40	0.38	1.8
Emmental	320	140	N[b]	0.44	0.05	0.35	0.10	0.09	2.0	20	0.40	3.0
Feta	220	33	0.50	0.37	0.04	0.21	0.19	0.07	1.1	23	0.36	2.4
Fromage frais	100	Tr[c]	0.05	0.02	0.04	0.40	0.13	0.10	1.4	15	N[b]	N[b]
Gouda	245	145	0.24	0.53	0.03	0.30	0.05	0.08	1.7	43	0.32	1.4
Gruyere	325	225	0.25	0.58	0.03	0.39	0.04	0.11	1.6	12	0.35	1.5
Mozzarella	240	170	0.16	0.33	0.03	0.31	0.08	0.09	2.1	19	0.25	2.2
Parmesan	345	210	0.25	0.70	0.03	0.44	0.12	0.13	1.9	12	0.43	3.3
Processed cheese[d]	270	95	0.21	0.55	0.03	0.28	0.10	0.08	0.9	18	0.31	2.3
Ricotta	185	92	N[b]	0.03	0.02	0.19	0.09	0.03	0.3	N[b]	N[b]	N[b]
Roquefort	295	10	N[b]	0.55	0.04	0.65	0.57	0.09	0.4	45	0.50	2.3
Stilton	355	185	0.27	0.61	0.03	0.43	0.49	0.16	1.0	77	0.71	3.6

[a] Adapted from Holland *et al.* (1989).

[b] The nutrient is present in significant quantities but there is not reliable information on the amount.

[c] Tr, trace.

[d] Variety not specified.

TABLE XV

MINERAL CONTENT OF SELECTED CHEESES, MG PER 100 G[a]

Cheese type	Na	K	Ca	Mg	P	Fe	Zn
Brie	700	100	540	27	390	0.8	2.2
Caerphilly	480	91	550	20	400	0.7	3.3
Camembert	650	100	350	21	310	0.2	2.7
Cheddar (normal)	670	77	720	25	490	0.3	2.3
Cheddar (reduced fat)	670	110	840	39	620	0.2	2.8
Cheshire	550	87	560	19	400	0.3	3.3
Cottage cheese	380	89	73	9	160	0.1	0.6
Cream cheese	300	160	98	10	100	0.1	0.5
Danish blue	1260	89	500	27	370	0.2	2.0
Edam	1020	97	770	39	530	0.4	2.2
Emmental	450	89	970	35	590	0.3	4.4
Feta	1440	95	360	20	280	0.2	0.9
Fromage frais	31	110	89	8	110	0.1	0.3
Gouda	910	91	740	38	490	0.1	1.8
Gruyere	670	99	950	37	610	0.3	2.3
Mozzarella	610	75	590	27	420	0.3	1.4
Parmesan	1090	110	1200	45	810	1.1	5.3
Processed cheese[b]	1320	130	600	22	800	0.5	3.2
Ricotta	100	110	240	13	170	0.4	1.3
Roquefort	1670	91	530	33	400	0.4	1.6
Stilton	930	130	320	20	310	0.3	2.5

[a] Adapted from Holland *et al.* (1989).
[b] Variety not specified.

Dairy products, including cheese, contribute little dietary iron (Table XV). Iron deficiency is commonly observed in both developing and developed countries, e.g., iron deficiency anemia in the United States has been estimated at 5.7% for infants, 5.9% for teenage girls, and 4.5% for young women (Dallman *et al.*, 1984). Hence, there has been interest in fortifying dairy products with iron to enhance their nutritional value. Cheddar and processed cheese have been successfully fortified with iron (Zhang and Mahoney, 1989a,b, 1990, 1991).

As discussed in Section IIIA6, NaCl serves several important functions in cheesemaking. A wide range of sodium levels are found in cheese due to different amounts of salt added during cheesemaking (Table XV). In general, the salt content of natural cheeses tends to be less than that of many processed cheeses.

Adults in the United States consume, on average, 4–6 g sodium/day (Surgeon General, 1988). Similar intakes are reported for other affluent Western Societies and are substantially above recommended safe and ade-

quate intakes, i.e., 1.1–1.3 g/day (Food and Nutrition Board, 1980). Considerable evidence exists that high sodium intakes can contribute to hypertension in a susceptible minority (20%) of individuals who are genetically salt-sensitive (National Research Council, 1989). Unfortunately, there is no simple diagnostic test to identify salt-sensitive individuals. Hence, dietary guidelines for the general public usually recommend that salt intake be restricted (National Research Council, 1989). However, even in countries with high consumption, cheese contributes only about 5–8% of total sodium intake (Renner, 1987).

G. NISIN AND OTHER ADDITIVES IN CHEESE

Sorbic acid and sorbates are used in several foods, including hard and semihard cheeses, to inhibit yeast and mould growth. It is a harmless and effective additive (Renner, 1987).

Nitrate is usually added to the milk for some varieties of cheese. It is reduced to nitrite which inhibits the growth of *Clostridium* spp. responsible for late gas blowing and flavor defects. Nitrite is rapidly destroyed in cheese so that the finished product contains only trace levels which pose no hazard to consumers. The contribution by cheese to the total intake of nitrite is negligible (Renner, 1987).

Consumer resistance to the use of synthetic additives in foods has stimulated interest in natural additives and preservatives. The principal natural additive used in cheese is the bacteriocin, nisin. Bacteriocins are peptides which inhibit a limited range of bacteria, usually closely related to the producer organism. The potential of nisin, produced by *Lactococcus lactis,* as a food preservative was first demonstrated using nisin-producing cultures in the manufacturer of Swiss-type cheese to prevent spoilage by clostridia (Hirsch *et al.,* 1951). To date, nisin is the only purified bacteriocin commercially exploited as a food preservative. It can be added to processed cheese products to prevent late blowing by clostridia, the spores of which, if present in the natural cheese, survive pasteurization (Barnby-Smith, 1992).

H. CHEESE AND DENTAL CARIES

A simplified description of the etiology of dental caries involves metabolism of sugars by oral microorganisms to acids which gradually dissolve tooth enamel. However, it is now recognized that a number of dietary factors and nutrient interactions can modify the expression of dental caries (Herod, 1991). The cariogenic potential of food is influenced by its composition, texture, solubility, retentiveness, and ability to stimulate saliva flow (Morrissey *et al.,* 1984).

In recent years, a considerable body of research has been conducted on the cariostatic effects of cheese. Early work (Shaw *et al.*, 1959; Dreizen *et al.*, 1961) demonstrated that the incorporation of dairy products into the diet greatly reduced the development of dental caries in rats. Reynolds and Johnson (1981) confirmed these findings. Later work (Weiss and Bibby, 1966; Jenkins and Ferguson, 1966) indicated that if enamel is treated with milk *in vitro* and subsequently washed, the solubility of the enamel is greatly reduced. This effect was attributed to the high levels of calcium and phosphate in milk (Jenkins and Ferguson, 1966) or to the casein (Weiss and Bibby, 1966). Later work supports both viewpoints.

Reynolds and del Rio (1984) found that both casein and whey proteins significantly reduced the extent of caries, with the former exerting the greater effect. Further evidence for the protective effect of casein was provided in a study on rats fed casein-enriched chocolate (Reynolds and Black, 1987). However, the palatibility of this innovative product was considered unacceptable for humans! Concentrates containing various levels of whey protein, calcium, and phosphorus, but negligible amounts of casein, significantly reduced the incidence of dental caries in rats (Harper *et al.*, 1987). Thus, there is evidence that milk proteins, calcium, and phosphate all exert an anticariogenic effect.

Rugg-Gunn *et al.* (1975) provided the first evidence that cheese consumption had an anticariogenic effect in humans. They showed that the consumption of Cheddar cheese after sweetened coffee or a sausage roll increased plaque pH, possibly due to increased salivary output. Similar effects were reported by Imfeld *et al.* (1978) who used a more sophisticated continuous wire telemetry procedure to monitor variations in plaque pH.

The effect of eating patterns on dental caries in rats was investigated by Edgar *et al.* (1982). Rats fed additional meals of cheese while on a high-sucrose diet developed fewer smooth surface carious lesions and exhibited increased salivary output (which buffers acid formed in plaque) and a reduction in numbers of *Streptococcus mutans*. Harper *et al.* (1983) suggested that the cariostatic effect of cheese in rats is due to its calcium phosphate and/or casein; the fat or lactose appeared to exert little influence. Further work by Rosen *et al.* (1984) on the effect of cheese, with or without sucrose, on dental caries and recovery of inoculated *S. mutans* in rats indicated beneficial cariostatic effects of cheese consumption but little effect on *S. mutans* numbers. These data suggest that the cariostatic effects of cheese may not be related directly to effects on *S. mutans*. Work on the protective effects of four different cheese varieties in an *in vitro* demineralization system suggested that most, but not all, of the protective effects of cheese could be explained by mass action effects of soluble ions, particularly calcium and phosphate (Jenkins and Harper, 1983).

An elegant study on the effects of Cheddar cheese on experimental caries in humans was reported by Silva *et al.* (1986) using an "intraoral cariogenicity test" (ICT). Demineralization and consequent reduction in the hardness of enamel monitored in this test is assumed to be typical of the early stage of caries development. Enamel slabs were polished and their initial microhardness determined using a Knoop Indenter. The slabs were then wrapped in Dacron and fastened on a prosthetic appliance made specifically for each subject to replace a missing lower first permanent molar. The subjects chewed 5 g of cheese immediately after rinsing their mouths with 10% (w/v) sucrose. Chewing cheese immediately after sucrose rinses resulted in a 71% reduction in demineralization of the enamel slabs, raised plaque pH, but caused no significant changes in the microflora of plaque compared with controls. Silva *et al.* (1987) investigated the effects of the water-soluble components of cheese on human caries using the ICT procedure and an experimental protocol which avoided salivary stimulus caused by chewing cheese. An average reduction of 55.7% in the cariogenicity of sucrose was reported, indicating the presence of one or more water-soluble anticariogenic components in cheese. Further evidence that cheese may inhibit dental caries in the absence of saliva was provided by Krobicka *et al.* (1987); rats that had their saliva-secreting glands surgically removed developed fewer and less severe carious lesions when fed cheese in addition to a cariogenic diet when compared to appropriate controls.

Trials on human subjects have confirmed that consumption of hard cheese leads to significant rehardening of softened enamel surfaces (Jenkins and Hargreaves, 1989; Gedalia *et al.*, 1991).

While more research is necessary to define the precise mechanism(s) involved in the cariostatic effects of cheese, there is ample evidence to support the consumption of cheese at the end of a meal as an anticaries measure (Herod, 1991). Gedalia *et al.* (1992) consider the most plausible mechanism for the protective effect of cheese to be related to the mineralization potential of casein–calcium phosphate of cheese and to the stimulation of saliva flow induced by its texture and/or flavor.

I. MYCOTOXINS

Mycotoxins are secondary metabolites of fungi which can cause acute toxic, mutagenic, teratogenic, and carcinogenic effects in animals. The fact that mycotoxins, such as aflatoxin, are among the most potent animal toxins and carcinogens known, warrants concern about the contamination of the human food supply, including dairy products, with mycotoxins.

The presence of mycotoxins in milk and dairy products may result from contamination of the cows' feedstuffs (indirect contamination) or contami-

nation of dairy products by mycotoxin-producing fungi (direct contamination) (van Egmond, 1989).

1. Indirect Contamination

Allcroft and Carnaghan (1962, 1963) first reported that intake of aflatoxin-contaminated feedstuff by dairy cows resulted in excretion of a toxic factor in their milk within a few hours. Subsequently, Allcroft *et al.* (1966) and Holzapfel *et al.* (1966) identified aflatoxin M_1 (the 4-hydroxy derivative of aflatoxin B_1) as the principal toxic metabolite in milk.

Studies (Shreeve *et al.*, 1979; Robinson *et al.*, 1979; Prelusky *et al.*, 1984) on the indirect contamination of milk with other mycotoxins such as ochratoxin A, zearalenone, T-2 toxin, sterigmatocystin, and deoxynivalenone have indicated that contamination of milk with these mycotoxins does not represent a significant public health issue.

An average of 1–2% of ingested aflatoxin B_1 is excreted in milk as aflatoxin M_1 (Rodricks and Stoloff, 1977; Patterson *et al.*, 1980; Lafont *et al.*, 1980). The carryover level varies between animals, from day to day and between milkings for the same animal.

The results of surveillance programs for aflatoxin M_1 in milk and milk products in many countries have been summarized by Brown (1982) and van Egmond (1989); the incidence and levels of aflatoxin M_1 in dairy products have decreased significantly in recent years, which has been attributed to the effect of legislation implemented in many countries on aflatoxin contamination of feedstuffs. However, a significant increase in aflatoxin M_1 levels in dairy products was noted in the United States in 1988–1989 as a result of feeding maize products contaminated with aflatoxin B_1 due to the severe drought in the U.S. Midwest in 1988 which created ideal conditions for aflatoxin production by *Aspergillus flavus.*

The surveillance programs discussed above contain few data on aflatoxin M_1 in cheese. In general, aflatoxin M_1 was not detectable or occurred at concentrations lower than the current legal limits (0.2–0.25 μg/kg) in a few countries for aflatoxin M_1 in cheese (van Egmond, 1989).

2. Fate of Aflatoxin M_1 during Cheese Manufacture and Ripening

The fate of aflatoxin M_1 in cheese milk is affected by the principal manufacturing steps. Early studies on the distribution of aflatoxin M_1 between the curd and whey gave contradictory results. Allcroft and Carnaghan (1962, 1963) reported that toxic activity was associated only with the rennet-coagulated curd. In contrast, Purchase *et al.* (1972) reported that Cottage cheese made by acid coagulation of naturally contaminated milk contained

no aflatoxin M_1, which was present in the whey. However, several other studies (below) have indicated aflatoxin M_1 present in milk partitions between the curd and whey in both acid-coagulated and rennet-coagulated cheeses.

Several investigators (Stubblefield and Shannon, 1974; Kiermeier and Buchner, 1977a; van Egmond *et al.*, 1977) have investigated the stability of aflatoxin M_1 during the conversion of milk to curd; recoveries of 88–111% of the aflatoxin M_1 in the milk were found for combined curd and whey fractions, indicating that aflatoxin M_1 is stable during cheesemaking. About half (40, 57, and 47%, respectively) of total aflatoxin M_1 was found in the curd fraction in these studies.

The partition coefficient of aflatoxin M_1 in water would suggest that most of the toxin should partition into the whey. The anomaly can be explained by data which suggest that aflatoxin M_1 tends to associate, possibly via hydrophobic interactions (Yousef and Marth, 1989), with casein micelles (Brackett and Marth, 1982c; Blanc *et al.*, 1983), causing a greater than expected concentration of the toxin in cheese curd.

The concentration of alfatoxin M_1 in Camembert and Tilsit (Kiermeier and Buchner, 1977b), Cheddar (Brackett and Marth, 1982a), and Brick cheese (Brackett *et al.*, 1982) increased during the early stage of ripening, followed by a gradual decrease to the concentration observed in the initial stages of ripening. In contrast, the concentration of aflatoxin M_1 in Gouda (van Egmond *et al.*, 1977) and Mozzarella did not vary significantly during ripening (Brackett and Marth, 1982b), while the concentration in Parmesan cheese decreased initially and then increased slowly (Brackett and Marth, 1982b). Despite these somewhat contradictory trends, it appears that aflatoxin M_1 is stable in cheese during ripening.

3. *Production of Toxic Metabolites in Mould-Ripened Cheese*

Cultures of *Penicillium roqueforti* and *P. camemberti* have been used for a long time in the manufacture of various types of blue-veined and white surface-mould cheeses. A report by Gibel *et al.* (1971), suggesting that these moulds could form toxic metabolites of potential public health significance, prompted a significant research effort on this topic. It was subsequently shown (Schoch, 1981) that strains of *P. roqueforti* and *P. camemberti* can produce a range of toxic metabolites. Some *P. roqueforti* strains can produce PR toxin, patulin, mycophenolic acid, penicillic acid, roquefortine, cyclopiazonic acid, isofumigaclavine A and B, and festuclavine. *P. camemberti* strains have been shown to produce only cyclopiazonic acid. Neither *P. roqueforti* nor *P. camemberti* have been reported to produce aflatoxins, either *in vitro* or in cheese (Engel and von Milczewski, 1977).

Cyclopiazonic acid, which is formed *in vitro* by all strains of *P. camemberti* examined to date, has been reported in samples of commercial Camembert and Brie (Le Bars, 1979; Schoch *et al.*, 1983). It occurs primarily in the rind and values of <0.5 mg/kg whole cheese are normally found in cheese stored in the cold but up to 5 mg/kg may be encountered if the storage temperature is too high. Evaluation of toxicological data currently available, together with data on the consumption of Camembert and Brie, indicates that these levels cause no appreciable risk to human health (Engel and Teuber, 1989).

Of the toxic metabolites produced by *P. roqueforti*, patulin, penicillic acid, and PR toxin have not been detected in commercial cheeses as the strains used for blue cheeses do not appear to produce these toxins in detectable amounts (Engel and Prokopek, 1979; Polonelli *et al.*, 1978). Mycophenolic acid has been reported in commercial cheese samples (Lafont *et al.*, 1979a; Engel *et al.*, 1982) but at levels well below those which pose a potential human risk. Roquefortine and isofumigaclavine A and B have been reported in commercial blue cheeses (Scott and Kennedy, 1976; Ware *et al.*, 1980) ripened at normal temperatures but the toxicity of these compounds is low and they are very unlikely to be hazards to human health (Engel and Teuber, 1989). The most compelling evidence that consumption of mould-ripened cheeses is not hazardous to human health is provided by studies in which *P. roqueforti* and *P. camemberti* cultures and mould-ripened cheeses were fed to rats and rainbow trout (Frank *et al.*, 1975, 1977; Schoch *et al.*, 1984); although the daily dose of mould was equivalent to human consumption of 100 kg cheese per day, no signs of toxicity were observed.

4. *Direct Contamination of Cheese with Mycotoxins.*

Unintentional mould growth on cheese during ripening and storage is a potential problem for manufacturers, retailers, and consumers; it results in financial loss, diminishes consumer appeal, and often necessitates trimming. However, mycotoxin production is a potentially more serious problem involving possible health risk. Such cheese has been reported to contain mycotoxins that are nephrotoxic (ochratoxin A, citrinin), teratogenic (ochratoxin A, aflatoxin B_1), neurotoxic (penitrem A, cyclopiazonic acid), carcinogenic (aflatoxins B_1 and G_1, ochratoxin A, patulin, penicillic acid, sterigmatocystin), or toxic antibiotics (patulin, penicillic acid, mycophenolic acid, citrinin) (Ueno, 1985).

Of the mycotoxigenic fungi isolated from cheese. *Penicillium* spp, are by far the most frequently reported, with *Aspergillus* spp. and others occasionally encountered. Moulds that develop on cheese during refrigerated storage are almost always *Penicillium* spp., which, in contrast to *Aspergillus* spp., can grow at low temperatures (Bullerman, 1981). However, not all *Penicil-*

lium spp. isolated from cheese are toxigenic. Toxicity in chick embryoes was induced by 30% of *Penicillium* isolates from Cheddar cheese (Bullerman and Olivigni, 1974) and 35% of *Penicillium* isolates from Swiss cheese (Bullerman, 1976). Toxicity in ducklings was induced by 47% of *Penicillium* isolates from South African Gouda and Cheddar (Luck *et al.*, 1976).

Cheese is generally a good substrate for fungal growth, given suitable conditions of temperature and humidity. Mycotoxin-producing moulds require oxygen and hence appropriate packaging of cheese is important; moulds are very unlikely to grow on vacuum-packed or wax-coated cheese. Good plant sanitation during manufacture and handling is also important in minimizing or preventing mould growth on cheese (Bullerman, 1977). Mycotoxins are unlikely to be produced during low temperature storage (Bullerman, 1981).

The presence of mould growth does not imply that mycotoxins are present in cheese. Bullerman (1976) analyzed mouldy Swiss cheese for the presence of penicillic acid and reported that 4 of 33 samples were positive. Lafont *et al.* (1979b) reported a low incidence of penicillic acid in 1 of 110 mould-contaminated Blue cheese samples, 3 of 48 samples of hard cheese, 5 of 39 semihard cheeses, and 2 of 18 goat-milk cheese samples. These investigators also reported a low incidence of patulin in hard cheese (1 of 48 samples), semihard cheese (4 of 39 samples), and goat-milk cheese (1 of 18 samples), and mycophenolic acid in hard cheese (4 of 48 samples) and semihard cheese (7 of 39 samples). However, high incidence of ochratoxin A and citrinin were reported in mouldy cheese samples in the UK (Jarvis, 1983). Richard and Arp (1979) found penitrem A in refrigerated mouldy cream cheese. Several studies on mouldy cheese for *Penicillium* mycotoxins gave negative results (Nowotny *et al.*, 1983; Williams, 1985; Fritz and Engst, 1981).

Work has also been conducted on the incidence of mycotoxins in cheese contaminated with *Aspergillus* spp. A low incidence of sterigmatocystin was reported in a number of studies (Lafont *et al.*, 1979b; Northolt *et al.*, 1980; Bartos and Matyas, 1982), but it was not detected in others (Steering Group on Food Surveillance, 1987; Nowotny *et al.*, 1983; Luck *et al.*, 1976; Bullerman, 1976). There is very little evidence that significant levels of aflatoxins are produced in cheese contaminated with *Aspergillus* spp. and many surveys have reported negative results (see Scott, 1989).

Some work has been undertaken on the ability of mycotoxins to migrate from the surface of cheese into the interior. Data on this topic are of significance in making objective decisions on whether or not to trim or discard mould-contaminated cheese. However, interpretation of much of the data is difficult since relatively high incubation temperatures were used. The Health Protection Branch of Health and Welfare, Canada (Anonymous 1981) has recommended that if a hard cheese is contaminated with a patch

of mould growth, the cheese can be salvaged by removing the infected portion to a depth of 2.5 cm. In view of both economic and public health implications, more research on the migration of mycotoxins is required.

J. BIOGENIC AMINES IN CHEESE

Biogenic amines can induce significant physiological effects in Man and animals under certain conditions. Biogenic amines are found in a wide variety of foods, including cheese (Maga, 1978; Smith, 1981; Rice *et al.*, 1976; Chang *et al.*, 1985; McCabe, 1986; Joosten, 1988). In cheese, biogenic amines are produced via decarboxylation of amino acids during ripening. Levels vary depending on the ripening period, the intensity of flavor development, and the microflora (Renner, 1987). High levels of biogenic amines are most likely in cheese heavily contaminated with spoilage microorganisms (Joosten, 1987). The principal biogenic amines found in cheese are histamine, tyramine, tryptamine, putrescine, cadaverine, and phenylethylamine. Renner (1987) reported average values of histamine and tyramine in some cheeses (Table XVI).

The ingestion of amine-containing foods may cause food poisoning (Rice *et al.*, 1976; Smith, 1981). However, for most individuals, ingestion of even large concentrations of biogenic amines does not elicit toxicity symptoms since they are rapidly converted to aldehydes by mono- and diamine oxidases and then to carboxylic acids by oxidative deamination (Edwards and Sandine, 1981). However, if mono- and diamine oxidases are impaired either due to a genetic defect or administration of inhibitory drugs, adverse reactions may occur on ingestion of biogenic amines (Rice *et al.*, 1976; McCabe, 1986; Joosten, 1988; Voight *et al.*, 1974; Diamond *et al.*, 1987).

TABLE XVI

AVERAGE TYRAMINE AND HISTAMINE CONTENTS OF SOME CHEESE VARIETIES[a]

Cheese variety	Tyramine (μg/g)	Histamine (μg/g)
Cheddar	910	110
Emmental	190	100
Blue	440	400
Edam, Gouda	210	35
Camembert, Brie	140	30
Cottage	5	5

[a] Adapted from Renner (1987).

Histamine is a normal constituent of the body; it is formed from histidine by a pyridoxal phosphate-dependent decarboxylase and mediates several important body functions (Douglas, 1980). The concentration of histamine in blood is strictly regulated and orally administered histamine will cause poisoning only when the regulatory mechanism fails to counteract all the ingested histamine, e.g., ingestion of a very high dose or impairment of histamine metabolism by other toxic substances (Taylor, 1986). Toxic symptoms, sometimes referred to as scombroid poisoning because they often result from consumption of fish of the Scombroidae family, become apparent within several minutes to 3 hr after ingestion of the histamine-containing food. Initially, a flushing of the face and neck occurs, often followed by an intense throbbing headache. Other symptoms sometimes reported include cardiac palpitations, dizziness, faintness, rapid and weak pulse, gastrointestinal complaints, bronchospasms, and respiratory distress (Taylor, 1986).

Oral ingestion of up to 1 mmol (ca. 100 mg) of histamine does not elicit toxic symptoms in normal individuals (Motil and Scrimshaw, 1979). However, vasodilation and increased heart rate result on intravenous administration of 0.07 μmol, demonstrating the importance of histamine-metabolizing enzymes in the digestive tract. More research is needed to define nontoxic levels of histamine in foods which may contain other substances that potentiate the action of histamine, e.g., putrescine and cadaverine (Bjeldanes *et al.*, 1978). Construction of an overall biogenic amine index may be valuable for the establishment of regulatory limits (Joosten, 1988).

Most cases of histamine poisoning are associated with fish (Taylor, 1986) and only a few cases due to the consumption of cheese have been reported. Gouda containing 85 mg histamine/100 g cheese was implicated in an outbreak in Holland (Doeglas *et al.*, 1967). Salt-tolerant lactobacilli, which contaminated the rennet, were considered the most likely factor responsible for the high levels of histamine (Stadhouders and Veringa, 1967). Two outbreaks of histamine poisoning have been reported in the United States. In 1978, 38 people exhibited histamine toxicity symptoms following consumption of Swiss cheese containing more than 9 mmol/kg of histamine (Chambers and Staruszkiewich, 1978) and in 1980, 6 people were poisoned by Swiss cheese containing 16.8 mmol/kg of histamine (Taylor *et al.*, 1982).

Unlike histamine, tyramine is normally present at very low concentrations in the body. If tyramine enters the bloodstream it causes a release of noradrenaline from the sympathetic nervous system (Joosten, 1988). Noradrenaline is important in many physiological reactions, on which tyramine can exert an indirect effect. The most common tyramine-induced responses include increased blood pressure, increased cardiac output, peripheral vasoconstriction and headache (Voight *et al.*, 1974; Rice *et al.*, 1976; McCabe, 1986; Diamond *et al.*, 1987).

In humans, monoamine oxidase (MAO)-catalyzed oxidative deamination to *p*-hydroxyphenylactic acid is the main tyramine degradative pathway. However, if a genetic deficiency of MAO exists or if MAO inhibitor drugs are administered, the normal route of tyramine degradation may be impaired and the toxicity symptoms outlined above may be manifested. Individuals whose MAO function is impaired are susceptible to the toxic action of tyramine, sometimes referred to as the "cheese reaction." This involves a hypertensive crisis, usually accompanied by severe headache and in certain cases could lead to intercranial hemorrhage, cardiac failure, and pulmonary edema. Fatal incidences have been reported (Asatoor *et al.*, 1963). It is important to note that other foods besides cheese, such as marinated herring, dry sausage, and marmite, may contain high levels of tyramine (Crocco, 1979).

The tyramine content of cheese varies with the degree of ripening, intensity of flavor development, and microflora (Renner, 1987) and may vary significantly within a block of cheese (Chang *et al.*, 1985). Unripened cheeses, e.g., Cottage cheese, contain negligible levels of tyramine (Voight *et al.*, 1974; Rice *et al.*, 1976; McCabe, 1986), while long-ripened varieties, such as Cheddar, may contain high levels (McCabe, 1986). If mature cheeses with a high tyramine content are used in processed cheeses, these cheeses may also have a high tyramine content. Whenever MAO inhibitor drugs are prescribed, patients should be advised to avoid intake of tyramine-rich foods. Food poisoning unambiguously caused by the consumption of tyramine-rich foods in the absence of MAO inhibitor drugs has not been reported (Joosten, 1988). The toxicity threshold for tyramine alone has been estimated at 3 mmol (ca. 400 mg) and it has been concluded that healthy individuals can tolerate the consumption of large amounts of tyramine-rich cheese (Grind *et al.*, 1986).

X. PERSPECTIVE

Cheese and wine are the outstanding examples of food biotechnology. Both originated at the dawn of civilization, perhaps 10,000 years ago. Both are surrounded by a certain mystique, have attained epicurean status, and are usually regarded as complementary. Cheese and wine are available in a great diversity of forms, both undergo long maturation periods, both have very complex flavor profiles, and, if properly produced, both improve with age. Although cheese and wine were discovered by accident, both would be regarded as outstanding biotechnological achievements if discovered today.

Although cheese production is a very ancient and traditional craft, cheese still has a very young image. It enjoys a consistent and substantial growth

rate, with a healthy and positive image. It could be regarded as the original convenience food which may be consumed as the main component of a meal, as a dessert, as a snack, as a condiment, or as a food ingredient. This latter application has become a major growth area, e.g., it now represents ~25% of total cheese consumption in the United States, largely through the increasing popularity of pizza, a product now common in many countries. Another significant feature of the cheese industry is that although there are at least 500 varieties, new varieties continue to be developed, usually by hybridizing varieties, e.g., Jarlsberg (Norway), Leerdamer (the Netherlands), Araglin (Ireland), and Proosdij (the Netherlands); interestingly, the first three of these are variants of Swiss-type cheese.

Cheese is an exciting research subject; it is produced in a great diversity of varieties, is complex, dynamic, and vital, and offers a challenge to scientists from many disciplines, e.g., analytical and colloidal chemists, biochemists, microbiologists, rheologists, and nutritionists, as well as engineers and technologies. This broad scientific appeal, coupled with its economic and dietary importance, has led to an extensive scientific and technical literature on cheese.

In this review, we have described the current state of knowledge on many aspects of cheese production. The review has concentrated on the chemical, biochemical, and nutritional aspects, with only cursory coverage of such important physicochemical aspects as rheology and texture. The microbiological and public health aspects of cheese were not considered (apart from mycotoxins and biogenic amines). The technological and engineering aspects of cheese production were omitted also. These important topics were excluded mainly in the interests of homogenity of presentation.

It seemed pertinent to conclude this review by considering the directions research on the chemistry and biochemistry of cheese will probably take in the immediate future.

The enzymatic aspects of milk coagulation are now rather well established at the molecular level and it is not readily apparent what new work of major significance might be done in this area. Undoubtedly, information on the enzymatic reaction will be refined but the principal reactions are already well established.

While the mechanism of coagulation of the rennet-altered micelles is understood in general terms, the precise chemical or physicochemical interactions that lead to gelation remain obscure. Perhaps further advances must await further refinement of our knowledge of the structure of the casein micelle, especially detailed knowledge of its surface and the changes therein as a consequent of enzymatic hydrolysis of κ-casein. Postaggregation phenomena, i.e., development of the gel network, its strength, and syneresis of the gel when cut or broken, are probably a continuation of the aggrega-

tion phase, modified by changing conditions of temperature and pH. Further work on the physicochemical aspects of these phenomena is required.

Considerable progress has been made during the past decade on the isoelectric coagulation of casein micelles in the formation of Quarg and fromage frais-type products. It is likely that work will continue on the coagulation mechanism, rheology, and physical stability of this important and increasingly popular group of dairy products.

The principal features of the primary biochemical events in ripening, especially glycolysis and lipolysis, are well established. Proteolysis, especially in long-ripened cheeses, is much more complex than glycolysis and lipolysis; the initial proteolytic events are now well established and the great diversity of peptides and the enzymes that produce them are now being elucidated. It appears reasonable to predict that the proteolytic diversity, pathways, and kinetics in the principal cheese varieties will be established during the next 5–10 years.

Undoubtedly, the products of these primary biochemical events, i.e., fatty and other acids, peptides, and amino acids, contribute to cheese flavor, perhaps very significantly in many varieties and proteolysis certainly has a major influence on the various rheological properties of cheese, e.g., texture, meltability, and stretchability. However, the finer points of cheese flavor are almost certainly due to further modification of the products of the primary reactions. The most clear-cut example of this is the oxidation of fatty acids to methyl ketones in blue cheeses. Catabolism of amino acids leads to the production of numerous sapid compounds, including amines, carbonyls, acids, thiols, and alcohols. Many of these compounds may interact chemically with each other and the compounds of other reactions via the Maillard and Strecker reactions. At present, relatively little is known concerning the enzymology of amino acid catabolism in most cheeses and even less is known about the chemical reactions. It is very likely that research attention will focus on these secondary and tertiary reactions in the short-term future.

In spite of extensive and intensive research over the last 40 years, the flavor of cheese remains elusive. While very considerable qualitative and quantitative information is available on the aromatic and flavorful compounds in many cheese varieties is now available, it is not yet possible to fully define cheese flavor in chemical terms. Extensive comparative studies of the volatile and nonvolatile low molecular weight compounds, both of cheeses of different quality characteristics of the same variety and between varieties should be useful. Numerous studies on cheese flavor have been published but the number and diversity of cheese, both with respect to the range of quality attributes and cheese types analyzed in such studies, have been rather limited. Although expensive, such a large-scale study appears

warranted. To date, most studies on cheese flavor have concentrated on volatile compounds. The water-soluble, nonvolatile fraction, which contains small peptides, amino acids, various low molecular weight acids, and NaCl, has a definite savory, cheesy flavor. Any further large-scale study of cheese flavor should include analysis of both the volatile and nonvolatile fractions and the data should be subjected to multivariate analyses.

It is almost certain that the flavor of cheese develops through the action of microorganisms and/or their enzymes. The starter cultures now used in the principal cheese varieties are very highly refined, perhaps only a single strain. Although these strains perform very reproducibly in terms of acid production and produce cheese free from off-flavors, many authorities feel that overrefinement has led to the production of cheese with a low flavor intensity. Apart from acid production, the mechanism of which is well understood, the precise contribution of the starter to flavor development is not yet known although many of their principal enzymes have been characterized at the biochemical and genetic level. However, the key compounds in cheese flavor, and hence the enzymes responsible for their production, are not yet known precisely. When these key reactions have been identified, the ability to modify the genetic make-up of starters will enable the engineering of strains that overproduce the key enzymes.

Phage infection is still the principal problem faced by cheese makers. Mechanisms by which lactic acid bacteria resist phage infection are known and work is in progress to engineer strains with superior phage resistance.

At present, many of the techniques used to genetically engineer starter bacteria are not food-grade but it is almost certain that food-grade techniques will be developed which will enable super tailor-made strains to be developed. Very exciting developments can be expected in this area.

Improved hygiene on the farm and at the factory, pasteurization of cheesemilk, and the use of enclosed, automated cheesemaking equipment has reduced the numbers and diversity of nonstarter bacteria in cheese. While this has led to greater uniformity of cheese quality, it has probably also reduced flavor intensity. To offset this, NSLAB are sought which will permit the production of highly flavored cheese consistently and predictability. Although NSLAB are not yet well characterized at the genetic level, it will probably soon be possible to genetically engineer NSLAB to produce cheeses with a desired type and intensity of flavor.

In this review we did not discuss the possibility of grading cheese objectively. As discussed in Section V, cheese is graded subjectively by trained graders who presumably reflect consumer preferences. Subjective grading is expensive and varies between factories and probably over time. Therefore, objective methods for quality assessment are being sought. While the maturity of cheese can be estimated fairly accurately by objective chemical and/

or physical methods, it is not yet possible to reliably assess the quality of cheese by such methods. However, as the depth of our knowledge on the biochemistry and flavor chemistry of cheese increases, it is very likely that it will become possible to objectively assess the quality of cheese.

Study of chemistry and biochemistry of cheese is at an exciting stage. It seems reasonable to predict that it will be possible in the not too distant future to describe completely the production and ripening of cheese at the molecular level.

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