N° 27/02
Chimie.

SYMPOSIUM: CHEESE RIPENING TECHNOLOGY

Texture Development During Cheese Ripening

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ABSTRACT

The texture of a cheese is determined primarily by its pH and the ratio of intact casein to moisture. The texture generally changes markedly in the first 1 to 2 wk of ripening as the hydrolysis of a small fraction of α_{s1}-casein by the rennet to the peptide α_{s1}-I results in a general weakening of the casein network. The relatively slow change in texture thereafter is determined mainly by the rate of proteolysis, which in turn is controlled largely by the proportion of residual rennet and plasmin in the cheese, salt to moisture ratio, and storage temperature. The rise in pH that occurs during ripening is also important in many cheese varieties. Cheese texture may be significantly changed by the use of coagulants other than chymosin, addition of neutral proteases, and incorporation of whey proteins. The stretching characteristics of natural cheese curd depend upon both its pH and the proportion of colloidal calcium phosphate that has been removed. Cheese containing residual coagulant loses stretchability very rapidly with age. Eye formation in Swiss-type cheese is dependent upon the cheese pH at the time of transfer to the hot room.

INTRODUCTION

The texture of cheese is important because it is this property by which the consumer first identifies and judges the specific variety. Overall appearance, the presence or absence of "eyes", and the mouthfeel of the cheese are all noted before its flavor is assessed. The textures of the various types of cheese are clearly so different that one might imagine that the subject could not be covered adequately in a single paper, particularly if texture is taken to include stretchability and eye formation. In reality, however, factors that determine changes in texture in all cheese varieties are basically the same. This is not really so surprising since the components of cheese—rennet, native milk enzymes, caseins, moisture, lactic acid, sodium chloride, fat, calcium—are the same for all cheese varieties. Only the proportions of these components differ.

EFFECT OF PROTEOLYSIS ON TEXTURE

Recent research in many countries has shown that the texture of cheese is largely dependent on its pH and the ratio of intact casein to moisture (Table 1). De Jong (7) reported good correlation between the firmness of a cheese and the quantity of intact α_{s1}-casein present. This is not surprising, because the breakdown products of the caseins are largely water soluble and cannot contribute to the protein matrix (48). Another feature of proteolysis, however, is probably significant (6). As each peptide bond is cleaved, two new ionic groups are generated and each of these will compete for the available water in the system. Thus, the water previously available for solvation of the protein chains will become tied up with the new ionic groups. Relatively low moisture cheese, such as Cheddar, tends therefore to become increasingly harder with age and more resistant to slight deformation (6, 40).

Two distinct phases in texture development take place during ripening. The first phase occurs in the first 7 to 14 d when the rubbery texture of young cheese curd is rapidly converted into a smoother, more homogeneous product. Because casein is the only continuous solid phase, this suggests that proteolysis of the network of caseins that make up the microstructure of the cheese is taking place. This casein network is greatly weakened when only a single bond in about 20% of the α_{s1}-casein is hydrolyzed by the coagulant to give the peptide

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Received July 14, 1986.
Accepted October 30, 1986.

TABLE 1. Changes in texture during ripening of cheese.

Phase 1:	Rapid change in first 7 to 14 d. Casein network greatly weakened when a single bond in about 20% of the α_{s1}-casein is hydrolyzed by the coagulant to give the peptide α_{s1}-I.
Phase 2:	Much more gradual change over following weeks determined by rate of proteolysis and rise in pH.

The texture of cheese at any specific stage of ripening is determined primarily by its pH and ratio of intact casein to moisture.

α_{s1}-I (6). This peptide is present, at least in the early stages of ripening, in all types of cheese.

The second phase involves a more gradual change in texture, as the rest of the α_{s1}-casein and the other caseins are broken down in a time scale measured in months rather than days. An examination of commercial Cheddar cheese in the United States, for instance, showed that 85% of the α_{s1}-casein was still intact in a 14-d-old cheese, and its primary hydrolysis product, α_{s1}-I, accounted for the remaining 15% (6). In 10-wk-old cheese, 90% of the α_{s1}-I had been hydrolyzed. Ninety-five percent of the β-casein, however, was still intact after 10 wk of ripening. The preferential degradation of α_{s1}-casein has been reported for a variety of other cheese types (33, 46).

Clearly, the lower the ratio of moisture to casein, the firmer will be the casein matrix of the cheese. Small changes in the moisture to casein ratio also result in relatively large changes in available moisture, since much of the moisture is bound to the caseins and their degradation products. Even small decreases in water activity greatly decrease the rate of proteolytic activity in cheese. In addition, the ratio of residual rennet to casein is less in low moisture cheese and the rate of change in texture will therefore be slower.

FACTORS AFFECTING THE RATE OF PROTEOLYSIS

Proteolytic activity in cheese is mainly determined by the levels of residual rennet and native milk proteinases present, salt to moisture ratio, temperature of ripening, type of coagulant used, and changes in pH during ripening. The redox potential and the calcium content of the cheese may also be important.

Rennet Retention in Cheese Curd

The influence of the rennet content on the rate of softening of relatively high moisture cheese was reported by de Jong (9). Rennet contents lower and higher than normal gave slower and faster rates of softening, respectively. When no active rennet was present neither α_{s1}-casein degradation nor softening occurred. Retention of rennet in Gouda cheese was related linearly to the proportion of rennet initially added to the cheese milk (44).

Most coagulant is lost in the whey at draining, but some remains in the curd, depending upon the pH and the proportion of whey retained in the curd. Holmes et al. (22) found that the distribution of chymosin between curd and whey was pH dependent, whereas that of milk-clotting enzymes from the *Mucor* spp. was not. This has been confirmed in a recent investigation (5) in which the pH of the curd was manipulated by the addition of acid to the cheese milk. The lower the pH of the curd at draining, the more rennet was retained in the curd and the greater the proportion of α_{s1}-casein hydrolyzed by the chymosin in calf rennet. However, distribution of a microbial rennet from *Mucor miehei* between curd and whey was not pH dependent. The extent to which α_{s1}-casein in the cheese was hydrolyzed was virtually the same, irrespective of the initial pH of the milk. Data covering eight separate trials are shown in Figure 1. The measurement of residual chymosin is not easy as indicated by the relatively high standard deviations.

Many of the conflicting reports on cheese texture likely arose from the lack of care to ensure that the level of residual chymosin in experimental cheese was the same as in control cheese. Use of a microbial rennet clearly has advantages over rennet in experimental studies on cheese texture, particularly as the percentage of chymosin in commercial rennet varies.

Plasmin Retention in Cheese Curd

The pH at draining also determines the proportion of plasmin in cheese. Plasmin is a native milk proteinase formerly known as

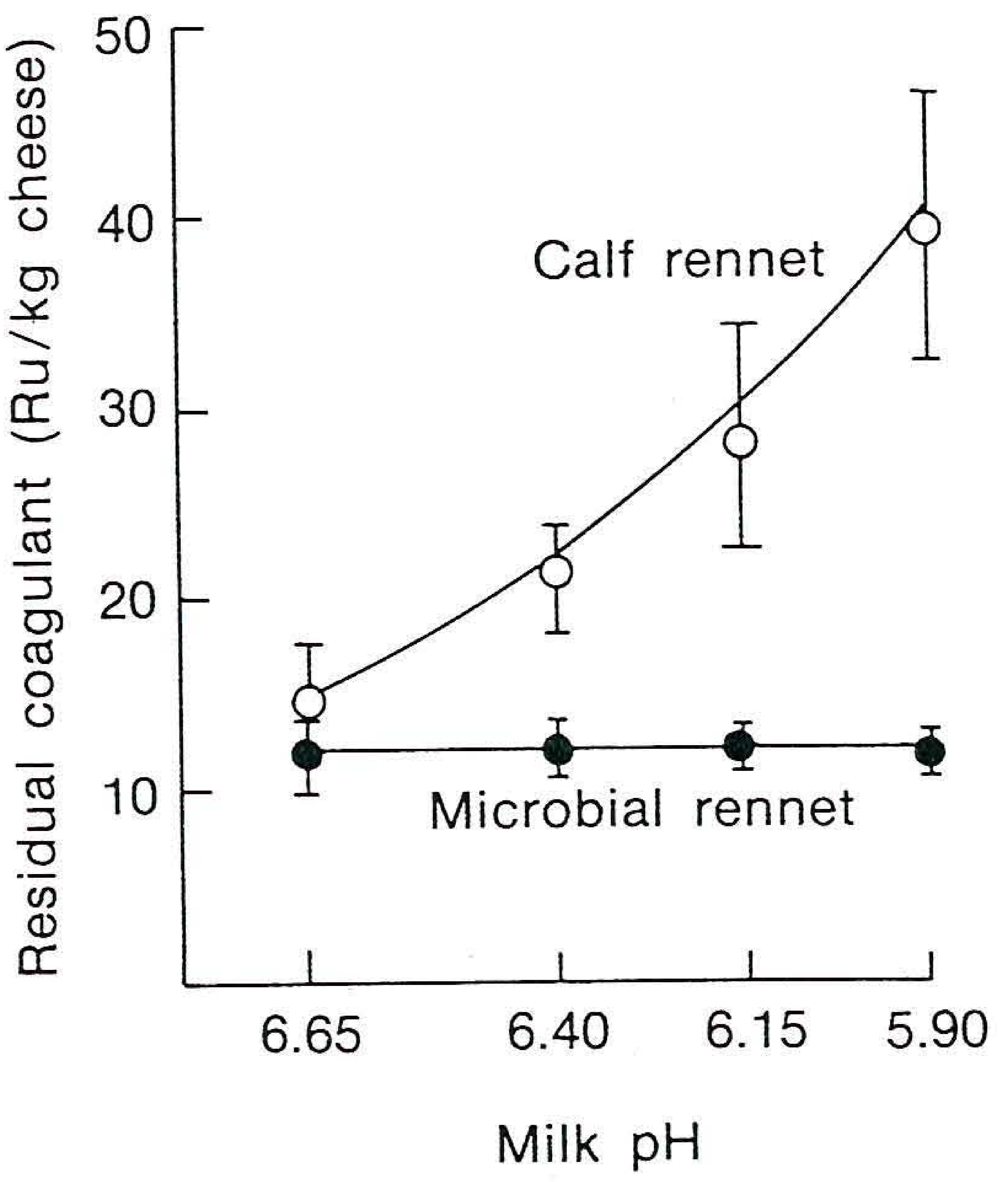


Figure 1. Variation in the coagulant concentration in cheese made with acidified milk during February to March 1983. The coagulant was either calf rennet (○) or Rennilase microbial rennet (●). Standard deviation is indicated by vertical bars (5).

alkaline milk protease. In fresh milk, plasmin is associated with the casein micelle (35), but it dissociates as pH is decreased (38). The proportion of plasmin is therefore greatest in cheese such as Swiss, in which the whey is drained from the curd at a relatively high pH, and least in acid cheese such as Cheshire. In Mozzarella and Swiss-type cheese, the high scalding temperatures used inactivate most, if not all, of the chymosin. Plasmin, however, is relatively heat resistant and casein breakdown appears to be largely due to plasmin.

Seasonal studies of the composition of milk in the Irish Republic (13) have shown that the concentration of β-casein decreases from mid-lactation to late lactation with a corresponding increase in the concentration of the three γ-caseins, suggesting that plasmin concentration increases during lactation. Recent investigations on New Zealand milk have confirmed that the concentrations of both plasmin and its precursor, plasminogen, increase two- to threefold throughout a lactational cycle (36). About eight times more plasminogen was present than plasmin. The conversion of plasminogen to plasmin is not well understood (47). Activators of plasminogen have been isolated in milk, but inhibitors of these same activators as well as inhibitors of plasmin are also present (Figure 2). The plasmin activity of Friesian milk was at least 50% higher than that of Jersey milk at a similar stage of lactation, suggesting that plasmin activity is also dependent on the breed of cow (36).

Another native proteinase in milk with an optimum pH of activity near 4.0 has been identified (32) and shown to produce α_{s1}-I under acid conditions from α_{s1}-casein and also β-I and β-II from β-casein (24). The suggestion that this acid protease might play some role in cheese protein degradation would be difficult to prove, however, since its action is masked both by that of chymosin and of plasmin.

Effect of Salt to Moisture Ratio

The rate of proteolysis during ripening is markedly affected by the salt to moisture ratio (S:M) in the cheese. This was demonstrated in Cheddar cheese in which an approximately linear S:M gradients was established across single 20-kg blocks (41). After ripening at 10°C for 28 d, only 5% of the α_{s1}-casein but 50% of the β-casein was still intact in that part of cheese with a S:M of 4.0%. At 6% S:M, 30% α_{s1}-casein and 80% β-casein remained intact. At 8% S:M, 60% of the α_{s1}-casein and 95% of the β-casein was unhydrolyzed. The relationship between the rate of degradation of both caseins and the S:M level was almost linear (Figure 3). Differences in the textures of plugs, drawn several months after manufacture, from

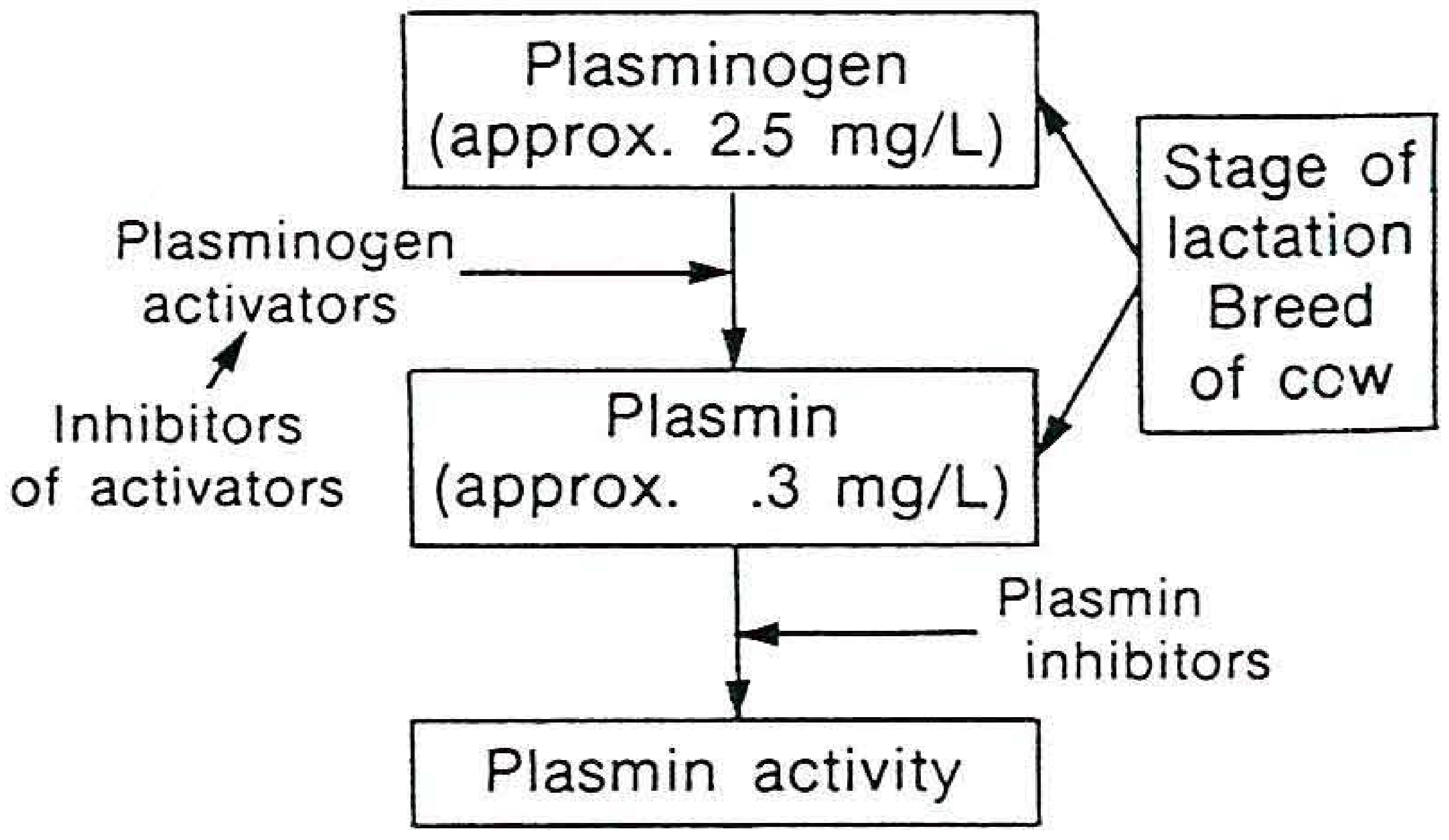


Figure 2. Factors determining the activity of plasmin in bovine milk (36).

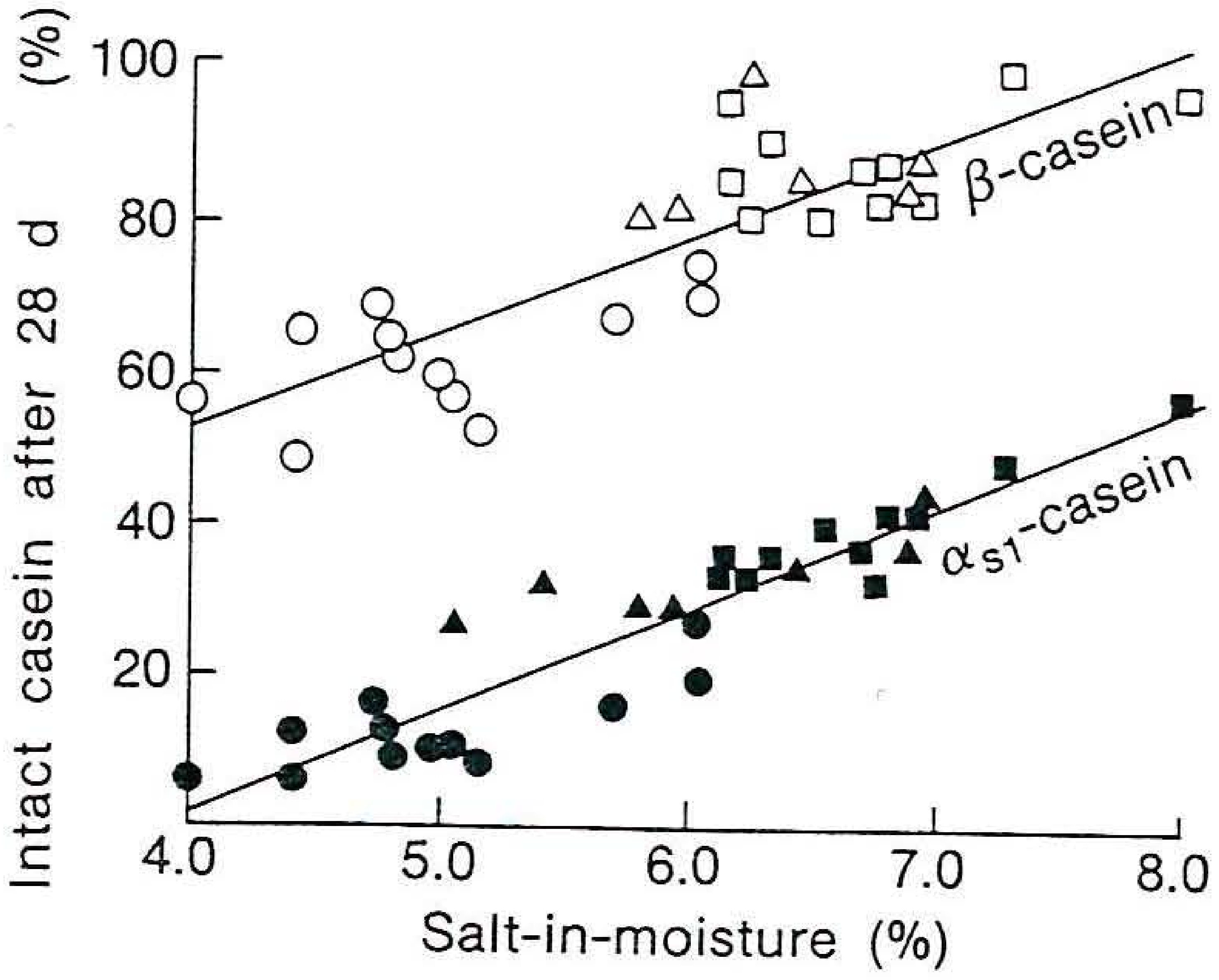


Figure 3. Effect of salt to moisture ratio on the extent of proteolysis of α_{s1}- and β-casein within three single blocks of Cheddar cheese after storage for 1 mo at 10°C. The amount of β-casein (○, △, □) and α_{s1}-casein (●, ▲, ■) remaining in the three cheeses is shown as a percentage of that originally present (41).

regions with different S:M levels in the same cheese block could be readily detected.

Effect of Temperature

Cheese shown in Figure 3 was ripened at 10°C, but the hydrolysis of β-casein in Cheddar cheese markedly decreases when the temperature of ripening is reduced to 6°C or less. The hydrolysis of α_{s1}-casein also decreases but to a much less extent. This may explain the observation that the textures of Cheddar cheese stored between 2 and 10°C are not normally strikingly different, because α_{s1}-casein is presumably a far more important structural element in the cheese framework than β-casein or the other caseins (10).

An increase in ripening temperature above 10°C has an effect on texture that is significant but not as great as the effect on flavor development. The higher the temperature, the greater is the extent of casein hydrolysis and change in texture. Cheddar cheese ripened at 15°C developed a texture in 8 wk equivalent to that developed in 16 wk at 8°C (17). The cheese became more brittle and less springy with increasing storage temperatures up to 20°C. Cheese proteolysis was significantly negatively correlated with cheese firmness and springiness, indicating softening of the cheese as the protein matrix was broken down. Similarly, the higher the storage temperature of rinded Emmental cheese, the more intense was proteolysis and the firmer and shorter was cheese body (14).

Effect of pH

Activity of rennet at different pH has been measured in cheese models (11, 32). The overall extent of proteolysis was reported to increase markedly in simulated cheese with pH greater than 5.8 (32). The relative rate of breakdown of α_{s1}-casein was greater at low pH than that of β-casein. At pH greater than 5.6, β-casein was more degraded than α_{s1}-casein, presumably as a result of increased plasmin activity. It is not easy to extrapolate from such studies to the situation in natural cheese, however, because the proteolytic activity of rennet is greatly influenced by the nature of the substrate (47).

Effect of Calcium

Differences in both the calcium dissolved in the cheese serum and the calcium bound to the protein have been considered (11, 33) to contribute to variations in the extent of proteolysis in cheese, although this has not been clearly defined. The calcium ion concentration of the cheese serum increases as the pH is decreased and also as the salt uptake increases. If the calcium in cheese is high at any specific pH, presumably calcium in the serum is also high.

It is difficult, however, to differentiate a direct effect of calcium on proteolytic activity from other factors that also influence the rate of proteolysis in cheese. Total calcium content of cheese is largely determined by the point at which the curd is drained from the whey. This key stage in the manufacture of all cheese also controls the proportion of residual chymosin and plasmin in the cheese. It is not surprising, therefore, to find a relationship between the total calcium content of the cheese and casein breakdown during ripening (27).

ROLES OF PROTEOLYTIC AGENTS IN CHEESE

The principal pathway of proteolytic degradation during cheese ripening appears to involve a relatively limited breakdown of the caseins by rennet or plasmin. The polypeptides so formed are then further degraded to small peptides and amino acids by the proteinase/peptidase systems of the starter and nonstarter bacteria present

(47). The coagulant plays the major role in the initial breakdown of α_{s1}-casein but, in the case of chymosin, other casein fractions are much more resistant to proteolysis (Table 2). Two factors are involved: a) the specificity of the enzyme and b) the accessibility of the peptide bonds to the enzyme. Chymosin cleaves predominantly leucine-X and phenylalanine-X bonds (47) but degrades α_{s1}-casein in cheese much more extensively than β-casein. This is a consequence of the cheese environment, especially the NaCl level, on the conformation of the protein. The extent of salt-induced aggregation of β-casein, and the consequent inaccessibility of the chymosin-sensitive bonds, increases as the S:M increases. Some unhydrolyzed β-casein is intact at the end of ripening in all cheese varieties except a few in which an internal mold is present.

κ-Casein and α_{s2}-casein contain cystine and exist as disulphide-bonded proteins in milk. The insensitivity of these caseins to chymosin attack in cheese may be related to the extent of crosslinking. The redox potential controls the ratio of disulphide to thiol in the proteins and thus the accessibility of susceptible bonds. In cheese containing little or no residual coagulant, plasmin appears responsible for the initial breakdown of caseins. Plasmin is a cystine-containing protein and its activity may therefore also depend upon the redox potential in the cheese. Plasmin is specific for peptide bonds at the C-terminal side of lysine or arginine residues, particularly in β-casein and α_{s2}-casein. The high susceptibility of α_{s2}-casein has been related to its relatively high content of lysine residues (47). Degradation of α_{s2}-casein in Swiss-type cheese has been shown to be directly related to plasmin content (37).

Caseins contain a greater number of proline residues than the majority of proteins (Table 2). The proteinases of *Streptococcus cremoris* are, almost without exception, specific for β-casein (18), but it has not yet been established whether this is related to the higher proportion of proline in β-casein than in the other caseins. The inability of almost all starter strains to degrade α_{s1}-casein makes it unlikely that starter proteinases contribute significantly to changes in cheese texture, at least in the early stages of ripening.

The individual proteinases also act together synergistically (45); for instance, the breakdown products of β-casein released by plasmin are further degraded by the starter proteinase/peptidase systems (46). Lactic acid bacteria contain up to three different proline-specific peptidases, of which the most important is generally prolyldipeptidyl-peptidase (2). The presence of aminopeptidase-P and proline-specific peptidases in all three species of group N streptococci appear to allow the complete hydrolysis of all polypeptides, derived from the primary degradation of the caseins, to amino acids (30).

TABLE 2. The relative roles of the different proteolytic agents in cheese.

Protein	Percent of cheese casein	Proline content[1]	Major proteolytic agents in cheese
α_{s1}-Casein	35.0	8.5	Chymosin (plasmin)
β-Casein	37.5	17.0	Plasmin,[2] starter (chymosin)
γ-Caseins	6.5	19.5	Plasmin, starter
α_{s2}-Casein[2]	13.0	5.0	Plasmin
Para-κ-casein[2]	8.0	11.0	Not attacked (?)
β-Lactoglobulin[2,3]	...	5.0	Not attacked
α-Lactalbumin[2]	...	2.0	Not attacked

[1] Proline as percentage of total amino acid residues.

[2] Cystine-containing proteins.

[3] Inhibits plasmin.

Although aminopeptidases from starter bacteria have a wide specificity, they are apparently unable to hydrolyze a peptide bond containing the imino group of proline. The liberation of a dipeptide by the dipeptidylpeptidase permits the continued action of the aminopeptidase. In any particular cheese the concentrations of the different bacterial peptidases will depend upon the strains used in the starter culture, the extent of their growth and lysis in the cheese, and the types and numbers of nonstarter bacteria present.

Effect of Coagulant Type

A strong relationship exists between the degree of proteolytic breakdown, particularly of α_{s1}-casein, and the consistency of cheese texture when calf rennet is used (7, 31, 33). This also holds for bovine pepsin, which has a specificity similar to chymosin but is less proteolytic. Trials have shown that cheese made with bovine pepsin contained less soluble nitrogen than calf rennet cheese and were correspondingly harder in texture (40). Microbial rennets attack α_{s1}-casein at rates similar to chymosin (6, 15) and their use results in cheese with textural characteristics in the early stages of ripening that are almost identical to calf rennet cheese.

There are many reports, however, that the texture of microbial rennet cheese, after long ripening periods, tends to be poorer, or at least different from calf rennet cheese (1). This has been attributed to the greater proteolytic activity of the microbial rennets, which are much less specific than chymosin toward the caseins and, unlike chymosin, readily hydrolyze α_{s2}- and β-caseins. In Cheddar cheese made with rennets from *Endothia parasitica* or *Mucor miehei*, β-casein was considerably more hydrolyzed than in calf rennet cheese; in cheese made with Endothia rennet, β-casein was even attacked in preference to α_{s1}-casein (15). The electrophoretic patterns of casein breakdown by the Mucor rennets are quite different from those obtained with chymosin (5, 15), showing that cleavage of the casein molecules is specific to the coagulant used.

The extent of proteolysis also increases when neutral microbial proteases are added to cheese to accelerate ripening (25). General proteolysis of Cheddar cheese was increased by about 20% in 2 mo with addition of a *Bacillus subtilis* proteinase. The cheese was softer bodied and more brittle than untreated cheese of the same age, apparently as a result of excessive breakdown of β-casein. This problem can be minimized by using a reduced amount of the neutral proteinase in combination with an exopeptidase preparation of *Streptococcus lactis* (26). Cheese ripening is, however, a complex, balanced process and accelerated ripening is likely to affect the traditional texture of a cheese unless the relative rates at which the caseins are broken down remain the same.

Effect of Incorporation of Whey Proteins

Cheese made from UF milk generally has a smoother consistency than that made from whole milk (12), the degree of smoothness presumably depending upon the proportion of whey proteins incorporated. Ultrafiltered cheese ripens more slowly (12, 21) than traditionally made cheese, apparently because β-lactoglobulin, the major whey protein, inhibits plasmin activity (47; C. T. Pedersen et al., 1986, unpublished data). In addition, undenatured whey proteins are resistant to the action of chymosin and other proteinases normally found in cheese (12, 23). Increased breakdown of β-casein in UF cheese, and also hydrolysis of the incorporated whey proteins, can be achieved, however, by either the addition of a neutral proteinase from *B. subtilis* (16) or the use of the proteinase from *Endothia parasitica* as coagulant.

The UF cheese are still marketed under traditional names even though the normal pattern of casein breakdown is changed and the textures and flavors therefore tend to be different. These new characteristics may well prove to be very acceptable commercially, and it may be more logical to develop a new range of cheese varieties rather than to attempt to duplicate exactly the properties of traditional cheese.

EFFECT OF pH ON CHEESE TEXTURE

The role of pH in cheese texture is particularly important because changes in pH are related directly to chemical changes in the protein network of the cheese curd. As the pH of cheese curd decreases there is a concomitant loss of colloidal calcium phosphate from the casein submicelles and, below about pH 5.5, a progressive dissociation of the submicelles into smaller casein aggregates (10, 20, 39). Nevertheless, the structural units in the protein matrix of Gouda, which has a pH of about 5.2 after brining (Figure 4), appear to be essentially in the same globular form as in the original casein submicelles in the cheese milk (10, 20). As the pH of the cheese curd approaches that of the isoelectric point of casein, the protein assumes an increasingly more compact conformation and the cheese becomes shorter in texture (6, 49). At pH below about 4.8, the aggregates appear to exist only as nonlinear strands. These are 3 to 4 nm in width, roughly the diameter of a spherical casein molecule, and up to 15 nm in length.

After about 14 d ripening, i.e., after the first phase of proteolysis, cheese can have a texture ranging from springy through to plastic to noncohesive, depending primarily on the pH of the curd and, to a lesser extent, its calcium content. This is irrespective of whether the cheese has been brine salted or dry salted. Historically, cheese that possessed a certain texture were given specific names, such as Cheddar or Gouda. Figure 4 has been constructed from the interpretation of electron micrographs of different cheese varieties (10, 20). Although this representation is undoubtedly simplistic it does help one to visualize what occurs during ripening. Thus, Gouda becomes increasingly more Cheddar-like in its texture during ripening, presumably because the submicelles are being degraded to give a range of casein aggregates that are normally associated with Cheddar. Similarly, as Cheddar ripens, proteolysis results in a texture that becomes increasingly less cohesive. Cheddar cheese also melts more readily as it ages but this is not so readily explained.

Relationship Between pH, Moisture, and Texture

An overlap between texture types occurs (Figure 4) since the effect of pH can be modified by other compositional factors, particularly the moisture, salt, and calcium contents. Between pH 5.5 and 5.1 much colloidal phosphate and considerable casein dissociate from the submicelles (39). These changes in the size and characteristics of the submicelles significantly increase their ability to absorb water. The swelling of casein submicelles in renneted milk is greatly increased in the presence of sodium chloride but is markedly inhibited if the brine solution contains ionic calcium (4, 19). It is not surprising that various types of texture can be obtained between pH 5.3 and 5.1 since a wide range of casein aggregates is present and differences in the sodium and calcium ion concentrations markedly affect the extent of swelling of the submicelles. This explains why a definition for the texture of Cheddar is particularly difficult. It may seem odd to describe the texture of Cheddar as "Cheddary" but other terms such as "intermediate" or "pseudo-plastic" are even less appropriate.

It is reasonable to speculate that the extent of swelling of the submicelles increases as the proportion of water to casein increases. The more moisture present at any pH, the softer in texture is the cheese. Hard and semi-hard cheese do not soften during ripening in the same way as the higher moisture cheese but the same changes in structure occur (10), as would be expected, since the rate of conversion of α_{s1}-casein into α_{s1}-I has been shown to be the same for a range of cheese varying in moisture from 40 to 60% (9). The pH and degree of casein degradation in Cheddar- and Gouda-type cheese are normally such that softening of the partly broken down curd would occur if the ratio of moisture to casein were high enough.

The rheological properties of cheese with a similar pH and sodium ion concentration, and at a similar degree of α_{s1}-casein degradation, are therefore regulated by their moisture contents. The firmness of Gruyère and Emmental (14, 30), Gouda (9), and Cheddar (6) is reported to be influenced markedly by relatively small variations in moisture. The role of water in the texture of very high moisture cheese, however,

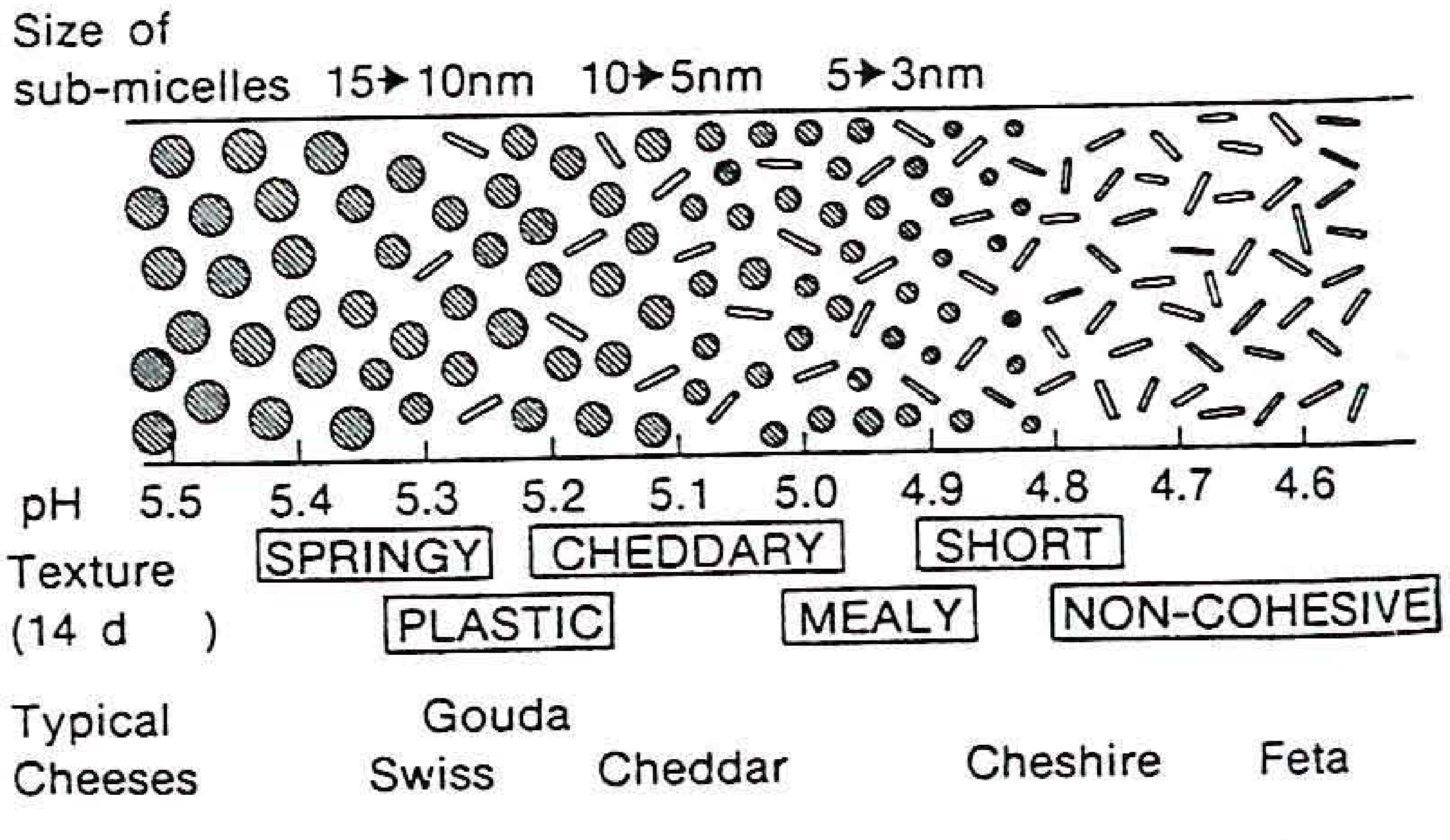


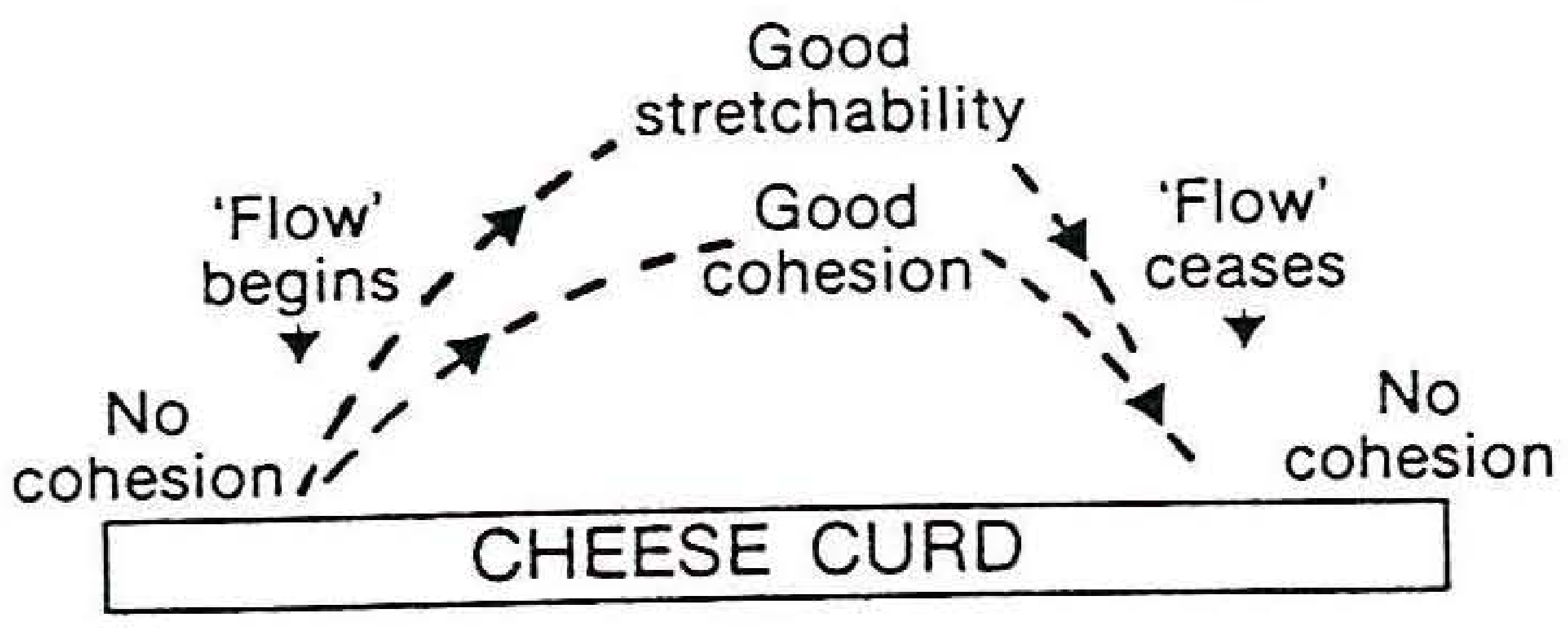
Figure 4. Effect of pH on cheese texture.

may be more complicated than simply a variation in the proportion of solid matter present since X-ray studies (48) show that the casein network is indeed modified by excess water.

Cohesion and Stretchability

No fusion of curd particles occurs until near pH 5.8 (Figure 5). Cohesion then increases to a maximum at about pH 5.2. There is a corresponding increase in the stretchability of the curd, and both effects are clearly related. The stretchability of natural cheese curd depends both on its pH and the proportion of colloidal calcium phosphate that has been removed. At about pH 5.2 conditions are usually optimum, that is, relatively small casein aggregates are present, from which about 75% of the colloidal calcium phosphate has been lost. Such aggregates bind together in long chains when heat is applied. Below about pH 4.8, when the casein aggregates have lost their identity, curd cohesion and stretchability are also lost. At pH 4.6, cheese still contains about 140 mmol calcium/kg, but it is all present in the cheese serum.

It follows that very young Cheddar curd with a pH of about 5.2 will stretch as well as Mozzrella but its stretchability is rapidly lost as the residual rennet breaks down the casein framework of the cheese. This is not surprising since the stretching characteristics of Mozzarella-type cheese appear related to the relatively high concentrations of intact casein and large peptides in the cheese (3). Even with Mozzarella, the stretching properties slowly decrease with age as the α_{s1}-casein is slowly degraded by the native milk proteinases present.



pH	6.0	5.8	5.6	5.4	5.2	5.0	4.8	4.6
Cheese calcium (mmol /kg)			240	220	200	180	160	140
Calcium in serum (percent of total)			55	65	75	85	95	100

Figure 5. The relationship between cheese pH, ionic calcium in cheese serum, and stretchability of cheese.

EFFECT OF MANUFACTURING PROCEDURES ON TEXTURE

The manufacture of all cheese varieties involves three factors that influence the subsequent change in texture during ripening: 1) the pH at which the whey is drained from the curd, since this determines the proportions of chymosin and plasmin in the cheese, 2) the S:M that controls, together with the ripening temperature, the activity of the residual rennet and plasmin in the cheese, 3) the pH of the cheese after salting, which is the single most important factor that influences texture. To illustrate these points, four different cheese types are briefly examined.

Cheddar Cheese

Because much of the acid production occurs at the vat stage for dry-salted cheese, the

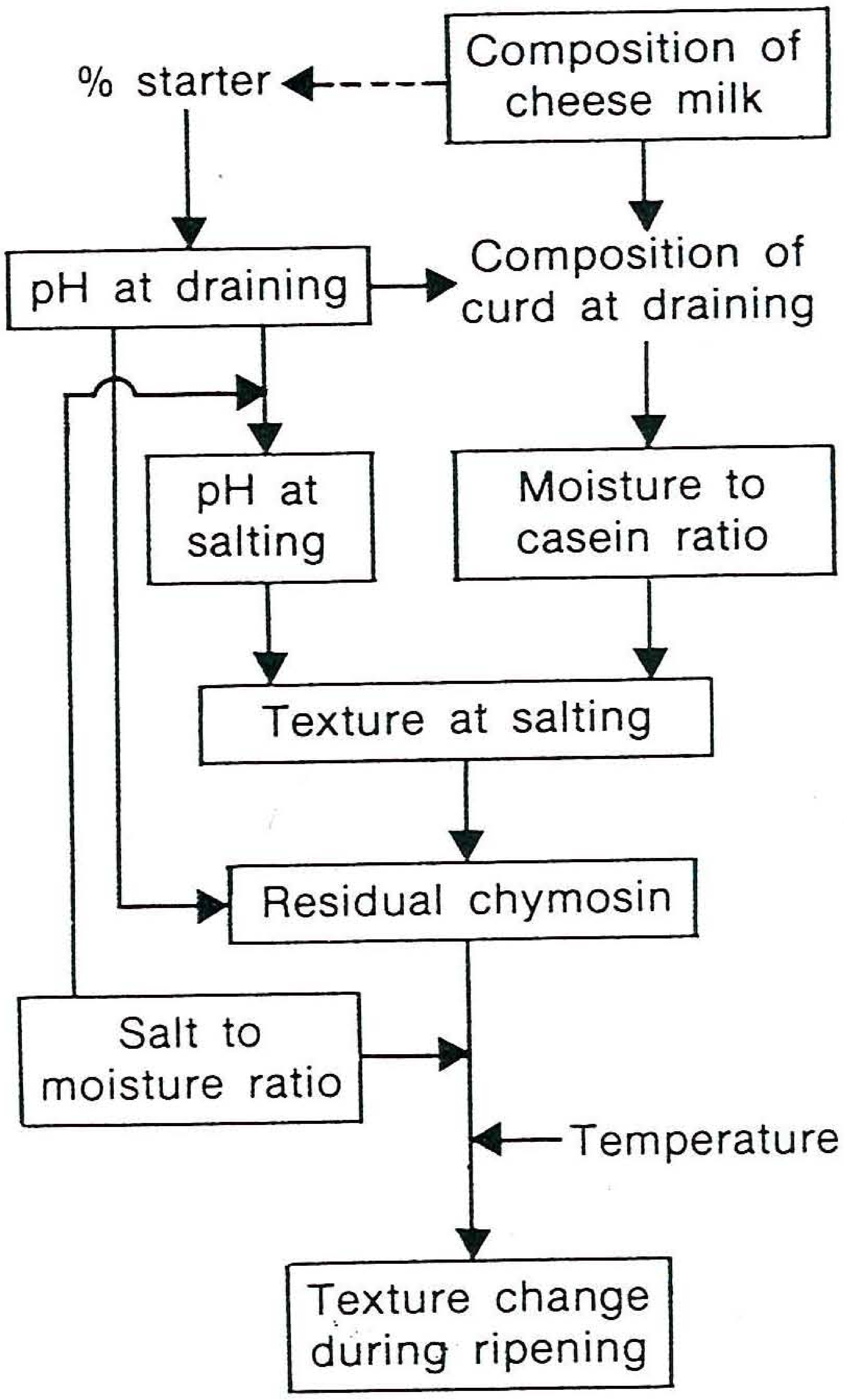


Figure 6. Main factors that determine the change in texture of Cheddar cheese during ripening.

activity of the starter up to draining is more important than for brine-salted cheese. The pH at draining is relatively low and chymosin retention therefore is high. Chymosin activity is much more important than plasmin activity during the ripening of Cheddar.

The S:M ratio in dry-salted cheese is particularly important because it controls the final pH of the cheese and the residual lactose, as well as the activity of the residual chymosin (Figure 6). The initial texture 1 d after manufacture is determined by the pH and the ratio of moisture to casein. Any required rate of texture (and flavor) development can be achieved by using appropriate combinations of S:M and ripening temperatures. In general, however, Cheddar cheese is still ripened at temperatures below 10°C to minimize the potential risk of off-flavors from the metabolism of the residual lactose.

The pH of Cheddar cheese normally decreases slightly in the first 14 d of ripening as the residual lactose is metabolized and thereafter increases only slightly, usually by about .1 pH unit after 6 mo. The texture of Cheddar cheese therefore normally changes relatively slowly between 1 and 6 mo of ripening. A significant increase in the proportion of rennet added to the cheese milk increases the "smoothness" of the cheese, particularly in the first few weeks of ripening, but may lead to bitterness and other flavor defects.

Gouda Cheese

In the manufacture of brine-salted cheese, most of the acid is developed after the whey has been drained from the curd and the pH at draining is higher than in Cheddar manufacture. One would therefore expect slightly more plasmin and less chymosin to be retained in Gouda than in Cheddar and evidence for this has been reported (3). Nevertheless, the contribution of plasmin to the degradation of α_{s1}- and β-caseins in Gouda is considered to be small in relation to those of rennet and bacteria (46).

The pH after brining is controlled independently by adjustment of the proportion of residual lactose in the curd. This is achieved by replacement of part of the whey in the vat with water. In the data shown in Figure 7, for instance, a constant proportion of whey (35%) was removed in each trial and replaced with sufficient water (15 to 25%) to ensure that the initial pH after brining was between 5.15 and 5.2.

The salt concentration in the interior of brine-salted cheese is initially much lower than near the surface but this apparently does not affect the pattern of decomposition of α_{s1}- and β-casein. Rate of casein degradation is faster in the interior in the 1st or 2nd wk, but the proteolytic products are the same (11). In a sense, however, brine-salted cheese can be said to ripen from the interior of the cheese to the outside. Rinded cheese tend to increase in firmness during ripening as the loss of moisture through the rind results in an increase in the casein to moisture ratio. This often overrides the normal decrease in firmness due to the hydrolysis of the casein matrix.

In contrast to Cheddar, the pH of Gouda rises relatively rapidly during ripening, but this is unlikely to be the direct result of proteolytic activity. It may be coincidental that the increase in pH is greatest in March, toward the end of the lactational season in New Zealand (Figure 7), when the plasmin content of the milk is at a maximum. It is appreciated that most countries do not have marked lactational changes in milk supply, but it is possible that some of the

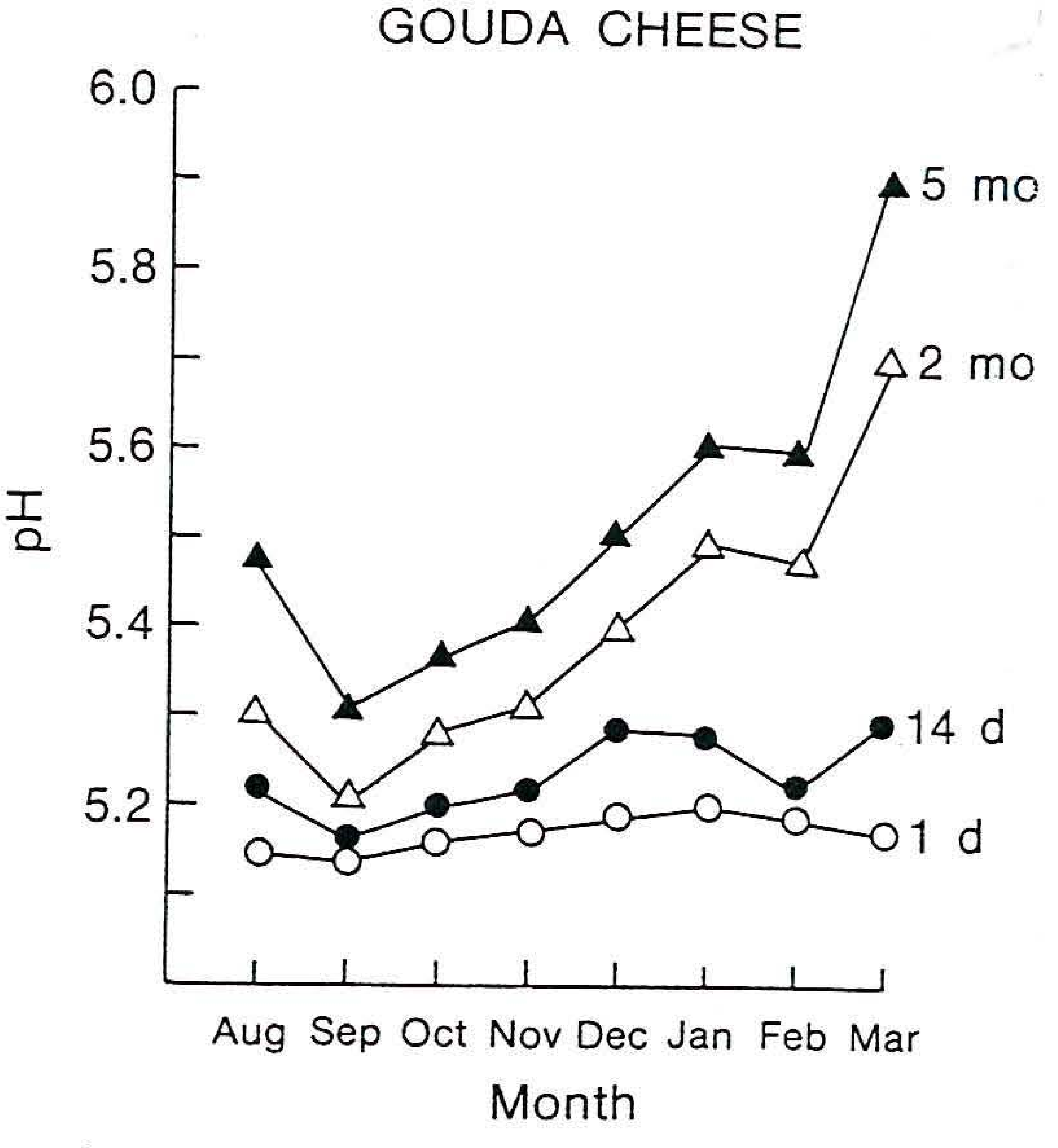


Figure 7. Increase in pH during the ripening at 10°C of Gouda cheese manufactured over a lactation. Each point represents the average pH obtained from four separate trials carried out each month.

variation experienced throughout the year in any country may be traced to the stage of lactation.

Swiss Cheese

In Swiss-type cheese the pH at draining is determined by the proportion of *Streptococcus thermophilus* added (Figure 8). The pH after brining is controlled independently by the Lactobacillus strain in the starter culture (27). The high scalding temperature used inactivates the chymosin in the curd but not the more thermostable plasmin. Little acid is produced up to the draining stage (dipping) and a relatively high proportion of the plasmin is therefore retained in the curd.

The pH of the cheese at the time of transfer to the hot room is critical for normal eye development because of its effect both on the texture of the cheese and the growth of propionibacteria (27, 29). As with Gouda, the pH normally increases slightly in the first 14 d after brining (43). Casein breakdown by plasmin, in conjunction with starter proteinases, may possibly be important at this stage, since eye formation is poor in the early part of the lactational season when the plasmin concentration in the milk is lower than usual. Eye formation is also more likely to occur in relatively high moisture cheese, possibly because the more rapid salt diffusion results in greater plasmin activity (11) or the development of a more suitable texture. The relatively low activity of plasmin toward α_{s1}-casein (46) presumably accounts in part for typically firm texture of Swiss cheese.

Camembert Cheese

The importance of pH changes during ripening on the texture of cheese is most readily demonstrated by surface mold-ripened cheese such as Camembert and Brie. Initially, the pH of the cheese is 4.7 or less and the texture is correspondingly crumbly throughout. Rennet degrades α_{s1}-casein as rapidly at pH 4.7 as at pH 5.2, but the cheese does not soften at the lower pH (7, 31, 33). The casein is degraded in such a way, however, at pH 4.7 that softening occurs as soon as the pH rises to about 5.2. The required increase in pH is brought about by the growth and subsequent deacidifying activity of the surface mold. The metabolism of lactic acid by the mold at the cheese surface and, to a lesser extent, the production of ammonia lead to the diffusion of lactic acid from the interior of the cheese to the surface (34). At 17 d, the pH just below the crust was about 6.0, whereas the pH of the cheese center was still only 4.8. After about 25 d, when the pH in the center had risen to about 5.2, the texture throughout the whole cheese was soft (28). Plasmin activity increases as the pH rises, particularly near to the cheese surface (32, 42), since plasmin is much less affected than chymosin by high salt concentrations (33, 46).

The role of the mold is essentially to establish a pH gradient but, in addition, a mineral gradient is also set up. The calcium and phosphorus content of Camembert is initially uniform throughout the cheese, but as cheese ripens, a large part of the minerals migrates rapidly to the surface (28). A crust of mold and calcium phosphate forms on the surface since calcium phosphate is less soluble as pH increases.

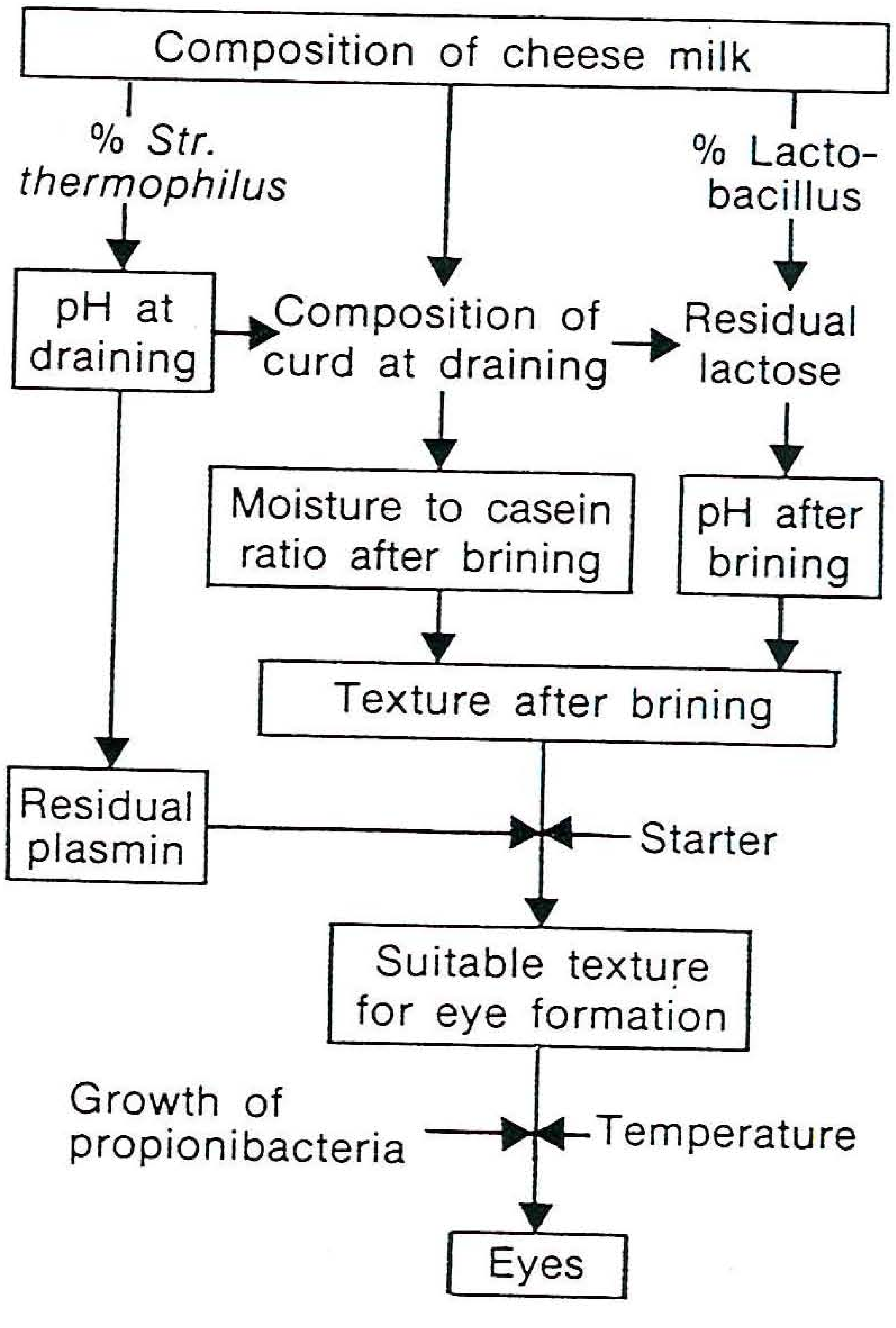


Figure 8. Main factors that determine the formation of "eyes" in Swiss-type cheese.

About 75% of the calcium and 33% of the phosphate migrated from the center of Camembert cheese to the surface in 17 d. It is not yet certain whether this migration of calcium and phosphate to the crust also plays a role in the softening of the texture. The swelling of cheese curd in brine is reduced, however, when calcium is present (19). It seems likely therefore that the migration of calcium to the crust further accelerates the softening of the cheese interior since swelling of the casein micelles will be increased by a reduction of calcium in the cheese serum.

In blue-veined cheeses, both the deacidifying and proteolytic activity of the internal flora of molds is likely to be responsible for the increase in pH and consequent change in texture. With smear-ripened cheese, such as Limburger or Gruyère, the proteolytic activity of the surface flora sets up a shallow pH gradient between the surface of the cheese and its center, and this may contribute to the characteristic texture of such cheese. The smell of ammonia coming off smear-ripened cheese in commercial ripening rooms is a certain indication that surface proteolysis is taking place.

Stabilized Cheeses

The procedures used in the manufacture of so-called "stabilized" cheese (Pâte stabilisée), which are now produced commercially in France, illustrate well the importance of pH and proteolysis in texture development. The shelf-life of cheeses with a relatively high moisture content, such as Camembert and Saint Paulin, can be considerably increased, simply by reducing the amount of residual rennet in the cheese (Figure 9). A low level of rennet is added to the cheese milk and the setting temperature raised appropriately to get a firm coagulum.

A uniformly smooth texture is then assured by so reducing the rate of acid development that the pH does not fall below 5.2. This can be achieved by addition of only a very small inoculum of starter, by the use of proteinase negative strains, or by use of thermophilic starters at a temperature that is well below that of their optimum growth. The activity of such slow acid-producing starters is regulated by their salt resistance, the speed of penetration of salt into the cheese, which will be influenced by the moisture content of the cheese, the final S:M, and storage temperature. If conditions are such that acidification is allowed to continue, for instance, if the S:M in the cheese is too low, then pH will continue to drop and texture will become firmer (31). Further control over pH can be achieved, if required, by washing the curd.

Because the consistency of cheese of a given pH and moisture content is determined by the rate of hydrolysis of α_{s1}-casein (8), the degree of softness required in a stabilized cheese in a specified time can be readily achieved by controlling the residual rennet level. The surface may be treated with a white mold if a stabilized Camembert or Brie is required, but the role of the mold is now largely cosmetic. It no longer has to establish the pH gradient that is so essential in the manufacture of traditional Camembert and Brie. The proteolytic activity of the mold is also less at pH 5.2 than 4.6. In the case of stabilized reproductions of cheese,

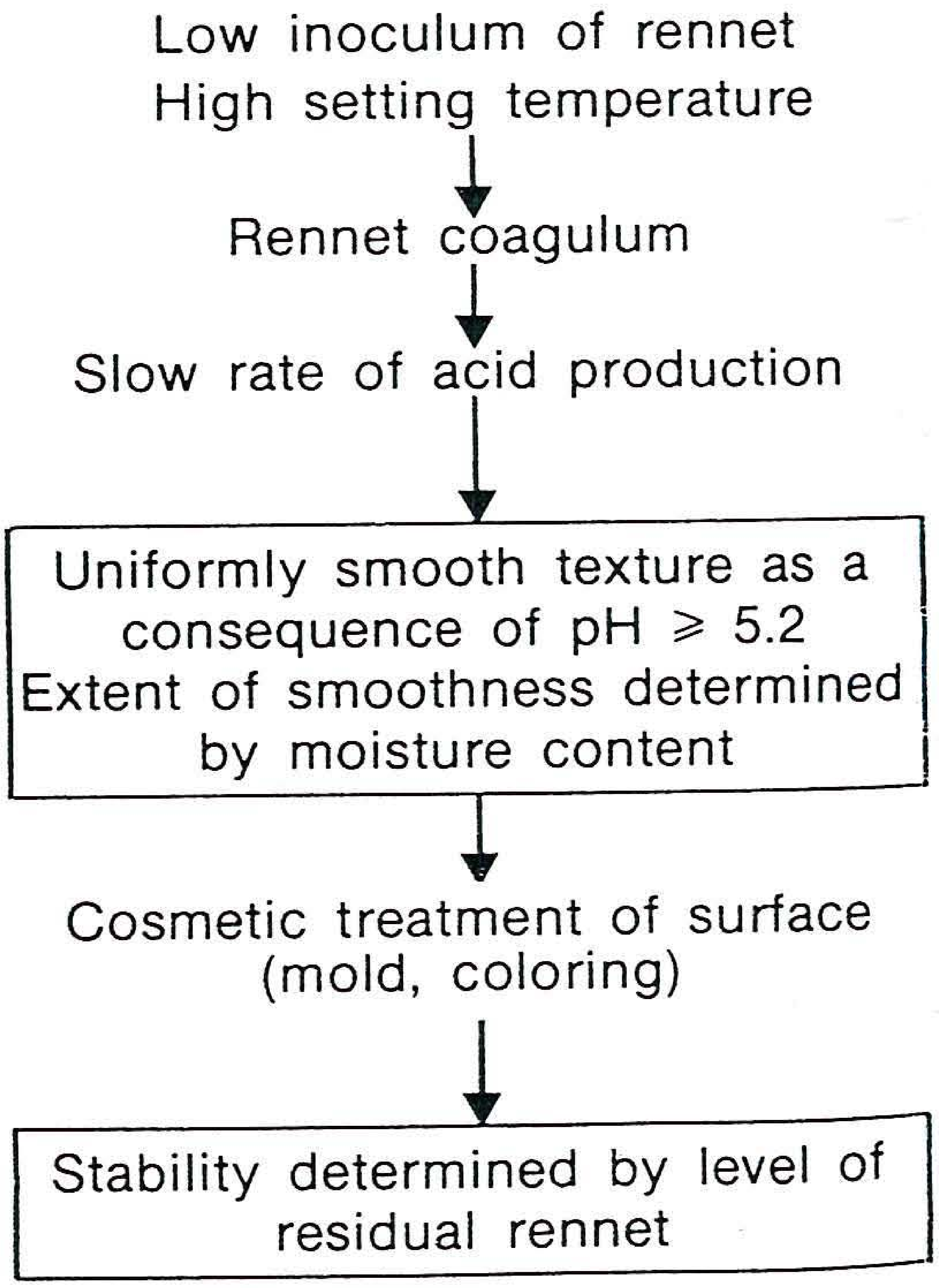


Figure 9. Factors that determine the stability and texture of stabilized cheese.

such as Brick or Saint Paulin, the surface is often just painted with a food dye. The use of a biological smear affects the flavor but not the texture of a stabilized cheese.

ACKNOWLEDGMENT

Grateful acknowledgment is made to K. N. Pearce, F. M. Martley, and B. C. Richardson for helpful discussions concerning this paper.

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Enzyme-Modified Cheese Technology

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ABSTRACT

Enzyme-modified cheese is derived from cheese by enzymatic means. Enzymes may be added during the manufacture of cheese or after aging. An incubation period under controlled conditions is required for proper flavor development. The mechanism of flavor development in enzyme-modified cheese may be related to the curing of cheese. Although many of the mechanisms for flavor development in cheese are not well understood, carbohydrates, proteins, and fat undergo enzymatic degradation during cheese aging, and these reactions are important in the development of flavor in cheese and enzyme-modified cheese. In some instances, the flavor profile or intensity is proportional to the degree of lipolysis and release of low molecular weight free fatty acids as with Romano or Provolone cheese. In other cases, a similar free fatty acid profile enhances both Cheddar flavor and Swiss cheese flavor but is not characteristic for either.

Enzyme-modified cheeses are generally added to foods at levels of .1 to 2.0%, although they can be used at 5% of the formulation to add dairy or cheesy notes to foods and to reduce the requirement for aged cheese in food formulations.

INTRODUCTION

Cheese that has been treated enzymatically to enhance the flavor or a significant portion of the flavor profile can be called enzyme-modified cheese. Enzyme may be added during the manufacture of the cheese or after the cheese curd has been pressed or even after a period of aging. Enzyme-modified cheese may have a texture similar to or slightly modified from the texture of the variety of cheese it represents or it may be in a paste form.

Flavor development in cheese is a subject of intense study. The complex enzymatic and nonenzymatic reactions that occur during curing are not well understood. Several hundred compounds have been identified as important components of cheese flavor yet few of them characterize a particular cheese flavor.

Enzymes may be added at various stages during manufacture of cheese or to melted cheese or cheese curds. The nature of the product is affected by the choice of enzymes, conditions of incubation, and the stage at which the enzyme is added.

A standard of identity cheese having a typical flavor profile can be produced by the addition of enzymes to milk or fresh curd. The production of this cheese can be accelerated by subjecting it to a controlled aging process. Alternatively, the same process may be used to produce a highly flavored cheese by alteration of the conditions of manufacture. Generally, this cheese also has a modified texture and body. Several months of aging are usually required for flavor development in each case.

High flavor intensity, enzyme-modified cheese can also be produced in a matter of days by careful addition of enzymes to the curd or by addition to melted cheese followed by a controlled incubation process.

A wide variety of flavors can be produced using enzyme technology and, in fact, a number of different enzyme-modified cheese flavors are commercially available including mild, medium, and sharp Cheddar, as well as Colby, Swiss, Provolone, Romano, Mozzarella, Parmesan, and Brick. Flavor profiles can be tailored to fit an individual customer's needs.

Flavor Compounds of Cheese

The chemistry of cheese flavor is very complex. Over 180 compounds have been identified as components of Cheddar cheese

Received August 11, 1986.
Accepted December 4, 1986.

1987 J Dairy Sci 70:1761–1769