# A Controlled Approach to Cheese Technology[1]

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## ABSTRACT

The single most important factor in the control of cheese quality is the acid production in the vat, because this largely determines the characteristic basic structure of the cheese and, for most cheese varieties, pH. Any specific cheese variety can be classified by its normal range of calcium content and pH. The pH at draining also determines proportions of residual chymosin (calf rennet) and plasmin in the cheese. Activities of these two enzymes play a major part in degradation of cheese caseins during ripening and in consequent development of characteristic cheese flavor. Rate of proteolysis also is influenced by the ratio of moisture to casein and the ratio of salt to moisture. Uniform cheese quality can be achieved routinely only by cheese made within specified ranges of chemical composition. These should include specification for the normal range of calcium content to be expected in a traditional variety, because this, together with pH, will be an indication that acid production up to the draining stage was normal. The use of calcium data in cheese specifications should improve the prediction rate when young cheese is graded to assess its probable quality at maturity. Acid production can be under complete control only if defined starter systems are used. The development in New Zealand of the "multiple strain" and "single pair" concepts is described.

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## INTRODUCTION

Investigations in New Zealand on cheese starters over the past 15 yr have created much more interest than our research on cheese itself. This paper is an attempt to redress the balance a little by discussing principles that have been developed for manufacture of cheese in New Zealand.

Cheese making is a relatively simple matter: the removal of moisture from a rennet coagulum. The three major factors involved are extent of acid production, proportion of fat in the curd, and scalding temperature rate (Figure 1). To achieve uniform cheese quality in large commercial plants, manufacturing procedures must be as consistent as possible. The first requirement is uniformity of raw milk. This is achieved by bulking milk in a silo to even out differences in milk composition from the various districts supplying milk to the cheese plant. For preference, milk should be bulked the day before use and kept under cold storage so that its milk fat content can be standardized accurately. For any specific cheese variety the milk should be standardized to a desired ratio of casein to fat. The more fat in the milk for cheese making, and, therefore, in the rennet coagulum, the more difficult it is to remove moisture for the same manufacturing conditions, because fat interferes mechanically with the process of syneresis (30). Capacity of cheese vats should be related to the throughput of the rest of the processing plant so that a vat can be discharged in about 20 min to minimize variations from vat to vat. The proportion of rennet added should be the minimum necessary to give a firm coagulum in 30 to 40 min. To achieve a similar firmness of coagulum throughout the season may involve addition of calcium chloride and increase of temperature of the milk at which rennet is added. Scalding temperature also should be kept constant (for instance, at 38°C for Cheddar cheese) throughout the entire cheese making season.

Given that these manufacturing variables are standardized, the most important factor by far

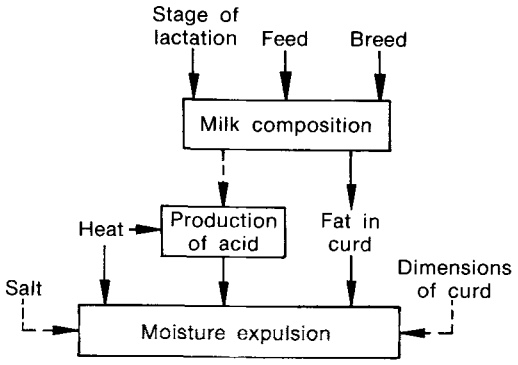


Figure 1. Main factors in expulsion of moisture from a rennet coagulum.

in producing cheese of uniform quality is the extent of acid production in the vats. To compensate for seasonal changes in milk composition it is normally only necessary to vary the percentage inoculum of starter added to achieve the required acidity at draining. This simple picture (Figure 1) applies to all cheese varieties. Strangely, however, text books on cheese, even the best of them, tend to divide cheese varieties up into tight groups, often using superficial characteristics such as proportion of moisture in the cheese or whether the cheese is surface mould-ripened, and so forth. It is, however, more rewarding to concentrate on similarities between cheese types, the basic principles that are common to all cheese varieties.

## CLASSIFICATION OF CHEESE TYPES

Over the past 20 yr our group has analyzed in detail 25 varieties of cheese. Large numbers of cheeses from both the experimental unit at the Institute and also from commercial cheese plants have been examined. However, it was the famous British astronomer, Sir Thomas Eddington, who said that one should not put too much confidence in experimental data until they have been confirmed by theory. In this paper, therefore, a general hypothesis that accounts for characteristic textures and flavors of all cheese varieties will be described.

The extensive data that have been collected suggest that cheese types are best classified by their calcium content and pH. This classification is an extension of the suggestion by Schulz (31) that cheese can be differentiated by the calcium content of their nonfat (NaCl-free) cheese solids. The four types of cheese in Figure 2 can be taken as representative of most of the common cheese varieties. Cheshire cheese, for instance, is representative, at least to some extent, of those cheese types for which the first stage of manufacture is production of a curd with low pH. Historically, cheeses of different macroscopic appearance that have a mineral content and pH within certain ranges have been identified by specific names – Cheddar, Gouda, and so forth.

Swiss, Gouda, and Cheshire traditionally have fairly narrow ranges of calcium and pH, but the range for Cheddar is relatively wide. It is probably for this reason that Cheddar cheese is a popular cheese to manufacture because pH and calcium can vary considerably but still be within a texture range that the consumer is willing to accept as characteristic of Cheddar. Gouda falls between Swiss and Cheddar in texture characteristics, and traditionally one expects to see small eyes in Gouda. In contrast, eyes are never found in Cheddar, no matter how much gas develops.

## FACTORS AFFECTING BASIC STRUCTURE OF A CHEESE

Differences between various cheese types – Cheddar, Gouda, Cheshire, and so forth – are to a considerable extent differences in basic structure. The basic structure of a cheese is determined essentially at the point at which

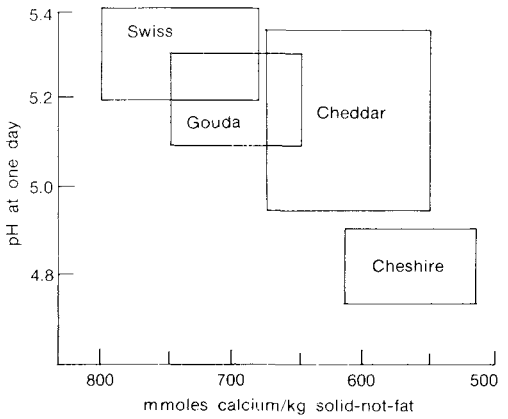


Figure 2. Classification of traditionally manufactured cheese varieties by their characteristic ranges of ratio of calcium/solids-not-fat and pH (18).

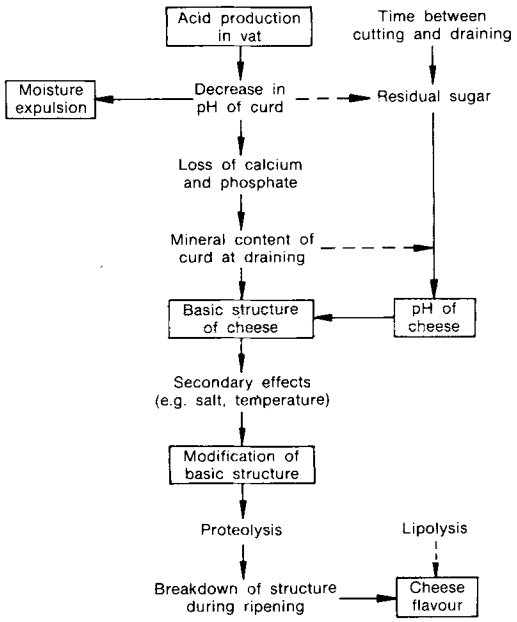


Figure 3. Relationship between extent of acid production up to the draining stage, basic structure of the cheese, and production of flavor.

curds and whey are separated because this largely determines the mineral content of the curd, and from the residual sugar content, the lowest pH that the cheese can attain (17). Each cheese variety thus has its own characteristic range of mineral content and pH, as a consequence of differences in acid production in the vat up to the draining stage. The mineral content of a cheese is determined by the quantity of calcium phosphate lost from the curd, which in turn is mainly dependent upon the acidity developed before the whey is drained off. This loss of calcium phosphate determines the extent to which the casein submicelles that were originally in the milk will be disrupted and thus determines in large part the basic structure of the cheese (Figure 3). There is, therefore, a continuous spectrum of basic structures in the different varieties of cheese. At one end of the spectrum are the cheese with a relatively high mineral content such as Swiss. At the other end are the acid cheese, such as Fetta, Cheshire, and mold-ripened cheeses, with a relatively low mineral content, in which the casein submicellar units have been disrupted.

Scanning electron microscopy of the four cheese types clearly differentiates changes of the protein matrix (6). The structural unit in the protein matrix of Swiss or Gouda is essentially in the same globular form (10 to 15 nm in diameter) as in the original cheese milk (Table 1). In contrast, the protein aggregates in Cheshire are much smaller (3 to 4 nm) and are apparently in the form of strands or chains; that is, the original submicellar protein aggregates appear to have lost almost all their identi-

TABLE 1. Relationship between extent of acid production at the draining stage and size of the protein submicelles in the subsequent cheese. The relative importance of the type of milk (breed and stage of lactation) and the relative activity of the residual calf rennet and plasmin are shown together with the predominant type of nonstarter bacteria likely to be present. Scale: 5+ = high; + = low.

| | Cheshire | Cheddar | Gouda | Swiss |
|---|---|---|---|---|
| Acid production | 5+ | 3+ | 2+ | + |
| Size of protein aggregates (nm) | 3–4 | 4–10 | 10–15 | 10–15 |
| Importance of milk type | + | 2+ | 3+ | 5+ |
| Relative activity[a] during ripening | | | | |
| Calf rennet | 5+ | 3+ | 2+ | + |
| Plasmin | + | 2+ | 3+ | 5+ |
| Nonstarter[b] (predominant) | Ped | Lb<br>Ped | Lb | Prop<br>Lb |

[a]Activity as shown by gel electrophoresis.

[b]Ped = pediococci; Lb = lactobacilli; Prop = propionibacteria.

ty. Cheddar is intermediate between Gouda and Cheshire; i.e., much of the protein in Cheddar is in the form of smaller globules than in Gouda.

Mineral losses after the draining stage are small under normal circumstances, despite the further decrease of pH. However, the pH of the cheese affects its basic structure to greater or less extent (Figure 3). In high acid cheese varieties, conformation of caseins changes markedly as pH approaches their isoelectric point with consequent obvious changes of the texture of the cheese. Similarly, in Camembert-type cheese, ammonia produced by the surface mold rapidly raises the pH, resulting in a soft, smooth texture, despite the relatively low proteolysis (A. Noomen, personal communication).

A number of other factors, such as addition of salt, the moisture and fat in the cheese, and purely physical effects such as heating or cheddaring the curd, modify the basic structure but are, nevertheless, unlikely to change significantly, the spatial arrangement of casein components. This matter has been discussed in detail in (18).

It would be best to measure the amount of calcium or phosphate directly associated with the casein, but this is not possible in practice, and the ratio of calcium to solids-not-fat must be used. The major part (about 85%) of the solids-not-fat in cheese consists of casein and a significant part of the minerals, which constitute about another 10%, is also associated with the casein. There is, therefore, a relationship between casein in cheese and solids-not-fat. A further advantage is that solids-not-fat is easily derived from commercial data, because fat and moisture are routinely measured in commercial cheese (16). In general, there is a close correlation between ratios of calcium and phosphate retained in the different types of cheese. One, therefore, usually needs to determine only the calcium to obtain an assessment of the overall mineral content of a cheese.

#### Effect of Milk Composition

The mineral content of a cheese serves as an indicator of the extent to which the structure of the casein submicelles has been disrupted. It follows, therefore, that the less acid that is produced up to the draining stage, the greater will be the influence of the type of milk. Thus, milk composition has progressively more influence on the basic texture of the cheese variety going from Cheshire through to Gouda and Swiss (Table 1). One can make a very acid cheese, such as Cheshire for instance, from milk of any breed of cow and at any stage of lactation. In Swiss cheese manufacture, however, the proportion of starter added is relatively small and the time in the vats before dipping, i.e., before separating the curd from the whey, is also relatively short. As a consequence, mineral losses from the curd are small and the identity of the casein submicelles in the Swiss cheese is almost unchanged from that of the original milk. One can predict, therefore, that composition of milk will be of great importance in Swiss cheese making, and, indeed, this is the case (Table 1). In New Zealand, for instance, it is possible to make good quality Swiss cheese from Friesian milk at any stage of lactation. Swiss cheese can be made satisfactorily from Jersey milk during the second half of the lactation but not in the first 3 to 4 mo. This would only be apparent in a country such as New Zealand where the majority of the cows are Jersey types, calving in the spring and drying off in the autumn, and are pasture-fed year round. As a result, composition of milk varies markedly from beginning to end of the season.

#### Manufacture of Cheese from Ultrafiltered Milk

If the concept of a basic structure in Figure 3 is correct, theoretically it ought to be possible to replace, to a large extent, the traditional acidification stage in the vats by suitable ultrafiltration (UF) procedures. It is easy to appreciate, however, why in practice it has proved to be so difficult to make traditional hard and semihard cheese varieties from UF milk. In normal Cheddar cheese manufacture, for instance, the ratio of mineral to casein (basic structure) is dependent only upon the extent of acid production in the vats. For UF milk, however, there are four distinct stages in the process (preacidification of milk, ultrafiltration, diafiltration, and acid production in the coagulum) at which mineral loss can occur (18). The ratio of minerals to casein, therefore, may need to be adjusted appropriately at each of these four stages if a basic structure equivalent

to that produced traditionally is to be achieved.

It long has been recognized that a Cheddar or Gouda cheese with poor texture almost invariably will result in a poor-flavored cheese. Unless a basic structure characteristic of the traditional variety is produced, cheese made from UF milk is likely to be atypical in texture and to develop an atypical flavor.

## CONTROL OF pH DURING CHEESE MANUFACTURE

The four cheese types in Figure 2 each exemplify one of the four main methods of achieving a required pH in cheese.

### Cheshire Cheese

A relatively high starter inoculum and a low scalding temperature (about 32°C) ensure that the extent of acid production is high, much calcium is lost, and a characteristic crumbly, open texture is formed. The clean acid flavor initially produced usually swamps most off-flavors that may be formed during ripening. Very acid cheeses such as Cheshire, Fetta, and the mold-ripened types are, therefore, technically relatively easy to manufacture.

### Gouda Cheese

Brine-salted cheeses such as the Gouda types also present few problems, because pH can be controlled by the proportion of lactose that is left in the curd after whey has been removed and water added (Figure 4). There is, of course, no salt in the curd to inhibit the starter. Because the lactose content of the cheese-milk varies during lactation, it is normal commercial practice to drain off the same proportion of whey throughout the season but to vary the ratio of water used to wash the curd. This ensures that the lactose content of the cheese curd before brining is no greater than that required to achieve the desired pH. It is possible, therefore, to manufacture Gouda to relatively precise specifications of calcium and pH. Residual lactose can have a marked effect upon quality of brine-salted cheeses. If too much lactose is present, the starter will grow to high numbers, and bitterness may result.

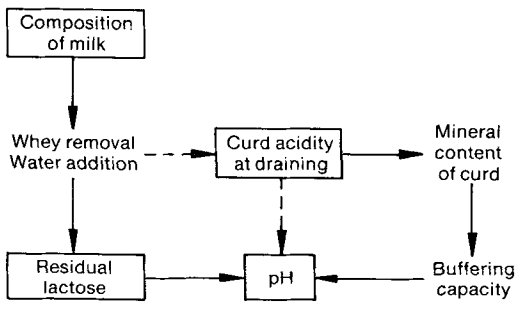


Figure 4. Main factors that determine pH of Gouda cheese.

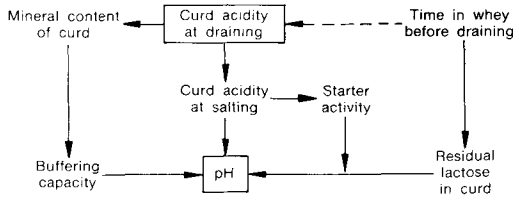


Figure 5. Main factors that determine pH of Cheddar cheese.

### Cheddar Cheese

Dry-salted cheese varieties such as Cheddar are in some ways relatively difficult to produce. They rely mainly on starter activity to remove lactose but do so at a temperature that may affect adversely the starter bacteria. The cheese maker, therefore, must exert judgement in balancing these two opposing effects. Acidity developed at draining controls both mineral content of the curd and, to a large extent, acidity of the curd at which the salt is added (17). These are the two most important factors in determining the pH at 1 day of dry-salted cheeses (Figure 5). The potential for further decrease of pH after salting depends upon residual lactose. In practice, however, the ratio of salt to moisture (S/M) in dry-salted cheeses determines the actual pH reached by controlling the extent of starter activity after salting. Thus, a S/M of 6% will inhibit activity of all *Streptococcus cremoris* strains, the starter organisms of choice for Cheddar manufacture (20). The proportion of residual lactose that remains unmetabolized in such cheese will be high. In a cheese with a S/M of 4.5%, however, the starter will not be inhibited completely and the lactose will be metabolized rapidly (34).

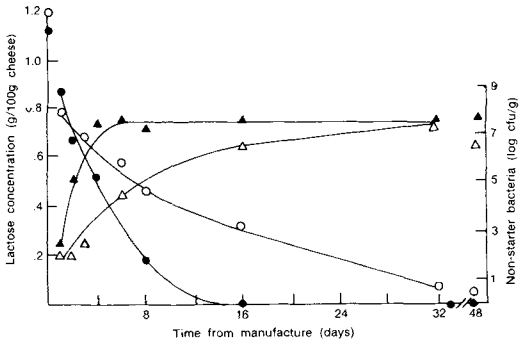


Figure 6. Relationship between ripening temperature (closed symbols 22°C; open symbols 12°C), metabolism of lactose (●, ○), and growth of nonstarter bacteria (▲, △) in Cheddar cheese (34).

This explains why the pH of 1-day Cheddar cheese may range from 5.3 (which is about the pH of the curd at salting) down to pH 4.9.

If the ratio of S/M is low (<4.5), starter numbers will reach a high level in the cheese, and the chance of off-flavors due to starter is increased greatly (25). For this reason cheese makers normally aim for a ratio of S/M in Cheddar cheese between 4.5% and 6% (16, 17). Within this range of S/M the rate of metabolism of lactose is controlled by a second factor, the temperature of cheese during the first few days of ripening, because this controls rate of growth of nonstarter bacteria such as lactobacilli and pediococci (4). For instance, when a cheese with a ratio of S/M of 5% was kept initially at 22°C for 7 days (Figure 6), the lactose disappeared rapidly and could not be detected after 16 days (34). The nonstarter count reached $5 \times 10$ cfu/g in about 4 days. In contrast, an identical cheese with the same S/M of 5% made from the same vat of milk, which was cooled immediately to 12°C after pressing, still had about .15% lactose remaining after 32 days. It took about 32 days to reach a nonstarter count of $5 \times 10^7$ cfu/g. The decrease of lactose correlated with the increase of numbers of nonstarter organisms (Figure 6). An initial temperature of 22°C is not unusual for cheese that is placed immediately after pressing in bulk bins, a common commercial practice these days.

Although nonstarter bacteria do grow on sources other than lactose in cheese, the presence of lactose encourages their rapid growth. This tends to result in a more heterolactic metabolism of lactose, usually with the production of off-flavors. The initial numbers of nonstarter bacteria in the salted curd can be controlled by attention to hygiene during manufacture. Thereafter their rate of growth, particularly in the first few days of ripening, should be kept to a minimum, and this is controlled largely by the temperature of the cheese.

An alternative to determining the actual numbers of nonstarter bacteria in cheese is to measure a compound such as acetate, because this is an index of the heterofermentative growth that may have taken place (T. F. Fryer, personal communication). When such growth is extensive, cheese quality is often less than satisfactory because of fermented and sour off-flavors, and one of the compounds responsible is undoubtedly acetic acid. More recently it has been shown that the L-lactate originally in the cheese is racemized slowly to D-lactate (32). Racemization, which normally takes about 90 days, represents a significant change during Cheddar cheese ripening, because about half of the lactate (.7% of the cheese mass) is transformed. All pediococci that have been isolated from Cheddar cheese have had racemizing activity, and this activity was up to seven times as great as in the small proportion of *Lactobacillus* isolates that could turn L-lactate to D-lactate (32). The salt of DL-lactate is less soluble than the calcium salt of L(+) lactate. Racemization, therefore, will encourage formation of undesirable white deposits in cheese.

### Swiss-Type Cheese

In Swiss-type cheeses a secondary fermentation by propionibacteria is responsible for the characteristic appearance and flavor. Manufacture of these cheeses consists of two distinct stages. The first is to develop a curd, the texture of which has sufficient elasticity to accomodate the gas that is formed (Figure 7). The second stage is production of a curd with a chemical composition that will allow propionibacteria to grow preferentially over any other bacteria that may be present. Failure to achieve either of these conditions is only too obvious, because the traditional large eyes will not develop. The cheese will either be "blind" or fractured.

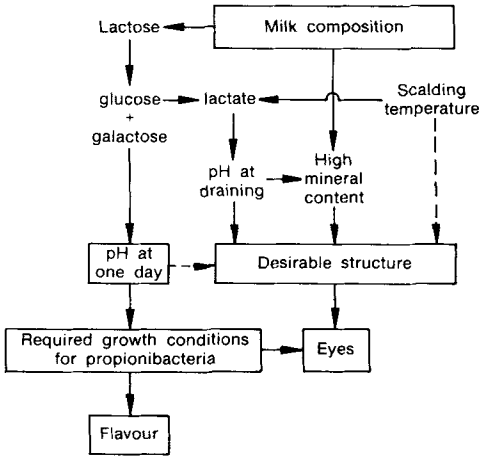


Figure 7. Factors that determine formation of flavor and "eyes" in Swiss-type cheese.

The elasticity of texture required in Swiss cheese is controlled by ensuring that the mineral content of the curd is relatively high. The proportion of acid produced before the whey is drained off has to be correspondingly small. This is achieved by a low percentage of starter and a high scalding temperature (50 to 52°C). Historically the use of high temperature evolved as a form of in-process pasteurization, but its more important role these days is to regulate the rate of acid production by *Streptococcus thermophilus,* which is one of the two species used in the starter culture. Indeed, with New Zealand milk the chance of good eye formation increases significantly if the pH at dipping is greater than 6.2. If the scalding temperature is kept constant throughout the season, the mineral content of the curd can be controlled by varying the quantity of *S. thermophilus* added. A secondary effect of the high temperature used in Swiss cheese manufacture is to plasticize the cheese. It is likely, however, that the structural elements in the casein framework remain in much the same relationship to each other after the heat treatment as before (18).

*S. thermophilus* hydrolyzes lactose to glucose and galactose but further metabolizes only the glucose moiety to lactic acid. The proportion of easily fermentable sugar available in the curd is thus effectively halved. The way in which lactose metabolism is controlled in the curd is an important feature in the manufacture of the different cheese varieties and the use of *S. thermophilus* is an elegant method of achieving this control. The galactose moiety however, is, metabolized subsequently, relatively slowly, by the *Lactobacillus bulgaricus.* It is the extent of fermentation of residual galactose to lactic acid that largely determines the final pH of the curd. Swiss cheese makers sometimes talk loosely about adding a bucketfull of coccus and a cup of the rod. However, the quantity of streptococcus added should be changed in a controlled way, as milk composition throughout the season changes, to ensure that the pH at dipping is the pH required. Similarly, the pH at 1 day should be maintained independently by changing the quantity of lactobacilli.

It is also important that the rate of growth of the propionibacteria is such that gas is produced at the required rate. This is determined both by the temperature at which the cheese is ripened and the pH at 1 day. Below pH 5.2 the chance of getting good eye formation falls markedly and the incidence of "blind" cheese increases (Figure 8). Above pH 5.4 the chance of fracturing increases significantly because of excessive gas production. Propionibacteria are also very sensitive to salt, and the ratio of S/M in Swiss-type cheese is traditionally low, usually less than 2%.

## GENERAL SCHEME FOR PRODUCTION OF ANY CHEESE VARIETY

It is clear from the examples given for a range of cheeses that there are two distinct stages in cheese manufacture (Figure 9). The first is development of the basic structure

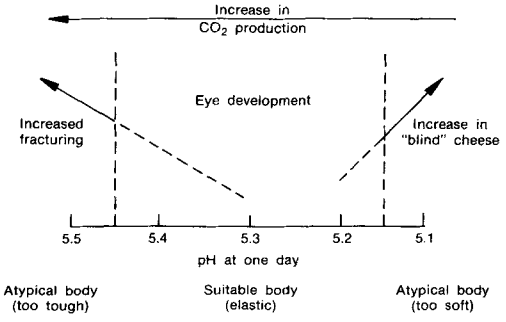


Figure 8. Relationship between eye development in Swiss cheese and pH of cheese at 1 day.

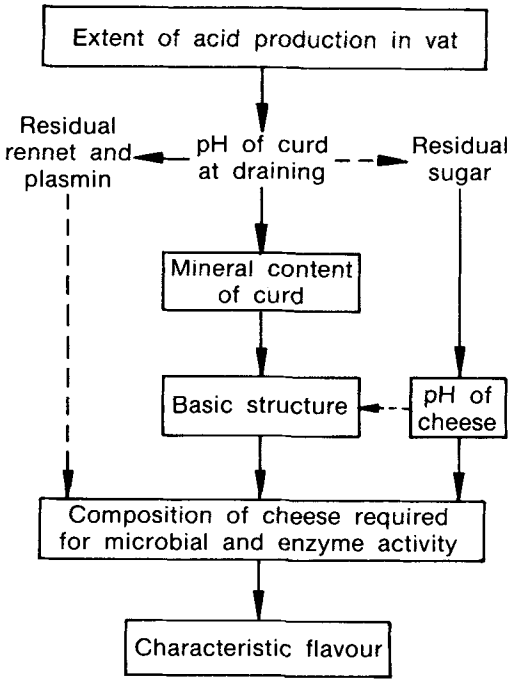


Figure 9. Main factors that determine characteristic basic structure and flavor of a particular cheese variety.

characteristic of a particular cheese variety. This is determined largely by the pH of the curd at draining, which is also important in that it controls proportions of both chymosin and plasmin that will remain in the curd. Holmes et al. (11) found that the distribution of calf rennet (chymosin) between curd and whey was pH dependent whereas that of the coagulants derived from *Mucor pusillus* was not. This finding has been confirmed in (18). More chymosin is retained in the acid Cheshire curd than in Cheddar made from the same milk supply (Table 1). Moreover, the activity of the chymosin is greater at the lower pH.

In some cheeses, of course, other factors are also important. Thus, in Swiss cheese the relatively high scalding temperature inactivates a high proportion of the chymosin (27). However, even small quantities of active chymosin are likely to produce significant proteolysis under the conditions of pH, temperature, and salt concentration that occur during manufacture and ripening of Swiss cheese. The proportion of residual chymosin is also relatively low in washed curd cheeses such as Gouda and Colby, because the water added washes out some of the chymosin.

The importance of plasmin, a native proteinase in milk formerly known as alkaline milk protease, in cheese ripening has been largely overlooked until recently (2). In fresh milk, plasmin is associated with the casein micelle, but it dissociates as the pH is decreased. Both the proportion of plasmin and its activity, therefore, will be greatest in the cheeses with higher pH. Indeed, in Swiss cheese the protein breakdown is largely due to plasmin (29). In contrast, relatively little plasmin activity is found in an acid cheese such as Cheshire (Table 1). In most cheese varieties, the activity of chymosin and plasmin, and thus their relative importance during cheese ripening, is determined by pH, the ratio of salt to moisture (33), and the ratio of moisture to casein in the cheese (18).

The final stage in cheese manufacture (Figure 9) is to ensure that the chemical composition of the cheese curd encourages the microbial and enzymic activity required to produce the typical flavor of the cheese variety. As one would expect, each cheese variety has a chemical composition that is suited for growth of particular organisms (Table 1). More specifically, the pH of the cheese plays a major role in determining the type of flora likely to be present (T. F. Fryer, personal communication). Thus, in Cheshire cheese the predominant nonstarter flora tend to be pediococci, whereas lactobacilli tend to be dominant in Gouda. In Swiss cheese propionibacteria are usually predominant, but lactobacilli are also normally present. Curds of different cheese varieties should be considered as preferential media in which particular organisms will grow much better than others. For example, it is not a coincidence that with mold ripened cheeses, such as the Camembert and Blue cheese types, the first stage is to produce an acid curd with a pH at 4.7 or less, because at this pH the molds used will grow better than any competitive organisms.

Throughout this paper the importance of pH measurements, both at draining and at 1 day, has been emphasized. This has been overlooked generally in the past, probably because it is relatively difficult to measure pH of cheese accurately. This has led to lack of appreciation of the significance of small changes of pH. In

addition, a pH per se is sometimes difficult to interpret unless considered in conjunction with calcium in the cheese (16).

### Cheese Flavor

One of the still outstanding problems with almost all cheese varieties is that it is not known yet which chemical compounds actually contribute toward characteristic flavors. Any organism that grows in the cheese, whether starter or nonstarter, and any active enzyme that may be present, such as chymosin or plasmin, must influence subsequent cheese flavor. Research in New Zealand has established, however, that if starter and nonstarter growth are controlled so as not to reach concentrations that give discernable off-flavors (4, 26) and if as little chymosin as possible is used (5, 25), the flavor that develops in Cheddar cheese is likely to be acceptable to most consumers. It is probable that a similar situation exists in all cheese varieties.

The role of plasmin in Cheddar cheese flavor has yet to be elucidated. Plasmin activity in cheese can be distinguished fairly easily from that of calf rennet (and microbial rennets) because plamin specifically breaks down $\beta$-casein to $\gamma$-casein and to proteose peptones. It may be relevant, however, that research workers at Ohio State University have reported that rate and extent of characteristic flavor development in Cheddar cheese slurries appeared to be related directly only to the degradation of $\beta$-casein (7). Plasmin may prove to be an enzyme of considerable importance in development of cheese flavor.

The flavor of Cheddar cheese varies widely, but fortunately most flavor variations are normally acceptable to the consumer. As the intensity of flavor becomes more pronounced with age, however, the cheese becomes less acceptable to a greater number of people. Because flavor is such a distinctly subjective matter, each person has a different concept of what the perfect Cheddar cheese should be. It follows that a major problem in cheese research is choice of a control cheese against which to measure any experimental cheese.

## CHEMICAL COMPOSITION AND CHEESE QUALITY

There is a considerable circumstantial evidence that the main factor in production of characteristic flavor for hard and semihard cheese varieties is breakdown of casein (1). This is supported by the finding that the ratios of moisture to casein and of salt to moisture are critical factors in cheese quality (5, 16), because both affect rate of proteolysis in a cheese (33). Traditionally in New Zealand, cheese makers described cheese in terms of its absolute moisture content. The problem was how to persuade them to switch to the much more important concept of the ratio of moisture to casein. Because commercial plants do not measure casein but only fat and moisture in cheese, the solution was to reintroduce the terms MNFS, i.e., the ratio of moisture to nonfat substance. The nonfat substance is not the same as casein in cheese but is equal to moisture plus solids-not-fat. The solids-not-fat consists of ash (about 4%) and protein, of which approximately 85% is casein. There is not, therefore, a strong relationship between the ratio of moisture to casein and MNFS. However, changes of MNFS for any particular cheese variety correlate closely with changes of the ratio of moisture to casein.

The MNFS in cheese gives a much better indication of potential cheese quality than the moisture content of the cheese in the same way that the ratio of S/M is a more reliable guide to potential cheese quality than is salt content of the cheese per se (16). In mechanized cheese plants, FDM (fat in the dry matter) is related to MNFS in a cheese (21), probably as a result of the relative inflexibility of procedures for control of moisture. This is of commercial interest because changing FDM is an effective way of controlling MNFS in cheese as composition of milk changes throughout the season.

The actual MNFS percentage for which a cheese maker should aim depends upon storage temperatures available and when the cheese is required to reach optimum quality. Experience has shown that if Cheddar cheese is to be stored at 10°C and the cheese is to be consumed after 6 to 7 mo, the MNFS of the cheese should be about 53%. The higher the MNFS percentage, the faster the rate of breakdown. Thus, if one anticipates that the cheese will be consumed after 3 to 4 mo, the MNFS percentage can be increased to about 56%. However, the higher the MNFS, the more rapidly Cheddar cheese will deteriorate in quality after reaching its optimum. The same is true for a Cheddar

cheese with a relatively low S/M, i.e., less than 4%, or with a high acid content (i.e., a low pH, <4.95). One has little control over such cheeses after they have reached maturity, because they tend to develop gas and sulfide-type off-flavors.

Recent developments in cheese marketing have resulted in demand for cheese of greater uniformity of composition than in the past. Such uniformity is achieved best by a grading system based on compositional analysis (5). Any such system will be relatively complex even when the FDM percentage has been standardized, because three other variables, MNFS, S/M and and pH, must be taken into account. It also has not been recognized sufficiently in the past that rate and extent of acid production not only determine indirectly the composition of the cheese but also govern the degree of acceptability of the cheese to the customer. Grading cheese by composition and pH to a large extent overcomes this problem.

Ranges of composition for Cheddar cheese first were suggested in 1974 (5). Since 1978 all New Zealand export cheese has been graded by compositional analysis, although ranges in Figure 10 have since been tightened (16). As long as acid production during manufacture has been normal, Cheddar cheese with a chemical composition that falls within the inner square is almost certain to result in a cheese that is acceptable to the vast majority of customers. Cheese, the composition of which falls in the outer square, is less certain to mature well.

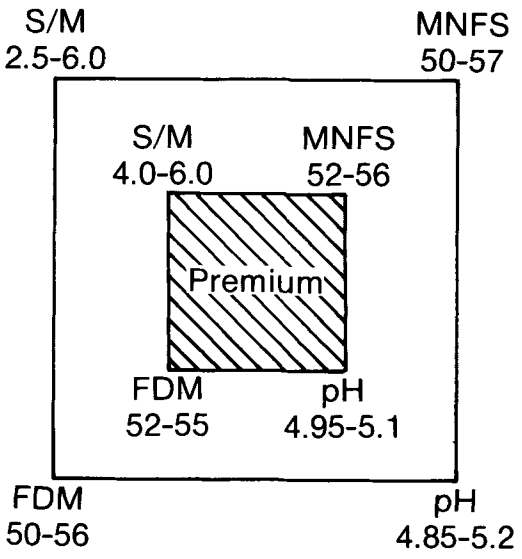


Figure 10. Ranges of salt in moisture (S/M), moisture in nonfat substance (MNFS), fat in dry matter (FDM), and pH proposed for Premium (shaded) and First grades of Cheddar cheese. Analyses 14 days after manufacture. The FDM minimum for New Zealand Cheddar cheese is 50% (5).

Large numbers of cheeses had to be evaluated to determine these ranges in composition. Cheese making is an exception to Rutherford's famous dictum that if the data need statistics, one should design a better experiment. With so many interrelated variables and with such relatively large changes of milk composition, both within a season and from season to season, one of necessity must seek to identify trends that are commercially significant. This is particularly true when assays themselves have inherent errors of precision; even simple sampling of a cheese presents many problems (17).

The necessity to introduce ranges of compositional analysis into the grading system follows from the simple fact that, no matter what system of cheese making is used, it is not yet possible to produce a completely uniform line of cheese within a day's manufacture. This does not mean necessarily, however, that some of the cheese produced during a day's run is of poorer quality than the rest. The rate of ripening will differ, but all of it is likely to be acceptable as long as the composition of the cheese is within the required compositional range. For instance, variations of moisture content and acidity of the curd before salting, of the quantities of salt delivered by salting equipment, and of the dimensions of physical structure of the milled curd will all result in considerable variation of salt uptake (17). It is not surprising that the S/M within a single 20-kg block of cheese tend to vary by as much as 1% or more. Nevertheless, as long as titratable acidity at salting is normal, an S/M between 4.5 and 6.0% will tend to result in acceptable cheese (16).

#### *Importance of Calcium*

The point at which the curd is drained from the whey is the key stage in the manufacture of any cheese, because it determines not only the basic structure but also controls to a large extent the proportions of residual chymosin and plasmin in the cheese, the final pH, and the

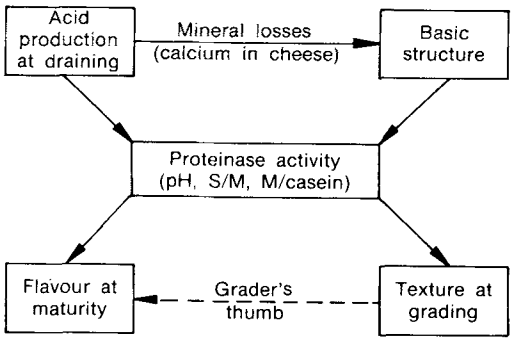


Figure 11. An explanation for the general validity of traditional sensory testing of cheese.

ratio of moisture to casein. All of these influence the rate of proteolysis in the cheese. It follows then that because calcium concentration is an index of acid production up to the draining stage (Figure 3), it should also be a rough indication of the rate of protein breakdown likely to occur during ripening (Figure 11). For instance, significant differences in calcium contents of Cheddar cheese manufactured on the same day suggest differences in proportions of residual chymosin and plasmin in the cheeses. Consequently, one would expect differences in rate of proteolysis and, thus, in production of flavor. Introduction of calcium into cheese specifications, therefore, should improve greatly the prediction rate of grading a young cheese, because a calcium concentration within a predetermined range would indicate that acid production up to the draining stage had been normal for the particular variety of cheese.

#### Grading of Cheese by Traditional Methods

It is now possible to explain why traditional sensory methods of grading young cheese are considerably more accurate than might be expected (Figure 11). Texture of cheese changes dramatically in the first few days of ripening. The simplest explanation (18) for this observation is that the cheese microstructure consists of an extensive network of $\alpha_S$-casein and colloidal phosphate. Creamer and Olson (3) found that cleavage by chymosin in calf rennet of just a few peptide bonds of $\alpha_{S1}$-casein greatly weakens this network in Cheddar cheese. This results in a relatively large change of the yield force. It is differences in this yield force, that a grader attempts to assess when he rubs down a plug of cheese between his thumb and forefinger. From his assessment of texture, after the cheese has been allowed to ripen for at least 7 days, the grader proceeds to predict what the quality of the cheese will be when it is mature. It is not possible normally to grade Cheddar varieties until at least 7 days after manufacture because the cheese is too curdy up to that stage.

There is some validity to the graders' method of prediction, because the rate of change of cheese texture during the first few days of ripening is determined by the same factors, i.e., pH at 1 day, ratio of salt to moisture and ratio of moisture to casein, which also influence quality of cheese at maturity. This includes production of its characteristic flavor (Figure 11). In addition, a Cheddar- or Gouda-type cheese with an atypical texture seldom, if ever, develops characteristic flavor. Unfortunately, however, the reverse it not true. A good-textured cheese does not always result in an acceptable flavor, because off-flavors still can be produced if manufacturing and ripening procedures are unsuitable (18). Essentially the grader's thumb is attempting to assess that acid production at draining was normal for the particular variety of cheese. However, this can be achieved more directly by measurement of calcium. Furthermore, a relationship does exist between calcium content of cheese, amount of residual chymosin retained in cheese, and protein breakdown during ripening (18).

## DEVELOPMENT OF DEFINED SINGLE STRAIN STARTER SYSTEMS IN NEW ZEALAND

Because this paper has emphasized the role of acid production as the single most important factor in cheese quality, a brief update of starter systems now being used in New Zealand may be of interest, particularly because so much has happened in the last 7 yr. There has been interest in the United States in the use of single strain starters for many years, but defined starter systems generally have not performed well, until recently, outside New Zealand.

Up until 1976 the New Zealand system had remained much the same for 40 yr, i.e., the use of a rotation of three or four pairs of phage-

unrelated single strains. Pair A/B would be followed by pair C/D and then by pair E/F (Table 2). The conclusion was reached, however, that rather than rotate three pairs of starter strains, it might be advantageous to use all six strains together. This defined multiple starter then would be used continuously, so that the cheese maker would need to become familiar with characteristics of only a single culture rather than three individual pairs. In addition, flavor of cheese would be more uniform because the use of different starter pairs tends to result in slightly different flavors in mature cheese.

The multiple starter system was introduced into New Zealand cheese plants in 1976 (22), and since then the concept has been adopted successfully also in North America and in Ireland. Multiple starters perform well in small, single-fill cheese plants, and three New Zealand plants still are using MS2 which was the first multiple introduced commercially. In all plants, however, four of the six strains in the original MS2 were attacked quickly by fast-replicating phages. These strains were replaced one at a time at yearly intervals by strains that were less sensitive to phage attack to give the multiple currently being used, i.e., MS6. The MS6 then contains only two of the original strains in MS2. None of the strains in MS6 is attacked by a fast replicating phage, but five of the strains are attacked by slow replicating phages. No one phage, however, will attack more than one starter strain. In practice this has been an ideal situation, as phage sensitivity can be used to monitor proportions of the six individual strains in the coagulated bulk starter. Multiples that contain more than one phage-insensitive strain, although initially giving greater protection, present practical difficulties in monitoring strains as well as the possibility that a phage may appear and attack more than one strain. Slow-replicating phages, particularly in a small cheese plant, are not normally a critical factor.

Most New Zealand cheese plants have increased considerably in throughput since 1976, and the multi-filling of vats throughout the day means that starters now have to be more uniform in acid production. Any phages, therefore, must be slow-replicating so that phage numbers do not build up significantly from one fill to the next. Phage numbers that resulted with the first two commercial multiples, MS2 and MS3, were too high to enable multi-fills of vats. It was decided in 1978, therefore, to revert to a rotation of three pairs (Table 2). Fast-replicating phages for two of the strains, D and F, soon appeared, and so a further decision was taken in 1979 to use strain B on all fills. This was in line with the long-held view that the fewer strains that are used in a cheese plant, the fewer phage problems will eventuate (15, 19). This move proved to be successful, and it only needed courage to suggest to cheese makers in New Zealand that the two strains, A and B, that were not at that time attacked by phage, should be used continuously.

This is the basis of a new concept in starter technology that has been called the "single pair" system and that was first introduced in 1980. Since then, most New Zealand cheese plants have been using this same single pair of strains; that is, every vat of cheese on every fill on every day has been made with the same pair A/B for at least 2 yr, and in some cases for 3 yr. In New Zealand a starter pair consists of one temperature-sensitive and one temperature-insensitive strain. Although a slow-replicating phage has now appeared intermittently in all cheese plants for the temperature-sensitive strain B, this has not been a sufficient problem to necessitate a change of strain. It will be more hazardous if a phage appears for the temperature-insensitive strain A as this strain controls rate of acid production after the curd has been "cooked". One, therefore, has to be in a position where starters currently in use can be replaced when necessary. Following commercial trials, proven replacement pairs have been stored away against the time that presumably

TABLE 2. Development of the "single pair" starter system in New Zealand (see text) for use in cheese plants filling each vat three times a day. Replicating phages that appeared are designated fast (○), slow (●), and very slow (*).

| Vats | 1978 | 1979 | 1980 |
|---|---|---|---|
| Fill 1 | A/B | A/B | A/B* |
| Fill 2 | C●/D○ | C●/B | A/B* |
| Fill 3 | E●/F○ | E●/B | A/B* |

inevitably will come when the current pair has to be replaced. A cheese company would be ill-advised to go over to the "single pair" system without such back-ups available.

The problem is that there is no way of predicting how long it will be before a phage does appear that will attack strain A. It may be 20 yr, or only a week before a phage for strain A appears. The ways in which a "new phage" might arise to attack a starter now are established fairly well. These include temperate phage from lysogenic starters (20), phage from wild strains in raw milk (10), modification of existing phages (28), host range mutants (12), and possibly recombinants of virulent and temperature phages. In old cheese plants there is also the likelihood of residual phage for previously used starters. In each instance only one active phage particle initially is required to establish infection. Although possible origins are clear, the actual origin of any particular "new phage" is not easy to determine. For example, although most lactic streptococci appear to be lysogenic (20), a recent investigation (13) suggests that temperate phages from the starter strains currently in use in New Zealand are unlikely to be the immediate source of phage in cheese plants. Regardless of its origin, however, phage in a commercial plant can be reduced greatly by removal of any strains that act as indicators for the phages present (9, 19).

The success of the "single pair" approach appears to be mainly due to two factors. The most important is improvements of the original method (8) of selecting strains likely to be insensitive to existing phages. There are four main morphological types of phage that attack lactic streptococci, and these phage types have been shown to be unrelated by DNA-DNA hybridization studies and by serology (14). A phage mixture containing high titers of some 60 variants of these four main types, almost all of which were isolated from commercial cheese wheys, has been assembled. A starter strain that survives laboratory screening against this potent phage mixture for some 7 to 10 cycles apparently has been exposed already to the current range of phage types that it is likely to meet in a commercial situation in New Zealand. This method of selection greatly reduces the probability that the two strains selected will be attacked by a phage, at least in the short term.

The second factor is that only the same two starter strains are ever available in the "single pair" system on which any phage can replicate. Cultures of lactic acid bacteria have a marked propensity to accumulate variants on repeated subculturing (23). Such variants may differ from the parent for phage sensitivity, proteinase activity, and ability to degrade lactose. An important safeguard, therefore, is to deep freeze cultures to prevent changes of these essential properties.

Although the present pair of strains has performed well over the past 3 yr, it would be an extraordinary coincidence if by chance the best possible pair had been chosen at the first selection. Different starter strains possess significantly different proteolytic systems. One can envisage that it may be possible to select starter pairs not only with proven phage insensitivity but with enhanced flavor-producing characteristics, in the same way for instance that increased starter activity, i.e., symbiotic growth, can be obtained by mixing together strains with complementary proteinase systems. In particular, the chance of defects that result from protein breakdown, such as astringency and bitterness, may be decreased. The search for such starter pairs is the main thrust of our present research.

Five years ago phage was still easily the most important factor affecting acid production in New Zealand cheese plants. However, now that the phage problem is effectively under control, any cases of slow acid production these days are likely to be caused by inhibitory materials in cheese milk. To overcome this, the activity of the starter coming out of the bulk starter tanks should be as active as possible. The simplest method (24) of ensuring this is to neutralize the coagulated overnight bulk starter back to pH 6.6 with a single addition of caustic soda solution and then allow the starter to grow again down to a preselected pH such as 5.2. This takes about 2 h. An effective procedure used by many cheese plants in the United States has been neutralization of the bulk starter continuously overnight with ammonia gas or liquid ammonia. Under New Zealand conditions, however, the one-shot neutralization step has been sufficient to guarantee active starter.

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