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A STUDY OF PROCESS PARAMETERS FOR ACCELERATING RIPENING OF CAMEMBERT CHEESE

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A STUDY OF PROCESS PARAMETERS FOR ACCELERATING RIPENING OF CAMEMBERT CHEESE

By

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ABSTRACT

A STUDY OF PROCESS PARAMETERS FOR ACCELERATING RIPENING OF CAMEMBERT CHEESE

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Twenty seven batches of loose curd Camembert cheese were prepared and changes in pH, volatile fatty acids, free fatty acids and soluble protein were followed during the 21 day ripening period. Maximum free fatty acid production was observed in cheese with low fat content, and maximum protein breakdown in cheese made from homogenized milk. Low salt concentration and high moisture content stimulates fat hydrolysis and protein breakdown during ripening. Cheese form was found to affect significantly the pace of ripening process. Determination of chemical composition of ripened commercial Camembert cheese showed that the loose curd cheese ripens faster. By electrophoresis it was demonstrated that 79% of α_{S1}-casein had disappeared at the end of the ripening period as compared to 41% observed for β-casein. Organoleptic evaluation demonstrated that the new process gave flavor intensity in 12 days of ripening that was comparable to commercial Camembert cheese ripened for 60 days.

To Eliana and our

daughter Edelweiss with

endless love and tenderness.

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INTRODUCTION

Mold ripened cheeses have been increasing in popularity and research has been done to improve their methods of manufacture and shelf-life. Particular attention has been paid to the use of Blue and Camembert cheese as an ingredient in processed cheese, salad dressings and chip dips. The labor requirements and special care in the manufacturing and ripening of mold ripened cheeses have encouraged the study of new procedures for making these cheeses, as well as ripen them in a shorter period of time, accelerating the formation of flavor. Much of the research has been related to the use of Blue cheese flavors as a substitute for the cheese. The innovations have included submerged culture fermentations, direct acidification and the quick ripening of the Blue cheese in a loose curd form.

The present work was undertaken to evaluate the development of Camembert cheese flavor in a loose curd form that could be incorporated directly in other Camembert flavored food products. Experiments were performed under different conditions to acquire information on the influences of milk pH, salt concentration, cheese fat content, the use of lipase and homogenized milk on the final characteristics of the cheese. Sensory evaluation was conducted

to evaluate flavor intensity in the cheese. The following changes occurring during a ripening period of 21 days were investigated:

1. Hydrogen ion concentration of milk used in cheesemaking.

2. Production of free fatty acids in cheese as a measure of lipid breakdown.

3. Protein degradation by soluble nitrogen assay.

4. Monitoring volatile fatty acids as indicators of general cheese flavor development.

5. Extent of degradation of α_{S1}- and β-casein by electrophoretical determinations in young and ripened cheese.

LITERATURE REVIEW

Cheese flavor may be described as a complex phenomenon which results from a multiplicity of chemical changes in the cheese system (Harper, 1959). The exact chemical nature of the flavor of any given cheese variety has not been completely elucidated; however much information is available which provides a partial understanding of cheese flavor and the complicated reactions responsible for its formation. According to Nelson and Richardson (1967) several mold species are the principal catalysts for imparting flavor to a wide variety of foods throughout the world; in western culture the predominant use of molds for flavor production - mold-ripened cheeses - is practiced under commercial conditions. The estimated hundreds of cheese varieties available throughout the world provide a broad spectrum of flavors for the human palate (Day, 1967). The normal and added microflora, and the procedure employed to ripen the cheese are also factors in determining varietal differences. Forss (1979) indicated that while information on flavor compounds may be very useful to manufacturers of imitation dairy products it may not help the dairy processors because it is often unclear how the chemicals were actually formed. Many conditions affect the final flavor of the

cultured dairy product. The most significant flavor compounds are produced by the metabolism of microorganisms and by the action of natural milk enzymes during the manufacture and ripening of the cheese (Blakely, 1970). If one has the right flora and the right conditions, the end product has the right flavor (Vedamuthu, 1979).

Mold-ripened Cheeses: Origins

Mold-ripened cheese dates back to the Roman era, when it was made in France. They are presently produced in major dairying countries around the world (Nelson, 1970). According to Nelson and Richardson (1967), the name "Roquefort" first appeared in the year 1070, but today there are many other cheese varieties in which typical flavor development is principally dependent upon the internal and/or external growth of mold, such as Blue, Gorgonzola, Stilton, Wensleydale, Gammelost, Nu-world, Camembert, and Brie. Kosikowski (1978) indicated that mold ripened cheeses are intrinsically considered to be Camembert, Roquefort and Blue cheese. Nelson (1970) reported that there are at least 10 distinct cheese varieties wherein typical flavor characteristics result primarily from the action of a mold species, usually a Penicillium. Concurrent with the increasing popularity of mold-ripened cheeses sold and consumed in natural form, there has been a growth and proliferation of food products flavored with, or in simulation of

mold-ripened cheese.

Origins of Camembert Cheese

The origin of Camembert cheese dates from 1791 when a farmer's wife, Marie Harel, who lived in the village of Camembert in Normandy, used to sell her cheeses in the market of the small town of Argentan, in France (Mocquot, 1955). According to Davis (1976) she perfected the method for making this type of cheese that remained a family secret for some time, but its manufacture soon spread and its fame grew. Kosikowski (1978) states that in 1791 Marie Harel produced a cheese which can be most closely associated with the present-day product. But there are conflicting reports on the origins of this cheese. Veisseyre (1975) reports that for many historians Marie Harel would have contributed just for a local development of the cheese, that existed already by the year of 1700.

Characteristics of Camembert Cheese

Camembert cheese can be made by many different methods. The cheese made by the traditional method (raw milk and no cutting) ripens faster and develops brown patches on the coat on account of the growth of Bacterium linens and similar types (Davis, 1977). The coat wrinkles and the odor of the uncut cheese is full and "ripe". The official definition of Camembert cheese, according to the French

government, states that "it is a cheese from uncut curd, round, with 10.5-11.0 cm of diameter, made exclusively from cow's milk, with surface molds, slightly salted and containing at least 40% fat in dry matter, and the total solids weight cannot be less than 110 g" (Veisseyre, 1975). According to Mocquot (1955) it is a flat cylinder, diameter 10-11 cm, height of 3 cm, weight of about 210 g, the percentage of dry matter being 52 percent. Two liters of milk would be needed to make one piece of cheese. Wilster (1980) suggests that an average composition of desirable market grades of Camembert cheese is approximately 28 percent fat, 20 percent protein, 50 percent moisture, and 2 percent salt and minerals. The finished cheese is generally 4 3/8 inches in diameter and from 1 to 1 1/2 inches thick. Kosikowski (1978) states that Camembert cheese of Denmark, Germany, and the United States show only Penicillium camemberti surface growth with a white surface mat. The flavor is nutty and the texture soft and creamy. According to the same author, the Camembert of France differed from these counterparts in that it generally had a mixed microbial vari-colored surface growth, although the white Penicillium camemberti still dominated and the cheese was very aromatic.

During the course of ripening, the cheese changes substantially. Davis (1976) reports that the early soft lactic curd first becomes firmer as it dries out and then, as the mold develops on the coat, it gradually changes from

snow white to a slightly greyish color, and the body becomes softer and more velvety or waxy. Wilster (1980) reports that below the rind there is progressive ripening which starts at the surface and moves toward the center. When the surface is becoming neutral, the red proteolytic bacteria begin to develop and the ripening proceeds to the centre of the cheese (Mocquot, 1955). According to Veisseyre (1975) the curd becomes yellowish and with a strong odor, as the cheese ripens, and if one wishes to keep the cheese for several weeks, must put it in a cooler, at about $1^{o}C$. Reports from other authors confirm these statements. It was observed by Davis (1976) that the central white layer steadily becomes thinner as the powerful proteolytic and lipolytic enzymes produced by the Penicillium camemberti diffuse inwards. Kosikowski (1977) suggests that Camembert cheese is ready for consumption when its surface is white, not brown; its interior, a lively yellowish color, not tan; and when the cheese, cut open, shows a faint hard white center core. A pronounced ammonia aroma, detectable through the package, indicates, along with other criteria, that the cheese is overripe and should be rejected.

Nomenclature of Penicillium

Camembert cheese is ripened by a white mold that grows at its surface. This mold is recognized as Penicillium caseicolum by some authors (Alais, 1974; Jacquet and Thevenot, 1961; Veisseyre, 1975), or Penicillium candidum by others (Davis, 1976; Mocquot, 1955). It was found by Desfleurs (1968) that the mold used in the making of Camembert cheese by Marie Harel, by 1791 was Penicillium camemberti, called at that time Penicillium album . Kosikowski (1978) reports that in the U.S.A. the white P. camemberti mold is required, whereas in France, lyophilized spores of P. caseicolum or P. candidum are used. According to Mocquot (1955) for many years the white and grey mixture of P. candidum and P. album was, as a rule, considered typical of the surface flora of Brie and Camembert cheese, but it is now more and more being replaced by the pure white color of P. candidum alone. Veisseyre (1975) reports that P. album has white spores that turn blue during ripening. Alais (1975) reports that both P. caseicolum and P. camemberti can be found at the surface of Camembert cheese, but only the first one, also called P. candidum, is sprayed on the cheese before ripening. According to the same author, P. camemberti white colonies turn grey after about 10 days. The organism has also been called P. album. Moreau (1979) indicates that the mold Penicillium commonly called "P. candidum" and "P. album" by fermented cheese manufacturers

corresponds to P. caseicolum Bain and P. camemberti Thom in the sub-section asymmetrica lanata, respectively. Both names have been considered as synonymous and the older taxon P. camemberti has been proposed for them. Dolezalek (1956) indicates that the biochemical properties of P. caseicolum, also called P. candidum, or P. album Epstein, are similar to those of P. camemberti Thom, but the colonies formed by P. caseicolum are more dense and deeper, presenting a definite white color.

Microbial Flora of Camembert Cheese

During the ripening of Camembert cheese, several microorganisms play important role in the formation of the final cheese characteristics. From the beginning, the pasteurized milk is inoculated with lactic bacteria (S. cremoris type) and P. camemberti or P. caseicolum (Olson, 1979). After about 8 days, a cottony, white mat of mold develops on the cheese.

According to Kikuchi (1966) spores of the inoculated molds were found at a concentration of 10^4 to 10^5/g of the Camembert cheese. Some yeasts were observed on the surface 20-30 days after production. Lenoir and Auberger (1966) presented results from their study on the Camembert flora: they found 100 strains of Streptococci, 110 strains of Micrococci and 79 strains of yeasts. Among the Streptococci 89 strains produced homofermentation (S. lactis 63,

S. diacetylactis 19, S. cremoris 7.) and 4 strains produced heterofermentation. Metche and Fanni (1978) found that during the ripening of Camembert cheese its external part shows a significant increase of its calcium and phosphorus concentrations, and that this process develops simultaneously with the exponential growth of P. caseicolum. Ergosterol, a precursor of vitamin D_2 normally absent in the sterols of milkfat has been demonstrated in white surface mold cheese like Camembert (Huyghebaert and de Moor, 1979). The ergosterol is formed by P. caseicolum during ripening. According to Lenoir (1963a and 1966) the fresh Camembert cheese is populated by lactic bacteria, and the cheese surface is dominated by the presence of yeasts such as of group Torula. Gradually Geotrichum candidum develops in the microflora and after 5 to 7 days the P. caseicolum appears on the cheese surface. Subsequently, as the curd approaches neutral pH Bacterium linens and yeasts become a significant part of the microflora. In his study Kikuchi (1966) reported that most of the isolated bacteria were identified as lactic acid bacteria and during the later stage of ripening the Micrococci group seemed to increase. During the early stage of ripening, Streptococcus cremoris was the predominant organism. This predominance was taken over by Leuconostoc dextranicum on approximately the 8th day, followed by Lactobacillus plantarum, Lactobacillus casei or Streptococcus lactis. According to

Sansonetti (1930) the Camembert surface is populated first by yeasts and molds and then by bacteria. Alais (1974) indicates that the first forms to appear are yeasts (Torulopsis) and molds (Oidium lactis and Geotrichum candidum). Veisseyre (1975) suggests that Micrococci (Micrococcus caseolyticum and Micrococcus conglomeratus) dominate on the surface of long ripened Camembert cheese. The total microbial population of a Camembert cheese is around 10^9 cells/g during ripening time (Lenoir, 1963a). Jacquet and Saingt (1952) isolated a microorganism from the Micrococci group (Micrococcus conglomeratus Migula) that they found responsible for the appearance of red stains on the cheese surface. In two studies, Schmidt and Lenoir (1978 and 1980) concluded that yeasts represent a substantial part of the bacterial flora of Camembert cheese. A total of 323 strains were isolated and identified. The yeast population seems to be dominated by Kluyveromices group and its imperfect forms constituting more than 60 percent of the population. Debaryomyces hansenii (12 percent) and Saccharomyces cerevisiae (9 percent), among others, were also found. In two new batches of the cheese, it was found that the yeast flora seemed to be dominated by S. italicus and also by Kl. lactis. Martley (1975) suggests that the proteolytic activity of the surface mold flora of Camembert may play the major role in producing non-bitter precursors, which are then degraded to bitter peptides by the proteases

of the lactic streptococci. It is believed that the bacterial population shifts and the transition of microfloral patterns are affected by changes in pH and the development of a growth factor as a result of casein decomposition during manufacture and ripening of Camembert cheese (Kikuchi, 1966).

The Proteolytic System of Penicillium Caseicolum

The enzymes prevailing in ripened cheese, except rennin and other added enzyme preparations if any, originate from the milk as natural tissue enzymes or become freed from the millions of bacteria, yeast or mold cells autolyzing in the cheese (Kosikowski, 1978). Penicillium caseicolum has been suggested to have two or more different proteases capable of hydrolyzing casein during cheese ripening (Tsugo and Matsuoka, 1963a; Lenoir and Choisy, 1971). It was demonstrated by Kikuchi and Takafuji (1971) that P. caseicolum protease shows an optimum pH at about 3.0, 6.0 and 9.5 and an optimum temperature of 35-40^{o}C. Tsugo and Matsuoka (1963) divided the proteases produced by P. caseicolum into 3 groups: acid, neutral and alkaline proteases with the optimum pH at 3.0, 6.8 and 10.5, respectively. The optimum temperature for acid, neutral and alkaline protease was 30, 35-40 and 35^{o}C, respectively. Alkaline proteases was rather unstable to heat and was inactivated by heating at a temperature in a range of 55 to 60^{o}C. Neutral protease,

however, was stable to heat. Gripon et al. (1977) showed that neutral protease from P. caseicolum induced large increases in pH 4.6- soluble nitrogen and non-protein nitrogen, but had little effect on production of free amino acids. However, the same protease plays a fundamental role in the proteolysis during cheese ripening. Penicillium caseicolum was found by Lenoir and Choisy (1971) to be largely responsible for the degradation of proteins in soft cheeses with moldy rind. The characteristics of the Penicillium caseicolum proteolytic system were determined on exocellular fractions obtained from cultures of 15 strains, by Lenoir and Auberger (1977a). They found that they produced the same proteolytic system, in a neutral medium with casein, showing an optimum activity at pH about 6.0 and a secondary optimum pH between pH 8.5 and 9.0. According to Veisseyre (1975), P. caseicolum has an optimum proteolytic activity at pH 5.5-6.0. Lenoir et al. (1973) studied the factors which control the synthesis of the proteolytic system of P. caseicolum. It was found that salts, especially Mg ions, some oligo elements and some forms of sulfur exhibited a noticeable effect on enzyme production. The optimum temperature and pH were 22-25^{o}C and 5.5-6.5, respectively. Lenoir and Auberger (1977b) demonstrated that the main component of the exocullular proteolytic system of P. caseicolum on a neutral medium was an enzyme with molecular weight of about 20,000 daltons. The optimum

pH and temperature of activity on casein was 6.0 and near 50°C, respectively. The heat stability of the enzyme was weak: at pH 6.0, after 40 min. at 55°C the activity was reduced to 10 percent of its initial value. In another study, Lenoir et al. (1979) found a second component of the exocullular proteolytic system of P. caseicolum grown in an acid medium at pH 4.0. The molecular weight of the enzyme was about 35,000 daltons, and the optimal pH and temperature of action were 3.5-4.0 and 45°C, respectively. The enzyme heat stability was also weak: at 60°C and pH 4.0, the activity was reduced to 10 percent of its initial value, after 13 min. The role of the neutral protease of P. caseicolum was studied by Desmazeaud and Gripon (1977) using aseptic curd admixed with this enzyme. A substantial increase in pH 4.6 soluble N and NPN was observed, without the appearance of amino acids. They indicate also that P. caseicolum has both endopeptidase and exopeptidase activities, the latter resulting in the release of large amounts of amino acids. According to Lenoir et al. (1973) the ability of P. caseicolum in producing proteolytic enzymes is rather low when compared to that of many other fungi. The study of its production factors shows that it is not easy to improve production of protease to a substantial degree.

The Lipolytic System of P. caseicolum

Penicillium caseicolum belongs to the surface flora of numerous soft cheeses and plays a vital part during the ripening phase, especially through its de-acidifying and proteolytic activities. Some lipolysis is also observed in these cheeses. Lamberet (1974) isolated 89 strains of P. caseicolum and studied their lipolytic activity. The optimum pH for activity was around 8.0 and the optimum temperature range between 20 and 35°C. The enzyme did not appear to be very heat-resistant. Proks and Cingrosova (1962) studied the lipolytic activity of P. camemberti and P. caseicolum and found that both species had a pronounced lipolytic activity, which was greater in the case of Penicillium caseicolum. Both species attacked mainly the lower chain saturated fatty acids and showed relatively little activity towards the unsaturated acids. Stepaniak et al. (1980) used regression equations to present interactions of the initial pH value of the medium, the cultivation time, the thickness of medium layer and the glucose content of the medium on the production of lipases and proteases by one strain of P. roqueforti and one strain of P. candidum. The equations obtained showed the possibility to control amount of lipases and proteases produced, as well as proportions between those enzymes by choosing cultivation conditions. The characteristics of the lipolytic systems

of P. caseicolum were tested by Lamberet and Lenoir (1976) from cultures of 8 strains showing different abilities to produce extra-cellular lipases. The systems revealed similar characteristics: the optimum pH was 8.5 at 30°C and at pH 4.5 nearly 45 percent of the maximal activity was still measured. The enzyme possessed low heat stability: at 37°C and pH 8.0 less than 15 min were needed to reduce the activity to 10 percent of the original. No lipase having an acid optimum pH from either endo- or exocellular preparation was found. In another study, Lamberet and Lenoir (1976b) isolated a P. caseicolum lipase from culture medium which probably represented the main exocellular lipolytic activity of the mold. The molecular weight of the lipase was 24,000 ± 1,000 daltons and the optimum pH and temperature were 9.0-9.6 and 35°C, respectively. The loss of activity was 60 percent after heating for 5 min at 40°C and pH 8.5.

X Protein Changes During Ripening

The cheese protein apart from the fat is the only solid phase in the cheese. Since the fat phase is discontinuous it cannot be expected to have a dominating effect on firmness. This would imply that the softening of the cheese is caused by the degradation of its protein phase (Jong, 1976). Ripening is induced by a complex mixture of microorganisms and their enzymes, i.e. proteases, exopeptidases, lipases,

deaminases, decarboxylases, etc. However, protein breakdown represents the primary phenomenon of the ripening process since it results in softening of the cheese-body and changes in its appearance (Desmazeaud and Gripon, 1973).

The general course of proteolysis is (Torres, 1979):

$$\underset{\text{(insoluble)}}{\text{Protein}} \xrightarrow{+H_2O} \underset{\text{(water soluble)}}{\text{Proteoses}} \xrightarrow{+H_2O} \underset{\text{(water soluble)}}{\text{Peptones}} \xrightarrow{+H_2O} \underset{\text{(water soluble)}}{\text{Peptides}} \xrightarrow{+H_2O} \underset{\text{(water soluble)}}{\text{Amino acids}}$$

Protein degradation varies with the variety of cheese. Primary protein hydrolysis to amino acids occurs to some extent in all cheese varieties. In certain types, such as Limburger and Camembert, proteolysis is much more extensive than in types such as Swiss (Harper and Kristoffersen, 1956). Decomposition of proteins is the most important phenomenon in cheese ripening, as they are partially converted from insoluble to soluble forms and are broken down to proteose, peptones, polypeptides, amino acids and finally, to ammonia (Singh and Ganguli, 1972). Desmazeaud *et al.* (1976) used an aseptic model curd to study the action of *P. caseicolum*. They found that *Penicillium* released great amount of amino acids and low molecular weight peptides. Their endopeptidase activity was also very important and they degraded simultaneously α_{S1}- and β-caseins. Singh and Ganguly (1972) studied changes in peptides and amino acids in Cheddar cheese made from cow's and buffalo's milk. They

found that free amino acids appeared in cheese within seven days of ripening. The maximum number of amino acids (17) appeared in cheese within 56 days of ripening in cow and 77 days in buffalo and during the rest of ripening there was no marked quantitative difference in the amino acid pattern. Soltys et al. (1973) studied the effect of milk pasteurization on protein and fat breakdown in Camembert cheese. It was found that in cheese ripened for 28 days, 45% of the casein had been broken down. Non-protein N amounted to 25% of total N, and ammonia N and amino N amounted to 20 and 10%, respectively, of soluble nitrogen. According to Lenoir (1963c) at the beginning of ripening, a Camembert cheese contains 10% of soluble N and this fraction is represented by 60% of N from proteoses and 30-35% N from peptides. In another study on the composition of N constituents in ripened Camembert, Saint-Paulin and Gruyere cheeses, Lenoir (1963b) reports that total soluble N amounted to 31-34% of total N in Camembert, 16-21% in Saint-Paulin and 28-32% in Gruyere. But the composition of this soluble N differed appreciably from one variety of cheese to another. Amino N and ammoniac N in Camembert amounted to 9-12% and 21-27% respectively, while the comparable figures for Saint-Paulin were 13-18.5% and 2.7-8.5%, and for Gruyere 32-37% and 10.5-14.5% (figures shown as percentage of total soluble nitrogen). Storgards and Lindqvist (1953) found that Camembert cheese contained

36.2% of its total N as soluble N.

Proteolytic Agents in Cheeses

It has been established that there are two major types of proteolytic agents in cheeses (Desmazeaud and Gripon, 1977):

1. Coagulating enzymes: rennet or rennet substitutes.
2. Proteolytic enzymes of the starter cultures: mesophilic and thermophilic lactic acid bacteria and fungal starters (Penicillium caseicolum and P. roqueforti).

The initial attack on the labile phenylalanine-methionine bond of κ-casein is accomplished by rennet, but the subsequent contribution of starter enzymes and rennet to the proteolytic breakdown is not certain (Dulley, 1974). According to Castberg and Morris (1976), cheese proteolysis, primarily of casein, is considered to result from several proteinase and peptidase activity, the main contribution coming from milk protease, rennet, starter organisms and organisms present as a result of random contamination. Ohmiya and Sato (1972) studied the contribution of intracellular protease of lactic acid bacteria to the casein hydrolysis during cheese ripening, as compared to rennin action. They found that as compared to rennin, the intracellular proteases released from the bacteria by autolysis contribute more to the hydrolysis of casein in cheese.

Reiter et al. (1969) studied the hydrolysis of fat and protein in small cheeses made under aseptic conditions and found that although rennet did not produce any amino acids in the mature cheeses it hydrolyzed some of the cheese protein to nitrogen compounds soluble in water. The proteolysis of casein under the action of certain bacterial endoenzymes was studied by Poznanski et al. (1965) and they suggest that compared to the "general proteolytic action" of rennin, the activity of the bacterial enzymes appears rather weak; but when the nature of the products formed during hydrolysis is considered, the situation looks different. While most of the products resulting from rennin activity are insoluble in trichloroacetic acid, most of those from the endoenzymes are soluble in the same reagent.

In a report on the breakdown of casein by rennet and microbial milk-clotting enzymes, Vanderpoorten and Wecky (1972) observed that the microbial rennets liberated more NPN from whole casein and from the α_s- and β-casein fractions than did veal rennet. It was established by Dolezalek et al. (1978) that microbial rennets stimulated the ripening of Camembert cheese, but no significant difference was established in the patterns of free amino acids and amines for different rennets.

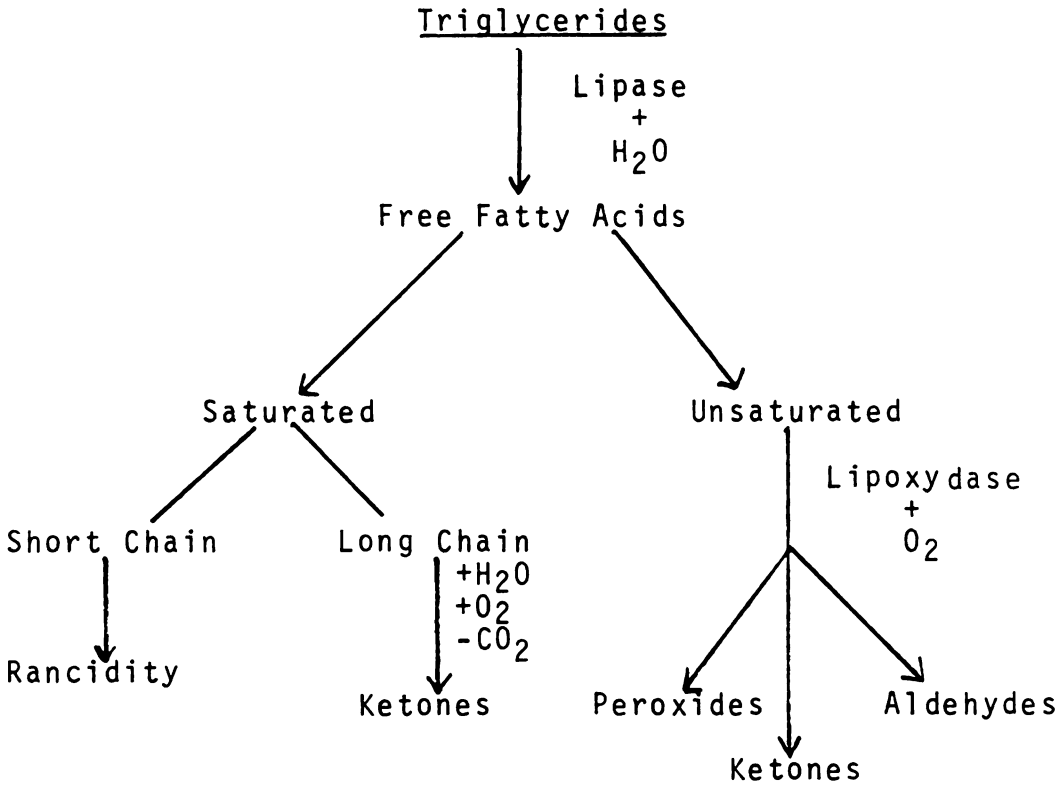
Concerning the role of milk proteases, the activity of these enzymes in cow's milk is generally considered to

be very low (Desmazeaud and Gripon, 1977). In cheese ripening, milk protease on its own liberates amino acids and low molecular weight peptides, but only in small amounts (Visser, 1977).

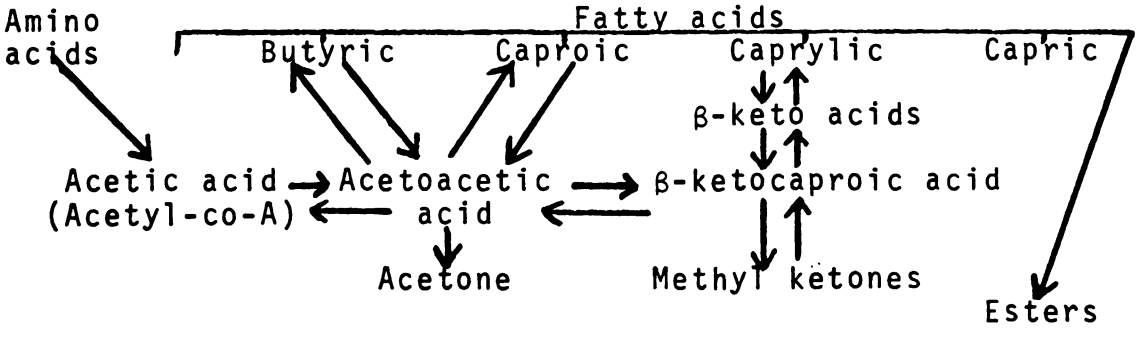
A very active proteolysis is generally observed in cheeses with surface mold or in Blue cheese. For ripened Camembert cheese, it was shown that pH 4.6-soluble N reached 32% of the total N and that amino N and ammonia N represented more than 10% of the total N. It may be concluded that Penicillium possesses a high endopeptidase activity (Desmazeaud and Gripon, 1977). The same conclusion was drawn by Gripon et al. (1977) in a study of the role of proteolytic enzymes of S. lactis, P. roqueforti and P. caseicolum during cheese ripening.

Lipid Changes During Ripening

Fat hydrolysis occurs to some extent in all cheese varieties but it is more important in some cheese varieties than in the others. Both primary and secondary degradation products of fat are powerful flavor and aroma components of cheese (Harper and Kristoffersen, 1956). The lipid changes in cheese can be shown in this scheme proposed by Alifax (1975):



Mature cheese usually contains a certain amount of free fatty acids (FFA) which affect its flavor. FFA may come from the fermentation and degradation of lactose, changes in amino acids and lipolysis of fat. Only free fatty acids from C_4 upwards can form as a result of the latter (Poznanski et al., 1968). Metabolic pathways and products of fatty acid catabolism in cheese were proposed by Harper and Kristoffersen (1956):



According to Stadhouders and Mulder (1957) the enzymes occurring in cheese and which may be involved in the process of fat hydrolysis, may originate from:

a) the rennet

b) the milk (true milk lipases secreted by the udder cells)

c) microorganisms, which comprise microorganisms from the milk, organisms from the interior of the cheese and organisms growing on the surface of the cheese.

Jensen (1966) reports that milk lipases are sensitive to pasteurization, heat, light, oxidation, heavy metals, formaldehyde, acidic conditions, aging, antibiotics and SH-blocking compounds. Wong (1980) states that milk lipases are normally inactivated at pasteurization temperatures. In a study on the hydrolysis of fat and protein in small cheeses made under aseptic conditions, Reiter et al. (1969) relate that milk lipase and bacterial lipase do possess activity at the low pH value of cheese, but milk lipase is inactivated during pasteurization at 63^{o}C for 30 min and that in the absence of a strongly lipolytic flora the FFA produced by the milk lipase are relatively important in cheese. By using aseptically drawn milk, which was pasteurized before cheesemaking, Stadhouders and Mulder (1960) suggested that by increasing the pasteurization temperature the greater part of the lipase was destroyed. They concluded that the low production of fatty

acids in a cheese made from milk pasteurized at very high temperatures was probably one of the causes of the lower quality of the cheese made from this milk. Dulley and Grieve (1974) studied the volatile fatty acid production in Cheddar cheese and concluded that with the exception of acetic acid, lipolysis was responsible for the production of most of the volatile fatty acids in the cheese. The fat hydrolysis by lactic acid bacteria in cheese was studied by Stadhouders and Veringa (1973) who found that starter bacteria are able to produce free fatty acids from mono and diglycerides in the cheese, during ripening.

According to Kuzdzal and Kuzdzal-Savoie (1966) lipolysis is a normal phenomenon in cheese, contributing to its flavor, and this action is specially intense in cheeses such as Bleu and Camembert. Molds can split fat on the cheese surface and are not only able to hydrolyze the cheese fat into glycerol and fatty acids, but at the same time they may be able to decompose the products of hydrolysis. Kowalewska et al. (1971) investigated the production of free fatty acids by lactic fermentation bacteria and molds on casein medium and found that microorganisms and enzyme preparations were able to produce FFA from casein substrate of pH 6.5 and 5.5, but molds failed to produce butyric acid. The free fatty acids of a Camembert-type cheese were determined by Hote-Baudart (1967), who found oleic acid being present in the greatest amount, followed

by the C_{16} and C_{14} acids. Volatile and straight-chain fatty acids were less abundant. Soltys et al. (1975) found that in a normal Camembert cheese, during ripening triglyceride content of fat decreased from 90 to 70% and content of free fatty acids increased from 1 to 10%. The volatile free fatty acids of Camembert cheese were determined also by Bonassi and Llisto (1978) who found that the cheese had low levels of all acids (288-434 mg/100 g) except butyric (399.2 mg/100 g). Veisseyre (1975) reports that a Camembert cheese may contain from 20 to 50 g of free fatty acids per kg. It may be said that the lipolytic activity of the studied species of Penicillia is rather intensive and both P. caseicolum and P. camemberti liberate the fatty acids, especially the saturated ones. Their influence on the unsaturated fatty acids is distinctly smaller and the lipolytic activity of P. caseicolum is much greater than the activity of both P. camemberti and mixed cultures.

Lactose Changes During Ripening

Carbohydrates are the most usually fermented group of compounds. Lactose is hydrolyzed to galactose and glucose. Either of these monosaccharides is readily fermented (Kosikowski, 1978). The changes in lactose occur primarily during cheese manufacture and during the first stages of cheese ripening. Regardless of the stage at which lactic acid is formed, most of the lactose originally present

disappears after 24 hours and only trace amounts of glucose and galactose are detectable for the next 7 to 14 days (Wong, 1980). The primary changes in the carbohydrate are initiated by the starter organisms. Lactic acid-producing bacteria are added to the milk to create proper acidic conditions in the cheese, since the conversion of lactose to lactic acid during and after manufacturing is essential in all cheese varieties (Harper and Kristoffersen, 1956). The decomposition of lactose in milk is for all cheese varieties very important for a normal ripening process, good keeping quality, and the formation of flavor. This decomposition occurs first at lactose level followed by the split products of disaccharides, galactose, and glucose, and finally calcium lactate is produced (Schormuller, 1968). According to Kosikowski (1978) there are six major sugar and citric acid fermentation reactions in milk, namely lactic acid fermentation, propionic acid fermentation, citric acid fermentation, alcohol fermentation, butyric acid fermentation and coliform-gassy fermentation. The lactic acid fermentation is the most important one in milk, for it is required in all instances. According to Schormuller (1968) the lactic acid developed from the fermentation of lactose depresses harmful and undesirable microorganisms, and regulates the pH level and the ion equilibrium, which is important to the physical reaction of paracasein. It is used also as a source of energy for

molds such as P. caseicolum growing at the surface of Camembert cheese, bringing about some neutralization of the curd (Veisseyre, 1975). The breakdown of sugar during Camembert ripening was studied by Berner (1970) who found that D-lactose and D-galactose were no longer detectable after 30 days, but the D-glucose content increased during storage. The rate of sugar breakdown proceeded differently in the internal and external parts of Camembert cheese. In another report, Berner (1971) discussed the degradation of lactic acid during ripening of Camembert. It was found that this degradation proceeded with varying rapidity in rind and core. The L-lactate content determined 32 days after a brine bath treatment, in the rind as well as in the core, was about 0.2%. The D-lactate exhibited relatively rapid degradation.

Factors Affecting Cheese Ripening

A ripening cheese is a virtual treasure house of micro-organisms, engaged in life and death activities, and enzymes catalyzing simple to complex reactions of fat, protein, sugar and their intermediate products (Kosikowski, 1978). There are many factors affecting the action of these ripening agents, that bring about important variations in the final characteristic of the cheese. Among those factors are the cheese and milk pH, storage temperature, moisture content and salt (NaCl) content of the cheese.

Milk and Cheese pH

The initial acidity of the milk as well as the subsequent rate and extent of formation of acid in the curd and whey is of major importance in cheesemaking. Certain kinds of cheese require comparatively fresh milk, as in the cases of Limburger, Bel Paese and Brick while certain other kinds, such as Cheddar, Camembert and Blue can be made from milk that has developed a slight acidity (Wong, 1980). Changes in pH level in the ripening cheese have a significant importance to the ripening process (Schormuller, 1968). Since after acidification by the lactic acid bacteria the pH of a young cheese is mostly about 5.0, the coryneform bacteria or gram negative rods can develop only after an increase of the pH. In this respect the fact that many yeasts and molds can break the lactic acid down into CO_2 and can hydrolyze the proteins to ammonia is of importance (Stadhouders and Langeveld, 1966). The ideal pH range for the growth of P. caseicolum on the surface of a Camembert cheese would be from 4.5 to 6.5 (Jacquet, 1956). In a study on the effect of acidity on the growth of Penicillium camemberti Kunz and Singer (1979) found that normal growth was obtained between pH 3.0-9.5 with optimum conidia formation at pH 4.5. The cheese pH also affects the lipolytic activity of the mold. Dolezalek and Minarik (1968) reported that lipolytic activity of Penicillium camemberti was highest at an initial pH value of 6.7. Eigel (1948)

obtained results indicating that the ripening process in Camembert cheese takes the outward-inward way, appearing highly probable that the superficial ripening process is some 8-9 days in advance of that of the core. These results seem to be in agreement with indications from Wong (1980) that in surface-ripened cheeses, such as Limburger or Camembert, acidity at the rind decreases during ripening until the reaction becomes almost neutral, while in the inside of the ripened cheese the pH value is usually between 5.2 and 6.2.

Ripening Temperature

The curing temperatures for most varieties of cheese are generally between 4.4 and 12.8^{o}C, with exceptions for certain varieties. Soft, high-moisture cheeses are cured at lower temperatures than hard cheeses, since a combination of high moisture content and high temperature promotes a development of inherent defects (Wong, 1980). According to Pettersson and Sjostrom (1975) cheese-ripening velocity is dependent on the total amount of ripening enzymes present in the cheese, as well as the ripening temperature. The temperature can not, however, be altered too much from customary ripening temperatures (10-15^{o}C) without risk of undesired fermentations. Solberg *et al*. (1953) studied the influence of temperature on the ripening of Gouda cheese and found that cheeses stored at 0^{o}C showed a decreased

fermentation when compared with cheeses stored at 10°C, the rate of proteolysis being especially reduced during the first months of cold storage. Veisseyre (1975) indicates that the optimum temperature for most enzymes acting on cheese ripening is 35-45°C, but that at 1°C the lipase of Penicillium caseicolum has only 50% reduction in its activity. The optimum temperature for the growth of molds at cheese surface would be between 20 and 25°C.

Salt Concentration

Sodium chloride is added to curd for most cheese varieties at the end of manufacture or during the early ripening stages either by direct addition to curd, by rubbing dry salt on the surface of the cheese or by immersion in brine. NaCl plays an important role in cheese ripening by virtue of its effect, probably selective, on the growth of microorganisms, on the activity of microbial and other enzymes, on the formation of certain substrates and by its direct contribution to flavor (Godinho and Fox, 1981).

Salt inhibits the growth of certain microorganisms more than others; thus the salting of Camembert cheese is said to aid in regulating the proper conditions of the organisms which effect the ripening (Wong, 1980). Davies et al. (1937) studied the effect of chemical substances in the ripening process of Cheddar cheese and found that the

absence of salt may increase the non-protein nitrogen by nearly 50 percent. The effect of salt on acid development in Cheddar cheese was also studied by Walter et al. (1958). They reported that, in general, acid production by most single strains of S. lactis was not inhibited by less than 1.6% salt. The acid production was not significantly affected by 1.6 to 2.0% salt, whereas most single strains of S. cremoris were inhibited slightly by 1.4%, definitely by 1.6% and almost completely by 2.0% salt. Rasic (1965) reported that the gradually increased concentrations of sodium chloride in the milk caused a more rapid growth and acid production of lactic streptococci cultures, than when the same salt concentrations were achieved at once. Studying the ripening of Blue cheese, Godinho and Fox (1981) found that lipolysis was delayed by higher salt concentrations. Kirchmeier (1972) states that some of the factors that influence the uptake of salt from brine by cheese are pore space, specific permeability to salt, and the exchange capacity of the para-casein for each of the various ions involved. The brine characteristics seem to influence the quality of Camembert cheese, according to Hardy and Weber (1978). They recommend that Camembert cheese be salted in brines at 12-15^{o}C and at pH of about 4.6. Schulz (1947) reported that the high concentration of NaCl could prevent the germinating process of P. camemberti on Camembert cheese.

Moisture Content

The influence of the moisture content on the consistency and protein breakdown of cheese was studied by Jong (1978). He found that the firmness of the cheese is closely related to its dry matter content, even if the protein breakdown and its influence on the firmness of the cheese is taken into account. Changes in dry matter content cause greater changes in firmness in a high dry matter cheese than a low dry matter cheese. Phelan and Guiney (1973) indicated that proteolysis in Cheddar cheese increased when the moisture levels were raised by adding water. Jong and Groot-Mostert (1977) stated that the most striking differences in physico-chemical conditions between cheeses were those of pH, salt and moisture content. Raadsveld (1953) demonstrated that the soluble N content was highest in the high moisture Edam cheeses studied. But, neither the moisture content nor the salt concentration in the water phase of the cheeses had any influence upon fat degradation. According to Alais (1974) and Veisseyre (1975) the higher the moisture content of a cheese, the faster the protein breakdown, at a given temperature.

In model experiments on the brining of cheese, penetration of NaCl into cheese and outward migration of water could be adequately described as an impeded mutual diffusion process (Geurts et al., 1974). The water activity of a medium (e.g. cheese) is defined as the water vapor pressure

of the medium relative to that of pure water at equal temperature and pressure (Veisseyre, 1975). In fact, the water activity indicates how far the water present is available for the microorganisms. The relation between the water activity and the moisture content of different cheeses was studied by Stadhouders and Langeveld (1966). They found that the salt content has a dominating effect on the water activity of the cheese. In a medium containing 0% NaCl and 0.992 water activity, P. candidum showed 100% growth; in a medium containing 5% NaCl (w/v) and 0.975 water activity, the growth was reduced to 80.9%. The water activity of a Camembert cheese was measured by Hardy (1979) after 40 and 120 min in the brine. In the center of the cheese the water activity was 0.970 and 0.955, after 40 and 120 min of brining, respectively. At the cheese surface, it was 0.962 and 0.947 after 40 and 120 min of brining, respectively, showing clearly the influence of salt in the water activity of the cheese.

Homogenized Milk in Cheesemaking

Homogenization of milk, which breaks the fat globules down to a smaller, more uniform size, results in a very strong activation of lipolysis. This is because much of the milkfat loses its natural protective membrane and becomes coated with a new membrane consisting largely of small casein particles. As the new membrane is less

structured and more permeable than the natural one the fat is much more vulnerable (Deeth and Fitzgerald, 1975). Generally the benefits of homogenized milk in the manufacture of cheeses are 1) lower fat losses in the whey, 2) higher yields of cheese, 3) lower shrinkage losses during ripening and 4) reduced fat leakage at elevated temperatures (Wong, 1980). The acceleration of lipase action in raw milk by homogenization is now generally accepted. This acceleration has been attributed by some to increased surface area afforded the lipase by the breakdown of the fat globules and by others to a re-surfacing of the fat globules by material more susceptible to lipolytic action (Gould, 1941). The immediate effect of homogenization is chiefly on the fat. The size of the fat globules is reduced and their surface area is increased from 10 to 30 times, thus greatly increasing the possibility of hydrolysis of the fat due to lipase activity (Wong, 1980).

According to Davis (1965) the homogenization process has an accelerating effect on rennet clotting, decreasing the curd tension, with less loss of fat in the whey. Iwaida and Tsugo (1962) found that increasing pressure during homogenization of milk, decreased the curd tension, but at the homogenization pressure of 200 kg/cm^2 or more, the degree of decrease in curd tension became very slight. It was found by Humbert _et al_. (1980) that curds from homogenized milk show less whey exudation as compared to curds

from non-homogenized milk. The feasibility of using homogenization of the milk in the manufacture of fat-rich Tvorog was studied by Mitskevichyus and Vaitkus (1973). It was found that increasing homogenization pressure impaired syneresis and adversely affected whey removal, but the fat and SNF losses in the whey were decreased, and total solids utilization increased from 54.38% for non-homogenized milk to 60-62.5% for milk homogenized at 75-150 atm. Lane and Hammer (1938) studied the manufacture of Blue cheese from homogenized milk and found that the cheese ripened in a relatively short time had a comparatively light color, and the body was unusually soft so that the cheese could easily spread with a knife. Wong (1980) reported that homogenization of the milk for Blue cheese causes considerable improvement in its ripening and flavor development. Growth of Penicillium roqueforti is accelerated, the cheese is lighter in color, the body is softer, the amount of volatile acidity and the acidity of fat are increased and the desirable sharp flavor develops more rapidly.

The Use of Enzymes in Cheese Ripening

Hydrolysis of milk fat catalyzed by enzymes has been applied in recent years as a useful, controlled process for the development of desirable flavors in various dairy products (Arnold et al., 1975). Lipolytic preparations of both animal and microbial origin are likely to be widely

utilized to intensify flavor and to reduce the ripening time of cheeses (Kornacki et al., 1979). Glandular enzyme preparations obtained from calves and goats have been used together with rennet extract, producing lipolysis and proteolysis in the cheese similar to the effect of rennet paste (Wong, 1980). The effects of addition of cheese slurry and enzymes mixture to buffalo milk Cheddar cheese was reported by Al-Fayadh et al. (1981) who found that the addition of enzyme mixtures produced excessive bitterness and rancidity in cheeses made with Mucor pusillus and Endothia parasitica at 7 months of ripening, but acceptable body and flavor in cheeses made with calf rennet and Mucor meihie coagulant. Babel and Hammer (1945) report that the addition of a small amount of rennet paste resulted in an increase in fat acidity of Cheddar cheese.

Blue cheese is a popular ingredient for salad dressings and cheese dips, and for this reason, much research has been done to improve and accelerate its flavor development. Fox (1980) indicates that treatment of Blue cheese curd with pre-gastric esterases improves and intensifies its flavor. Coulter and Combs (1939) suggest that since fat hydrolysis appears to be essential for the development of the characteristic flavor in the Roquefort-type cheese, the addition of enzyme preparations containing lipase appears to offer some promise of shortening the ripening period. The flavor development in pasteurized milk Blue cheese by animal and

microbial lipase preparations was studied by Jolly and Kosikowski (1975). It was found that by adding enzymes, lipolysis and proteolysis were accelerated, with increases in volatile free fatty acids and soluble nitrogen. A method for the production of quick-ripened Blue cheese was developed by Harte (1974) who found that the addition of a commercial (unspecified) lipase to the curd had no beneficial effect.

Electrophoretic Study of Cheese Ripening

Electrophoresis is frequently used as a quantitative and qualitative method for the evaluation of cheese ripening. Vanderpoorten and Weckx (1972) used polyacrylamide gel electrophoresis (PAGE) to study the breakdown of casein by rennet and microbial milk-clotting enzymes in Gouda cheese. They obtained results indicating that the microbial rennets liberated more NPN from whole casein and from the α_s- and β-fractions than did veal rennets. Jong (1975) presents the electrophoretic patterns of soft cheese after proteolysis (27 days) and reports that only a very small quantity of the degradation products of β-casein had an R7 value comparable with, though not identical to, that of α_s casein and only when β casein was very highly broken down. The products from α_s casein hydrolysis moved in front of the original band. Visser and Groot-Mostert (1977) studying the ripening process of Gouda cheese demonstrated that in

normal aseptic cheeses α_{s1}-casein was attacked rapidly, the degradation being nearly complete after one month of ripening. Beta-casein was more resistant to proteolysis: after 6 months of ripening about 50% was still intact. During cheese ripening κ-, β-, and α-caseins are degraded to compounds of lower electrophoretic mobility (Wong, 1980). During the ripening of Belgian Herve cheese PAGE studies showed that in the outer layer α_s and β-casein were broken down more strongly under the influence of the surface flora of this soft cheese (Weckx and Vanderpoorten, 1973).

Sodium chloride seems to have some influence on the proteolysis of casein by rennet and enzymes from starter, during ripening of cheese. Fox and Walley (1971) found that the proteolysis of β-casein by rennin and by pepsin was completely inhibited in the presence of 10% NaCl and was very significantly reduced by 5% NaCl during Cheddar cheese ripening. The rate of proteolysis of α_s-casein was maximal in the presence of 5-10% NaCl. These results are in agreement with those of Jong and Groot-Mostert (1977). Low concentrations of NaCl favored the breakdown of β-casein but 11% NaCl almost completely inhibited the proteolysis of this casein. Maximum breakdown of α_{s1} casein occurred at 3-5% NaCl; higher concentrations had an inhibitory effect but did not stop the reaction as in the case of β-casein. According to Phelan et al. (1973) β-casein is highly resistant to proteolysis in Cheddar cheese. A decrease in NaCl

concentration reduced its resistance, but even in the absence of salt the amount of proteolysis of β-casein was slight; the proteolysis in the cheese increased when the moisture levels were raised by adding water. The activity of proteolytic enzymes in a simulated soft cheese was studied by Noomen (1978). It was found that the degradation of α_{s1} casein was stimulated by NaCl concentrations in the moisture up to about 4%, and retarded by higher salt contents. The breakdown of β-casein was maximum in the absence of NaCl and was already considerably reduced at low salt concentrations.

Ledford et al. (1966) studied the residual casein fractions in several ripened cheese and found that in Camembert cheese most of the β-casein remains intact. Desmazeaud and Gripon (1977) report that PAGE of the pH 4.6 insoluble N fraction of an aseptic curd ripened by Penicillium roqueforti revealed a complete breakdown of α_{s1}- and β-casein and the occurrence of products having a low electrophoretic mobility. Desmazeaud et al. (1976) indicate that both P. roqueforti and P. camemberti have a very important endopeptidase activity and degrade simultaneously α_{s1}- and β-caseins.

The Flavor Components of Camembert Cheese

Cheese is a product which the consumer buys for its flavor more than anything else. For any type of cheese the ripening process can be described as a series of reactions related to lactic acid metabolism, lipolysis, oxidation and proteolysis which can be followed by reactions between the products formed in each pathway. The cheese flavor is highly related to the products from these reactions.

According to Adda et al. (1978), in Camembert cheese oct-1-en-3-ol plays a highly specific role and accounts for the pleasant mushroom note always present in that type of cheese. Dumont et al. (1974) studied the neutral volatile compounds in Camembert cheese and the main constituents present were identified as 3-methyl butanol, 2-heptanol, 2-nonanol, oct-1-en-3-ol (mushroom odor), 2-nonanone, 2-undecanone, 2-tridecanone, 2-heptanone, 2-phenyl ethanol and β-phenyl ethyl acetate. In another report, Dumont et al. (1977) isolated volatile components of Camembert cheese and detected several trace compounds such as bis (methylthro)methane, diethyldisulfide, 2.4.5.-trithia-hexane, anisole, 3-methylthro-propanol and 2.4-dimethyl-anisole. Some minor components in the aroma of Camembert cheese were identified by Moinas et al. (1975) as aromatic hydrocarbons and ketones, aromatic esters, an aldehyde containing sulfur and a nitrite. Comparisons of mature and

immature Camembert cheeses indicated that the latter contained more short-chain methyl ketones particularly acetone (20-40%) and low levels of oct-1-ene-3-ol (Moinas et al., 1973). By comparing Blue and Camembert cheese, Schwartz and Parks (1963) observed that all of the ketones found in the Camembert cheese were present in Blue cheese, but the latter contained much more ketone than does Camembert. A close similarity is observed by Moinas et al. (1973) between Camembert and Roquefort volatile aroma constituents which are characterized in common by methyl ketones and by primary and secondary alcohols.

Dumont et al. (1976) studied the influence of psychrotrophic bacteria on the quality of Camembert cheese and found that cheeses made from milk stored for 9 days at $5^{o}C$ had a bitter and rancid flavor and lacked the ammonia characteristic. These cheeses also contained more free fatty acids and 2-alkanones and 2-alkanols than cheeses made from milk stored for 2 days at $5^{o}C$. According to Martley (1975) the proteolytic activity of the surface mold flora of Camembert may play a major role in producing non-bitter precursors, which are then degraded to bitter peptides by the proteases of the lactic streptococci.

New Methods of Production of Camembert Cheese

The use of ultrafiltration concentrate from skim milk in Camembert cheese manufacture was studied by Kammerlehner (1977). Results showed that Camembert cheese of satisfactory quality could be produced from the concentrate under the following conditions: UF temperature 60-63^{o}C, degree of concentration 1:3 and pre-ripening of the concentrate at 10-12^{o}C for 10-16 hours to a pH of 6.2-6.0. Voss (1976) reported that Camembert cheese made from ultrafiltration concentrates (UFC) showed considerably higher yield due to the retention of whey proteins and that consistency and flavor corresponded to traditionally made Camembert. These results are in agreement with those of Prokopek et al. (1975) who found that it would be possible to produce a good quality Camembert cheese from ultrafiltration concentrates. According to Maubois (1973) the major advantage of the use of ultrafiltration in the manufacture of Camembert cheese is increased yield (by 16-20% of the SNF portion) due to very low losses of nitrogenous compounds. Besides, cheeses have more uniform weight and considerable savings are achieved in the use of rennet. Jepsen (1977) reports that by using membrane filtration for making Camembert cheese the product yield was increased and lower amounts of rennet and culture were required.

Krsev (1973) studied the use of concentrated frozen cultures in Camembert cheese manufacture. Best results with experimental cheeses were obtained when 0.8% of the concentrated frozen culture was used. Yield, composition and organoleptic quality of these cheeses was equal to those of customarily produced cheeses. It was observed by Peters and Knoop (1974) that Camembert cheese made from milk powder presented a porous structure that caused quicker penetration of the brine and quicker ripening of the cheese. Tsugo and Matsuoka (1963b) observed that when Camembert cheese was made from milk with acidity adjustment with lactic acid immediately following addition of lactic starter had a good flavor and showed the same texture as that of cheese made by the conventional procedure.

EXPERIMENTAL PROCEDURES

Preparation of Quick Ripened Camembert Cheese

For this study, Camembert cheese curd was produced from milk received by the Dairy Plant at Michigan State University. A total of twenty seven 39 kg lots of pasteurized milk ranging in fat content from 1.9 to 4.58% were used.

The manufacturing procedure was an adaptation of the methods given by Kosikowski (1978) and Veisseyre (1975). Milk was pasteurized at 65°C (149°F) for 30 minutes and cooled to 35°C (95°F). Direct-vat-set lactic culture from Chr. Hansen Laboratory, Inc. (5 ml) containing lactic acid producing bacteria, and 10 ml of single-strength rennet were then added to the vat and stirred for 2-3 minutes. Coagulation to the proper degree of firmness required about 50 minutes. The curd was cut with 1 cm horizontal and vertical knives. The curd cubes were then gently stirred while running hot water through the jacket of the vat to maintain the temperature at 35°C (95°F). After 20 minutes of continuous stirring all the whey was drained. The curd was dry salted (2.0%) by hand, placed upon a draining mat and left for 4 hours at room temperature (23°C). Then, the product was moved to a curing room maintained at 12.8°C

(55°F) and 95 percent relative humidity. After 16 hours the curd was sprayed with aqueous suspension of Penicillium caseicolum spores, obtained from the Laboratoires G. Roger, in France (Strain C). The cheese was held in the curing room for 21 days. After a ripening period of 8 days, the mold growth could be clearly seen at the cheese surface.

As the purpose of this study was to further refine and standardize the above procedure, many adaptations to the original method were attempted. These modifications involved ripening the milk to two different pH values, adding different salt concentrations, standardizing milk to different milk fat content, subjecting milk to homogenization and the use of an additional lipase preparation. The parameters of each modification are listed below. In general, the procedure described above was followed with the variables introduced as necessary for the study. All batches of cheese were made in duplicate or triplicate simultaneously in different vats arranged side by side.

1. CONTROL (duplicate)
   Milk fat - 3.5%.
   Total salt used - 2.0%.
   Milk pH - 6.7.
   Milk was not homogenized.
   No lipase preparation added.
2. MILK pH 6.43 (triplicate)
   Milk fat 3.5%.

Total salt used - 2.0%.

Milk pH - 6.43 (after ripening w/ starter).

Milk was not homogenized.

No lipase preparation added.

3. MILK pH 6.16 (triplicate)

Milk fat - 3.5%.

Total salt used - 2.0%.

Milk pH - 6.16 (after ripening w/ starter).

Milk was not homogenized.

No lipase preparation added.

4. LOW SALT CONCENTRATION (triplicate)

Milk fat - 3.5%.

Total salt used - 1.0%.

Milk pH - 6.7.

Milk was not homogenized.

No lipase preparation added.

5. HIGH SALT CONCENTRATION (triplicate)

Milk fat - 3.5%.

Total salt used - 3.0%.

Milk pH - 6.7.

Milk was not homogenized.

No lipase preparation added.

6. MILK OF HIGH FAT CONTENT (triplicate)

Milk fat - 4.5%.

Total salt used - 2.0%.

Milk pH - 6.7.

Milk was not homogenized.

No lipase preparation added.

7. MILK OF LOW FAT CONTENT (triplicate)

Milk fat - 1.9%

Total salt used - 2.0%.

Milk pH 6.7.

Milk was not homogenized.

No lipase preparation added.

8. USE OF ADDITIONAL LIPASE PREPARATION (triplicate)

Milk fat - 3.5%.

Total salt used - 2.0%

Milk pH - 6.7.

Milk was not homogenized.

Commercial preparation of bovine lipase (Lipase Powder no. 600, from Laboratory Miles) was added to the milk before setting (1 ounce/1000 lbs milk)

9. USE OF HOMOGENIZED MILK (triplicate)

Milk fat - 3.5%.

Total salt used - 2.0%.

Milk pH - 6.7.

No lipase preparation added.

After pasteurization, milk was homogenized at 1800 psi (1st stage) and 500 psi (2nd stage) at 54^{o}C (130^{o}F).

Based upon the information obtained from the above trials, the procedure was modified in order to test different

parameters to find an optimum method:

10. OPTIMUM PROCEDURE (duplicate)

Milk fat - 1.0%.

Total salt used - 1.0%.

Milk pH - 6.7.

After pasteurization milk was homogenized at 1800 psi (1st stage) and 500 psi (2nd stage) at 54°C (130°F). Commercial preparation of bovine lipase from the Laboratory Miles (Lipase powder no. 600) was added to the milk before setting (1 ounce/1000 lbs milk).

Chemical changes during curing of the curd in a loose form as well as in conventional disc form of Camembert cheese were monitored.

11. DIPPING THE CURD INTO MOLDS

The parameters were the same as for the optimum procedure (curd taken from same batches). After draining out the whey the curd was dipped into round molds, turned frequently for 30 minutes, dry salted and 4 hours later, taken to the curing room, along with the loose curd from optimum procedure.

Mold Maintenance and Inoculation

The commercial lyophilized spores of *Penicillium caseicolum* (Strain C, obtained from Laboratoires G. Roger, France) were suspended in 10 ml of sterilized distilled water and transferred to sterilized Czapek-Dox agar in

Petri dishes. The agar was incubated at $25\pm1^{o}C$ ($77\pm2^{o}F$) for 1 week and kept in refrigerator until used to prepare the aqueous spray solution. The culture was transferred every two weeks to a new agar.

Organoleptic Assessment

Sensory analyses were made separately by a panel of 4 to 5 experienced judges to evaluate flavor, general acceptability and overall preference of the samples. A commercial Camembert cheese, aged at least 60 days was used as the control. The experimental samples were evaluated after 12 days of ripening. Different code numbers were given to each sample for each panelist. Three digit codes were determined by throwing dice and those numbers were marked on plastic cups containing the sample ground in a mortar. For the presentation of the samples to the panelist, the arrangement was selected from a table of arrangements of 5 samples. A balanced Hedonic Scale (shown in Fig. 1) was used for rating each attribute. For instance, a score of 7 should be attributed to a sample presenting "very strong" flavor, and a score of 1 to one presenting "very mild" flavor, and so forth.

Figure 1. Sample form of questionnaire presented to the panel to evaluate flavor, general acceptability and overall preference of cheese.

Score Sheet for Camembert Cheese

Judge:_____ Date __/__/__

Instructions: Evaluate flavor, general acceptability and overall preference of each sample according to the appropriate 7 point rating scale for each attribute. Record results in table below each attribute.

	Flavor	General Acceptability	Overall Preference
	7 very strong	7 very acceptable	7 like extremely
	6 mod. strong	6 mod. acceptable	6 like moderately
	5 slt. strong	5 slt. acceptable	5 like slightly
	4 neither strong/mild	4 neither acceptable/unacceptable	4 neither like nor dislike
	3 slt. mild	3 slt. unacceptable	3 dislike slightly
	2 mod. mild	2 mod. unacceptable	2 dislike moderately
Sample Number	1 very mild	1 very unacceptable	1 dislike extremely

Comments:

ANALYTICAL PROCEDURES

Preparation of Samples

The cheese samples were analyzed 1 day after manufacturing for pH, total solids, fat content, Volatile Fatty Acids (VFA), Free Fatty Acids (FFA), Total Protein, Water Soluble Protein, and salt (as NaCl). Electrophoretic patterns of various samples were also determined. Then, at 7, 14 and 21 days of curing they were analyzed again for pH, protein degradation or soluble protein, free fatty acid titer and volatile acidity. Electrophoretic patterns were determined again at 21 days of curing. For the electrophoretic determinations the fresh and ripened cheese samples were sealed in Barrier bags and held frozen at -20°C (-4°F) until the time of actual analysis.

pH

The pH measurements were made with a CHEMTRIX Type 60A digital pH/mv meter equipped with an ORION model 91-63 pH electrode designed for surface measurements. Before testing the pH meter was standardized with standard buffer solutions, pH 4.01 and 7.0, and manually set for the temperature of the product. After each determination, the

electrode was moved to test two other areas of the same cheese sample. An average of the three pH readings is reported in the Results section.

Fat

Fat content in all samples was determined by the Roese-Gottlieb method with Mojonier modification for cheese (Milk Industry Foundation, 1959). Steps followed were those described by Mojonnier Bros. Co. (1925) for determination of fat in cheese.

First Extraction

1. About 1 g of cheese was weighed into extraction flask using a butter boat.
2. Eight milliliters of distilled water were added to the sample in the extraction flask and mixed thoroughly.
3. Then, 1.5 ml of ammonia was added and mixed thoroughly.
4. Ten milliliters of 95% alcohol were added and the bottle was shaken for 30 sec.
5. Twenty five milliliters of ethyl ether were added, the bottle was covered with cork and shaken vigorously for 20 seconds.
6. Twenty five milliliters of petroleum ether were added, the bottle covered with cork and shaken vigorously for 20 sec.

7. Flasks were centrifuged 30 turns, taking 30 sec.

8. The ether mixture containing the extracted fat was poured off into previously weighed fat dishes. Prior to use, the empty fat dishes were heated in the vacuum oven at 135°C for 5 min and then cooled in the cooling disiccator for 7 min.

Second Extraction

1. This time, neither water, nor ammonia was added.

2. Five milliliters of alcohol were added to the residue in the flask and it was shaken for 20 sec.

3. Then 25 ml of ethyl ether were added, the bottle covered with cork and mixed for 20 sec.

4. Twenty five milliliters of petroleum ether were added, the bottle covered with the cork and mixed 20 sec.

5. Flasks were centrifuged 30 turns, taking 30 sec.

6. The ether-fat solution was poured off into the same fat dish used in the first extraction. When it was necessary to raise the dividing line in the extraction flask, a proper amount of distilled water was added just before pouring. Care was taken not to pour off any of the residue below the ether solutions.

7. Ether was evaporated off from the fat dishes on the electric hot plate at 135°C for 5 min with no less than 20 inches of vacuum.

8. Dishes were cooled for 7 min in the desiccator.

9. Dishes were rapidly weighed, results recorded and used for calculations of the percentage of fat.

For each sample, the test was done in duplicate and the average was reported.

Moisture

Moisture content of cheese samples was determined in duplicate by Vacuum Oven Method, described by Kosikowski (1978) as follows:

1. Solid dish was prepared by heating in the vacuum oven at 100°C, for 1 hr., then removed and cooled in the desiccator for 30 min.

2. Cheese (2 g) was weighed into the prepared and pre-weighed solids dish.

3. The dish was placed on a hot plate at 180°C and heated until the first traces of brown color appeared.

4. Then the dish was transferred to the vacuum oven and held at 100°C for 4 hr., at 28 in. Hg.

5. The dish was then cooled in the desiccator for 5 min. and reweighed on an analytical balance.

6. The percentage moisture was calculated by the difference in weight of the original sample and the dried solids residue.

Salt

Salt was determined by the Modified Volhard Test described by Kosikowski (1978) as follows:

1. Cheese (3 g) was weighed and transferred to a 300 ml Erlenmeyer flask.

2. Twenty five milliliters of 0.1 N $AgNO_3$ were added from a burette.

3. Ten milliliters of halogen-free NHO_3 and 50 ml of distilled water were introduced into the flask.

4. The mixture in the flask was heated to boiling under a hood.

5. As it boiled, 15 ml of fresh 5% $KMnO_4$ solution were added in three 5 ml portions, each one introduced after the purple color of the mixture had changed to yellow.

6. Then, the digested yellowish solution was cooled to room temperature and distilled water was added to make the volume to approximately 100 ml.

7. After mixing, it was decanted, leaving precipitate behind, rewashed with 100 ml distilled water and decanted once again.

8. Then, 2 ml of saturated ferric ammonium sulfate were added.

9. The contents, while stirring, were titrated directly with 0.1 NKSCN to a brick-red end point.

10. The salt content was then determined by the

following formula:

$$\frac{(25 \text{ ml } 0.1N \text{ } AgNO_3 - \text{ML } 0.1N \text{ KSCN}) \times 0.0058}{3.0} \times 100 = \% \text{ NaCl}$$

For each sample the test was done in duplicate and the average was reported.

Total Protein

Total protein in cheese samples was determined by the Micro-Kjeldahl method described by the Association of Official Agricultural Chemists (1975) for cheese:

1. Approximately 0.05g of cheese was weighed and transferred to a 30 ml digestion flask.

2. Two grams of K_2SO_4, 40 mg of HgO and 2.3 ml of concentrated H_2SO_4 were added to the digestion flask along with boiling chips.

3. The samples were then digested for 90 min in the digestion rack heaters, under a hood.

4. After digestion, the flasks containing the samples were cooled and about 3 ml of distilled water were added to dissolve solids.

5. The digest was then transferred to the distillation apparatus and the flask was rinsed twice with 2 ml portions of distilled water.

6. Next, 10 ml of the sodium hydroxide-sodium thiosulfate solution (60 g NaOH and 5 g $Na_sS_2O_3 \cdot 5H_2O$ dissolved

and made to 100 ml with distilled water) were added to still.

7. A 50 ml Erlenmeyer flask containing 5 ml saturated H_3BO_3 solution and 4 drops of methyl red-methylene blue indicatorwas placed under condenser with tip extending below surface of solution.

8. Approximately 20 ml distillate was collected and titrated to end point with a 0.02 N HCl solution.

9. Simultaneously, a blank was run which consisted of no cheese but all reagents used in cheese protein analysis.

10. Formula used for calculations was:

% total proteins in cheese =

$$\frac{(\text{ml HCl sample} - \text{ml HCl blank}) \times 0.02 \times 14.007 \times 100 \times 6.38}{\text{weight of sample}}$$

For each sample, the test was done in duplicate.

Soluble Protein

Soluble protein in cheese was determined (Kosikowski, 1978) and in this procedure Sharp's extracting solution was prepared as described below:

A. Stock solution:

57.5 ml	glacial acetic acid
136.1 g	sodium acetate (3 H_2O)
47.0 g	sodium chloride
8.9 g	calcium chloride (anhydrous)

Add distilled water to make up to 1 liter.

B. Extraction solution:

Make 250 ml stock solution A up to 1 liter with distilled water. The pH of this solution is 5.5.

1. Three grams of cheese were weighed and placed in a porcelain mortar. A small amount of extracting solution at 50°C was added and the cheese was ground to a thick paste.

2. Diluted suspension of cheese was transferred to a 100 ml volumetric flask and more extracting solution was added to the 100 ml mark. The flask was placed in a water bath at 50°C and maintained at this temperature for 1 hr.

3. The suspension was then filtered through a Whatman No. 1 fluted filter paper and 4 ml of the filtrate was placed in a 30 ml digestion flask.

4. Digestion and distillation steps were conducted as for total protein given above. The formula used for calculations was:

$$\% \text{ Soluble Proteins in cheese} = \frac{(\text{ml HCl sample} - \text{ml HCl blank}) \times 0.02 \times 14.007 \times 100 \times 6.38}{\text{weight of sample in the aliquot}}$$

All the tests were run in duplicate.

Free Fatty Acid (FFA) Titer

FFA titer was determined by the rapid silica gel method for measuring free fatty acids reported by Harper et al. (1956). This method is outlined below.

Materials

1. A chromatographic column, 38 mm diameter by 230 mm in length with a perforated glass disc sealed into a 34/23 standard taper joint attached to a suction flask.

2. Silicic acid, 100 mesh powder.

3. 2 M phosphate buffer, pH 6.4. Stock solutions of 2 M KH_2PO_4 (27.2 g/100 ml) and 2 M K_2HPO_4 (34.8 g/100 ml) were prepared and mixed. The pH was checked and adjusted to 6.4 using a pH meter.

4. Buffered silica gel slurry. A stock solution of the slurry was prepared by mixing thoroughly 50 g of dry silicic acid with 30 ml of the phosphate buffer and 200 ml of USP chloroform. The slurry was stored in a tightly stoppered brown bottle at room temperature.

5. Eluant, 5% N-butanol in chloroform (v/v).

6. 20% H_2SO_4.

7. Titrating alkali, KOH solution, 0.01 N in absolute alcohol.

8. Phenol red indicator, pH 7-8 (0.1% phenol red dissolved in absolute alcohol).

Preparation of Cheese Sample

Five grams of the cheese sample were dissolved in 5 ml of H_2O and acidified to pH 1.8-2.0 (predetermined) with 0.3 ml of 20% H_2O_4 to liberate free fatty acids and to stop further lypolysis. Eighteen grams of silica gel were added and the mixture was ground thoroughly. Then it was transferred to the top section of the column.

Preparation of Chromatographic Column

The column was prepared in two sections:

1. Bottom Section

The column was attached to a 250 ml suction flask. A filter paper disc was placed on the sintered glass bottom of the column, and 25 ml of the silica gel slurry was poured. The suction was applied quickly to form a uniform bed of the buffered silica acid.

2. Top Section

The acidified sample and silica gel mixture was slurried with 50 ml of 5% n-butanol in chloroform (v/v) and transferred quantitatively on to the top of the bottom section of column. This was repeated with two more installments of 50 ml each of the eluant. In order to expedite the extraction, suction was applied so that the eluant flow was approximately 30 ml/min. Fifteen milliliters of methanol and 4 ml of phenol red indicator were added to the eluate and titrated with 0.01 N alcoholic KOH. The results

were expressed as net micromoles (mol) FFA/g cheese fat, after correction of the titer value for observed acidity of the blank sample. All samples were run in duplicate and results expressed as the average.

Volatile Acidity

Volatile acidity was determined by the rapid direct distillation method for determining the volatile acids of cheese (Kosikowski and Dahlberg, 1946).

1. A 10 g sample of cheese was weighed and thoroughly ground in a porcelain mortar with warm (50-55^{o}C) 10% H_2SO_4. The ground cheese mixture was washed quantitatively from the mortar into a 500 ml 2-neck distillation flask with a total of 50 ml H_2SO_4 solution, including both grinding and washing.

2. Ten Hengar granules (Hengar Co., Philadelphia, PA), for smooth boiling and 35 g of $MgSO_4 \cdot 7H_2O$ were added to the distillation flask and exactly 250 ml of distilled water at 25^{o}C were added. Antifoam was sprayed into flask.

3. Next, the flask was placed in heating mantle and connected to distilling tube. Powerstat was turned to setting 105 and flask heated rapidly to a boil.

4. Distillation was begun with the distillate passing through slightly moistened filter paper (Whatman No. 2) of 18.5 cm diameter and was continued until 250 ml distillate had been collected. The temperature ranged from 100 to 106^{o}C

and never over 110°C. Approaching the end of distillation (200 ml distillate collected) the powerstat setting was lowered to 90 to prevent burning of the sample. This distillate was the water-soluble portion.

5. This distillate was titrated with 0.05 N NaOH, using phenolphthalein as an indicator. The final results were expressed according to standard form as ml 0.01 N volatile acid per 100 g cheese.

6. Absolute alcohol (25 ml) was used to rinse the insoluble acids from the condenser through the filter paper and into a small Erlenmeyer flask. Alcohol rinsings were titrated in a manner similar to that employed with the water-soluble distillates. The sum of the titrations of the water-soluble distillates and of the alcohol rinses were considered the total volatile acidity of the cheese. Duplicates were run on all samples.

Discontinuous Polyacrylamide Gel Electrophoresis (Disc-PAGE)

Disc-PAGE was performed according to technique adapted from Ornstein (1964) and Davis (1964). All electrophoretic studies were performed in 6 mm I.D., 2 mm walled and 125 mm length glass tubes. The tubes were detergent washed, immersed in chromic acid, treated with Photo-Flo (0.5% v/v, aqueous solution) rinsed with distilled water and dried before use. Electrophoresis was carried out in a

water-cooled Bio-Rad Model 150A electrophoresis apparatus and power was supplied by a Bio-Rad Laboratories Model 500 power supply. Gels were stained for protein with Coomassie Brilliant Blue G250 (0.04%) for 15 hours and destained with a Bio-Rad Laboratories Model 1200A Electrohporetic Destainer.

Electrophoresis Solutions

1. Running gel buffer, pH 8.9, 0.38 M Tris-HCl, 7 M Urea:

-4.6018 g Tris and 42 g urea were dissolved in about 70 ml distilled water and HCl was added to pH 8.9; then, the solution was made up to 100 ml with distilled water, and filtered.

2. Running gel solution, 25% (w/v) acrylamide solution:

-0.32 g N,N-Methylenebisacrylamide (BIS) and 12.415 g acrylamide monomer were dissolved in 50 ml of running gel buffer and filtered.

3. Stacking gel buffer, pH 6.7, 0.062 M Tris-HCl, 7 M Urea:

-0.7508 g Tris and 42.0 g urea were dissolved in about 70 ml distilled water and HCl was added to pH 6.7; then, the solution was made up to 100 ml with distilled water and filtered.

4. Stacking gel solution, 6.2% (w/v) acrylamide solution:

-0.625 g BIS and 12.415 g acrylamide monomer were dissolved in 50 ml of stacking gel buffer and filtered.

5. N,N,N',N'-tetramethylethylene diamine (TEMED).

6. Electrode buffer, pH 8.3, 0.046 M Tris-glycine:

-16.71 g Tris were dissolved in about 2850 ml distilled water and glycine (2 M) solution was added to pH 8.3; next, the solution was made up to 3000 ml with distilled water and filtered.

7. Ammonium persulfate solution, 5.0% (w/v) 7 M Urea:

-0.625 g Ammonium persulfate and 5.25 g urea were dissolved in 12.5 ml of distilled water and filtered. The solution was prepared immediately before using.

8. Urea extraction solution, 7 M:

-42.04 g of urea were dissolved in about 60 ml of distilled water and volume made up to 100 ml, followed by filtration.

9. Bromophenol blue, 1% (w/v):

-0.1 g bromophenol blue were dissolved in about 8 ml stacking gel buffer and made up to 10 ml with the same buffer, followed by filtration.

10. Saturated Sucrose Solution

-Sucrose of high purity was dissolved up to complete saturation in 20 ml of stacking gel buffer, followed by filtration.

11. Staining solution

-250 ml 2-propanol, 100 ml acetic acid and 0.4 g Coomassie Brilliant Blue were mixed and made up to 1000 ml with distilled water, followed by filtration.

12. Destainer Solution

-80 ml acetic acid and 200 ml 2-propanol were mixed and made up to 2000 ml with distilled water, followed by filtration.

All solutions prepared were stored at $3^{o}C$.

Preparation of Gel Tubes

1. The dry tubes were marked with a felt tip pen at distances 10.0 and 11.6 cm from the bottom.

2. The bottom of each tube was fitted with a small square of plastic wrap held on with a rubber adapter. Each tube was then placed in the leveled rack.

3. Running gel solution (10.8 ml) and running gel buffer (18.84 ml) were mixed to yield 29.64 ml of solution of 9% acrylamide concentration when made to 30 ml.

4. Ammonium persulfate (0.36 ml) and TEMED (24 µl) were then added to the solution.

5. Using a 18 gauge needle, each tube was filled to the 10 cm mark.

6. Carefully, the gels were overlaid with distilled water using a 100 µl syringe and allowed to polymerize for 16 hours.

7. Following polymerization, the water was removed from the gel surface with a paper tissue and care was taken not to disturb the gel surface.

8. Then, the gel surface was rinsed by adding a small amount of stacking gel buffer with the 100 µl syringe.

The rinse liquid was removed by dipping a tissue paper in it.

9. To a 10 ml volumetric flask 5 ml of stacking gel solution and 1 g of sucrose were added and made to volume with stacking gel buffer. After mixing, the solution was transferred to a small beaker.

10. Ammonium persulfate (40 µl) and TEMED (10 µl) were then added to the solution.

11. Using the 18 gauge needle each tube was filled to the 11.6 cm mark.

12. Carefully the gels were overlaid with distilled water using the 100 µl syringe and allowed to polymerize for approximately 30 minutes.

Preparation of Samples

1. Fifty milligrams of ground cheese was dissolved in 2 ml of 7 M urea extraction solution and made to 3 ml with distilled water.

2. The mixture was gently shaken and kept in a water bath at $37^{o}C$ for 1 hr.

3. Then, two drops of 2-mercaptoethanol were added and the sample was kept in the water bath for an additional 45 minutes.

4. Right before electrophoresis, 5 µl of Bromophenol Blue and 125 µl of saturated sucrose solution were added to the sample.

Electrophoresis

1. The gel tubes were then inserted into the grommets of the electrophoretic chamber and electrode buffer was poured in both the cathodic (upper) and anodic (lower) chambers.

2. Using the 100 µl syringe about 200 µg of sample was added to the interfacial layer just on top of the gel.

3. The power supply was connected and current adjusted to about 2.0 mA/tube. Electrophoresis was carried out for about 6 hours, until the tracking dye (Bromophenol Blue) had migrated to the bottom portion of the tubes.

4. At the completion of electrophoresis, power supply was turned off and the gel tubes removed from the upper reservoir.

5. The gels were then removed from the tubes by rimming under water.

Staining

The gels were then placed in test tubes containing the staining solution (0.04% Coomassie Brilliant Blue) and stained for about 15 hours at room temperature.

Destaining

Electrophoretic destaining was performed for 60 min in a destainer solution containing 4% acetic acid and 10% 2-propanol.

Gel Densitometry

Gels were scanned using a Beckman DU Spectrophotometer, Model 2400 (Beckman Instruments, Inc., Fullerton, CA) equipped with a gel scanner Model 2520 and a photometer 252 by Gilford (Gilford Instrument Laboratories, Inc., Oberlin, OH). This system was summoned to an HP Integrator Model 3380 S (Hewlett Packard, Avondale, PA). The gels were scanned at a rate of 0.5 cm/min and a chart speed of 1.0 cm/min. Start delay and slope sensitivity settings were 0 and 3.0 mV/min., respectively. Attenuation was set at 128. Gels were scanned at a wavelength of 550 nm. The relative areas of the individual protein peaks were recorded.

RESULTS AND DISCUSSION

Due to the number of modifications that were attempted the results and discussion were arranged in the following manner. A general description of the results as a whole is given followed by a discussion of each modification. The data are presented in tables and many of them are plotted in figures for discernibility of trend in each case. In Figures 2, 3, 4, 5 and 6, the Volatile Fatty Acids (VFA) are expressed as ml of N/10 acid per 100 g of cheese, the Free Fatty Acids (FFA) are expressed as micromoles ($_{\mu}$mol) per gram of cheese fat, and water Soluble Protein expressed as percentage of Total Protein of the cheese. These results were statistically analyzed through Analysis of Variance and comparisons of all means to control mean were made at 5% level of significance by the modified Tukey's Test, according to Gill (1978).

Effect of Ripening Milk with Starter to Different pH Before Setting

The loose curd Camembert cheese was prepared from milk ripened to pH 6.43 and 6.16 with starter and compared to the control cheese whose pH was 6.7 at setting time. The

results are shown in Tables 2, 4, 6 and Figure 2. The chemical composition of the fresh cheeses is presented in Tables 1, 3 and 5. The initial acidity of the milk plays an important role in the manufacture and ripening of the cheese, and is due to the activity of lactic acid-forming bacteria. The development of acidity can be controlled within the most favorable limits by using pasteurized milk, by varying the proportion of starter and by permitting the milk to ripen for 1 hour after addition of starter (Wong, 1980).

In all cases there was an increase in the cheese pH during ripening. At 1 day of ripening control cheese had a pH of 5.08 and cheeses from milk pH 6.43 and 6.16 had a pH of 5.36 and 5.65, respectively. The lower pH in control cheese may be due to the fact that this cheese was kept longer at room temperature than the others, allowing more intense acidification. It may be seen that although the pH of cheese increased gradually from 1 to 21 days of ripening, no significant difference was observed at 5% level.

In the same tables and figures are presented the results of soluble protein during ripening. Cheese made from milk at pH 6.43 presented an increase from 5.13 to 37.75% in water soluble protein. The same trend was observed in cheese made from milk at pH 6.16, with water soluble protein increasing from 6.28 to 50.74% in 21 days, as compared to from 4.16 to 53.90% in the control cheese, in the same

Table 1. Chemical composition of fresh control cheese.

Batch	A	B	Mean
pH[4]	5.03	5.12	5.08
Moisture	47.03	51.17	49.10
Total Solids	52.97	48.83	50.90
Fat	21.84	17.06	19.45
Fat in Dry Matter	41.23	35.00	38.21
Volatile Fatty Acids[1]	8.30	6.75	7.53
Free Fatty Acids[2]	41.80	35.20	38.50
Total Protein (TP)	24.35	23.85	24.10
Soluble Protein (SP)	1.05	0.95	1.00
(SP/TP)x(100)[3]	4.31	3.98	4.15
Salt (NaCl)	1.50	1.94	1.72
Salt/Moisture	3.09	3.65	3.38

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]μmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

[4]All other data, except pH, expressed as percentage.

All data are the average of duplicate tests.

Table 2. Changes in pH, volatile fatty acids, free fatty acids and soluble protein of control cheese during ripening.

Batch	Time of Ripening (days)			
	1	7	14	21
		pH		
A	5.03	5.29	5.65	6.75
B	5.12	5.34	5.75	6.78
Mean	5.08	5.32	5.70	6.77
		Volatile Fatty Acids[1]		
A	8.30	8.94	32.28	33.20
B	6.75	7.94	30.47	34.10
Mean	7.53	8.44	31.38	33.65
		Free Fatty Acids[2]		
A	41.80	119.70	843.40	1205.30
B	35.20	110.20	995.50	1328.40
Mean	38.50	115.00	919.50	1266.90
		Soluble Protein[3]		
A	4.31	14.05	25.67	54.03
B	3.98	10.10	23.85	53.67
Mean	4.15	12.08	24.80	53.90

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]μmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

period. No significance at 5% level was found among these results. The increase in pH seems to be related to the increase in water soluble protein during ripening. The increase in pH is caused by the destruction of the lactic acid, formation of non-acidic transformation products and appearance of weaker or less dissociated acids like acetic and carbonic. The liberation of alkaline products of protein decomposition contribute further to pH rise (Torres, 1979). It was found by Stadhouders and Langeveld (1966) that many yeasts and molds can break the lactic acid down into carbon dioxide and can hydrolyze the proteins to ammonia.

A net increase in volatile fatty acids (VFA) and free fatty acids (FFA) was also observed, as can be seen in Tables 2, 4, 6 and Figure 2. Volatile fatty acids of cheese are those acids freed during cheese ripening and recovered by steam distillation. Neutral fat and lactose serve as the basic sources of volatile fatty acids (Kosikowski, 1978). It is expressed generally as ml of N/10 acid per 100 g of cheese. In control cheese, the VFA increased from 7.53 to 33.65 ml in 21 days. In cheese made from milk with pH 6.43 and 6.16 the VFA increased from 10.93 to 65.23 ml and from 8.65 to 54.64 ml respectively, in 21 days. A significant difference at 5% level was found between control cheese (pH 6.7) and cheese made from milk pH 6.43 and 6.16. The ripening of milk, bringing about a greater number of bacteria in the curd, may be the cause of this difference.

Table 3. Chemical composition of fresh cheese made from milk ripened to pH 6.43.

Batch	A	B	C	Mean
pH[4]	5.55	5.52	5.02	5.36
Moisture	57.07	57.52	53.03	55.88
Total Solids	42.93	42.48	46.97	44.12
Fat	16.05	16.46	22.76	18.42
Fat in Dry Matter	37.39	38.75	48.46	41.75
Volatile Fatty Acids[1]	11.99	11.41	9.40	10.93
Free Fatty Acids[2]	24.59	17.35	33.95	25.30
Total Protein (TP)	21.23	20.90	18.71	20.28
Soluble Protein (SP)	1.23	1.10	0.78	1.04
(SP/TP)x(100)[3]	5.79	5.26	4.17	5.12
Salt (NaCl)	1.86	1.98	2.03	1.96
Salt/Moisture	3.16	3.33	3.69	3.39

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]μmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

[4]All other, except pH, expressed as percentage.

All data are the average of duplicate tests.

Table 4. Effect of ripening of milk to pH 6.43 on pH, Volatile Fatty Acids, Free Fatty Acids and Soluble Protein contents of cheese during ripening.

Batch	Time of Ripening (days)			
	1	7	14	21
			pH	
A	5.55	5.29	6.39	7.01
B	5.52	5.29	6.12	7.02
C	5.02	5.41	6.43	6.80
Mean	5.36	5.33	6.31	6.94
			Volatile Fatty Acids[1]	
A	11.99	10.36	33.77	60.10
B	11.41	7.78	34.78	71.84
C	9.40	12.40	37.41	63.76
Mean	10.93	10.18	35.32	65.23
			Free Fatty Acids[2]	
A	24.59	84.70	657.45	958.70
B	17.35	73.27	663.44	896.20
C	33.95	98.40	747.20	1122.20
Mean	25.30	85.46	705.32	992.37
			Soluble Protein[3]	
A	5.79	16.60	22.80	36.74
B	5.26	12.10	24.64	36.40
C	4.17	15.34	33.67	40.10
Mean	5.13	14.68	27.04	37.75

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]µmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

Figure 2. Changes in pH, volatile fatty acids, free fatty acids and soluble protein during ripening of control cheese (C) and cheese made from milk ripened to pH 6.16 (A) and 6.43 (B).

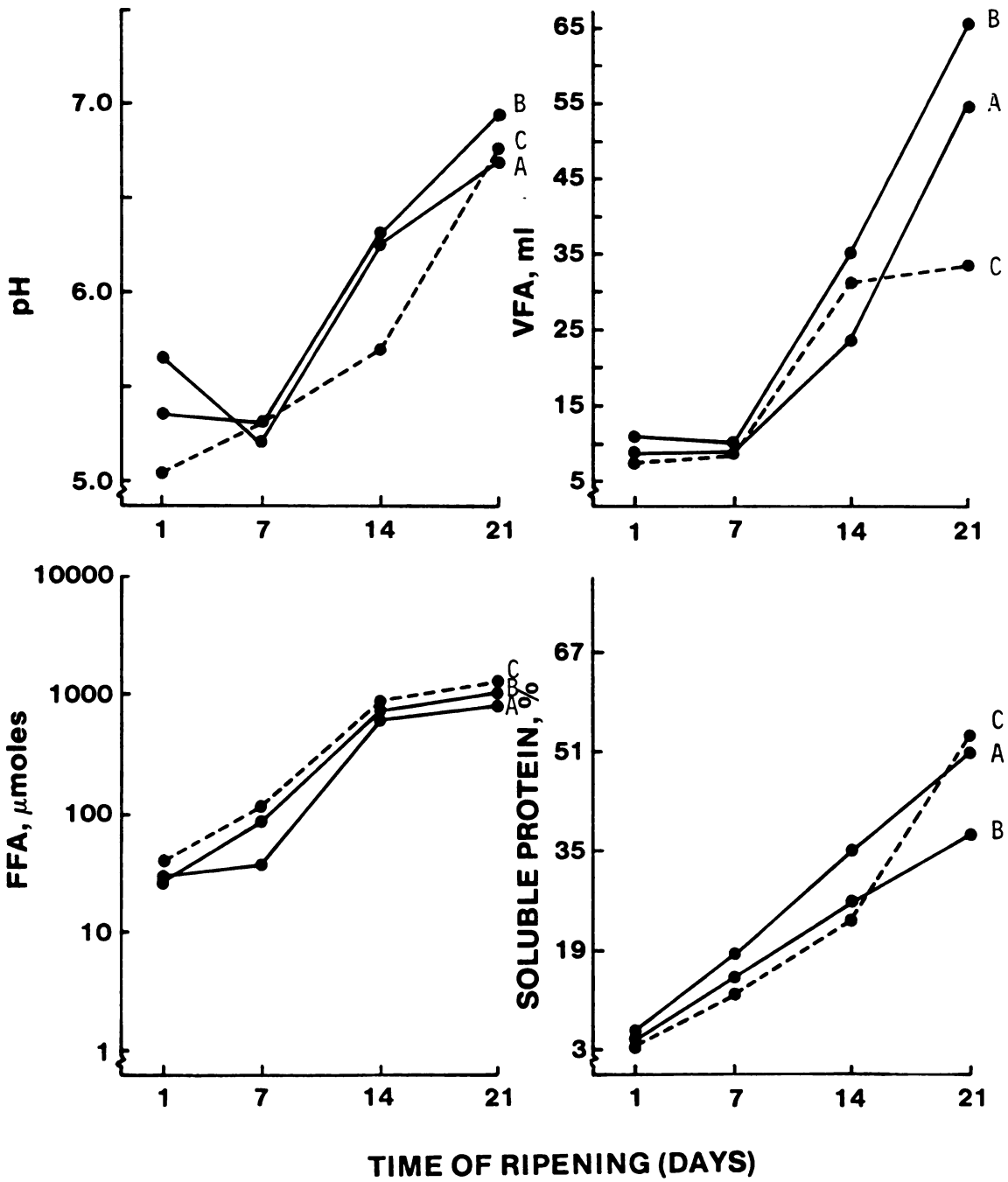

pH
7.0
6.0
5.0
B
C
A
VFA, ml
65
55
45
35
25
15
5
B
A
C
FFA, μmoles
10000
1000
100
10
1
C
B
A
SOLUBLE PROTEIN, %
67
51
35
19
3
C
A
B
1
7
14
21
TIME OF RIPENING (DAYS)

Table 5. Chemical composition of fresh cheese made from milk ripened to pH 6.16.

Batch	A	B	C	Mean
pH[4]	5.75	5.67	5.53	5.65
Moisture	50.59	60.13	59.74	59.82
Total Solids	40.42	39.87	40.26	40.18
Fat	21.21	18.36	18.66	19.41
Fat in Dry Matter	52.47	46.05	46.35	48.31
Volatile Fatty Acids[1]	8.87	8.41	8.68	8.65
Free Fatty Acids[2]	22.94	29.66	31.56	28.05
Total Protein (TP)	15.74	16.22	16.32	16.09
Soluble Protein (SP)	0.92	1.11	0.99	1.01
(SP/TP)x(100)[3]	5.84	6.84	6.07	6.28
Salt (NaCl)	2.21	2.29	2.48	2.33
Salt/Moisture	3.58	3.67	3.98	3.75

[1] Expressed as ml N/10 acid per 100 g cheese.

[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

[4] All other data, except pH, expressed as percentage.

All data are the average of duplicate tests.

Table 6. Effect of ripening of milk to pH 6.16 on pH, Volatile Fatty Acids, Free Fatty Acids and Soluble Protein contents of cheese during ripening.

Batch	Time of Ripening (days)			
	1	7	14	21
		pH		
A	5.75	5.38	6.08	6.58
B	5.67	5.12	6.27	6.94
C	5.53	5.15	6.36	6.66
Mean	5.65	5.22	6.24	6.73
		Volatile Fatty Acids[1]		
A	8.87	8.01	30.48	56.95
B	8.41	10.20	22.25	50.10
C	8.68	9.03	17.93	56.88
Mean	8.65	9.08	23.55	54.64
		Free Fatty Acids[2]		
A	22.94	30.45	578.29	859.46
B	29.66	39.26	786.71	823.40
C	31.56	37.81	563.93	769.73
Mean	28.05	35.84	642.98	817.53
		Soluble Protein[3]		
A	5.84	20.45	40.98	48.16
B	6.84	17.69	29.64	53.88
C	6.07	16.36	34.31	50.18
Mean	6.28	18.17	34.98	50.74

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]µmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

According to Wong (1980) about 95% of bacteria present while the whey is draining are contained in the curd cubes and not in the whey, indicating that early lactic acid fermentation occurs primarily within the cubes.

The ripening of the milk with starter in general does not appear to influence substantially the rate of formation of free fatty acids in the loose curd Camembert cheese during ripening, as shown in Tables 2, 4, 6 and Figure 2. The free fatty acids titer is expressed as micromoles per gram of cheese fat. In control cheese the FFA titer was 1266.90 μmoles/g cheese fat after 21 days, as compared to 992.37 and 817.53 μmoles/g of cheese fat for cheeses made from milk ripened to pH 6.43 and 6.16, respectively. No significant difference was found as compared to control results. Lipases in cheese made from pasteurized milk arise from different sources such as microorganisms, rennet pastes and molds. According to Reiter et al. (1969), the lactic acid bacteria have a weak lipolytic activity in cheese ripening. The native milk lipase is inactivated during pasteurization at 63°C for 30 min. From these results, it appears that there is a linear relationship between the formation of VFA and FFA, and the increase in the cheese pH during ripening. The higher the pH, the higher amounts of VFA and FFA were produced. The results are in agreement with the statement of Lamberet (1974) who found that the optimum pH for lipolytic activity of Penicillium caseicolum was around 8.0.

According to Stadhouders and Mulder (1959) molds can split fat on the cheese surface and are able to hydrolyze the fat into glycerol and fatty acids. Recent work by Al-Hir (1982) has shown the optimum pH to be around 9.0.

The fresh curds showed basically the same composition, except for moisture that was clearly lower in control cheese, as can be seen in Tables 1, 3 and 5. This must be due to differences in the treatment, since this curd was left longer at room temperature. Both in cheese made from milk with pH 6.43 and 6.16 it was observed a slight decrease in pH from 1 to 7 days of ripening. It may be conjectured that it is due to final fermentation of lactose left in the cheese after manufacture. According to Veisseyre (1975) 5 to 8% of milk lactose is transferred to cheese and is gradually fermented to lactic acid, lowering the pH in the first days of ripening.

Effect of Fat in Dry Matter (FDM) on the Ripening of Loose Curd Camembert Cheese

To study the effect of fat content in the ripening of loose curd Camembert cheese, the cheese was prepared with a low fat content in dry matter (average of 30.14%) and a high fat content in dry matter (average of 52.50%), and compared with control cheese whose fat content in dry matter was 38.21%. The fat plays an essential role in the cheese ripening, since products from its degradation are extremely important in the cheese flavor. According to Wong (1980) whereas milk proteins

Table 7. Chemical composition of fresh cheese with low fat content in dry matter (FDM)

Batch	A	B	C	Mean
pH[4]	5.32	5.34	5.22	5.29
Moisture	61.92	63.59	61.73	62.41
Total Solids	38.08	36.41	38.27	37.59
Fat	11.39	11.66	10.93	11.33
Fat in Dry Matter	29.91	33.69	28.56	30.14
Volatile Fatty Acids[1]	6.51	8.14	7.60	7.42
Free Fatty Acids[2]	56.57	49.45	52.08	52.70
Total Protein (TP)[3]	20.86	19.20	21.20	20.42
Soluble Protein (SP)	0.49	0.69	0.69	0.63
(SP/TP)x(100)[3]	2.35	3.59	3.25	3.04
Salt (NaCl)	2.18	2.13	2.13	2.15
Salt/Moisture	3.40	3.24	3.34	3.33

[1] Expressed as ml N/10 acid per 100 g cheese.

[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

[4] All others, except pH, expressed as percentage.

All data are the average of duplicate tests.

Table 8. Effect of low fat content in dry matter (FDM) on pH, volatile fatty acids, free fatty acids and soluble protein contents of cheese during ripening.

Batch	Time of Ripening (Days) 1	7	14	21
		pH		
A	5.32	5.94	6.61	6.68
B	5.34	5.98	6.73	6.65
C	5.22	5.87	6.57	6.82
Mean	5.29	5.93	6.64	6.72
		Volatile Fatty Acids[1]		
A	6.51	9.23	26.32	35.91
B	8.14	10.32	25.00	31.10
C	7.60	7.61	19.00	34.11
Mean	7.42	9.05	23.44	33.71
		Free Fatty Acids[2]		
A	56.57	429.86	1310.61	2823.90
B	49.45	603.72	1325.44	2763.50
C	52.08	392.19	1217.40	2960.00
Mean	52.70	475.30	1284.50	2849.10
		Soluble Protein[3]		
A	2.35	21.24	44.00	71.43
B	3.59	22.08	48.80	77.71
C	3.25	21.42	40.14	65.09
Mean	3.04	21.55	44.20	71.20

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]µmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

Figure 3. Changes in pH, volatile fatty acids, free fatty acids, and soluble protein during ripening of control cheese (C) and cheese with low (L) and high (H) fat in dry matter.

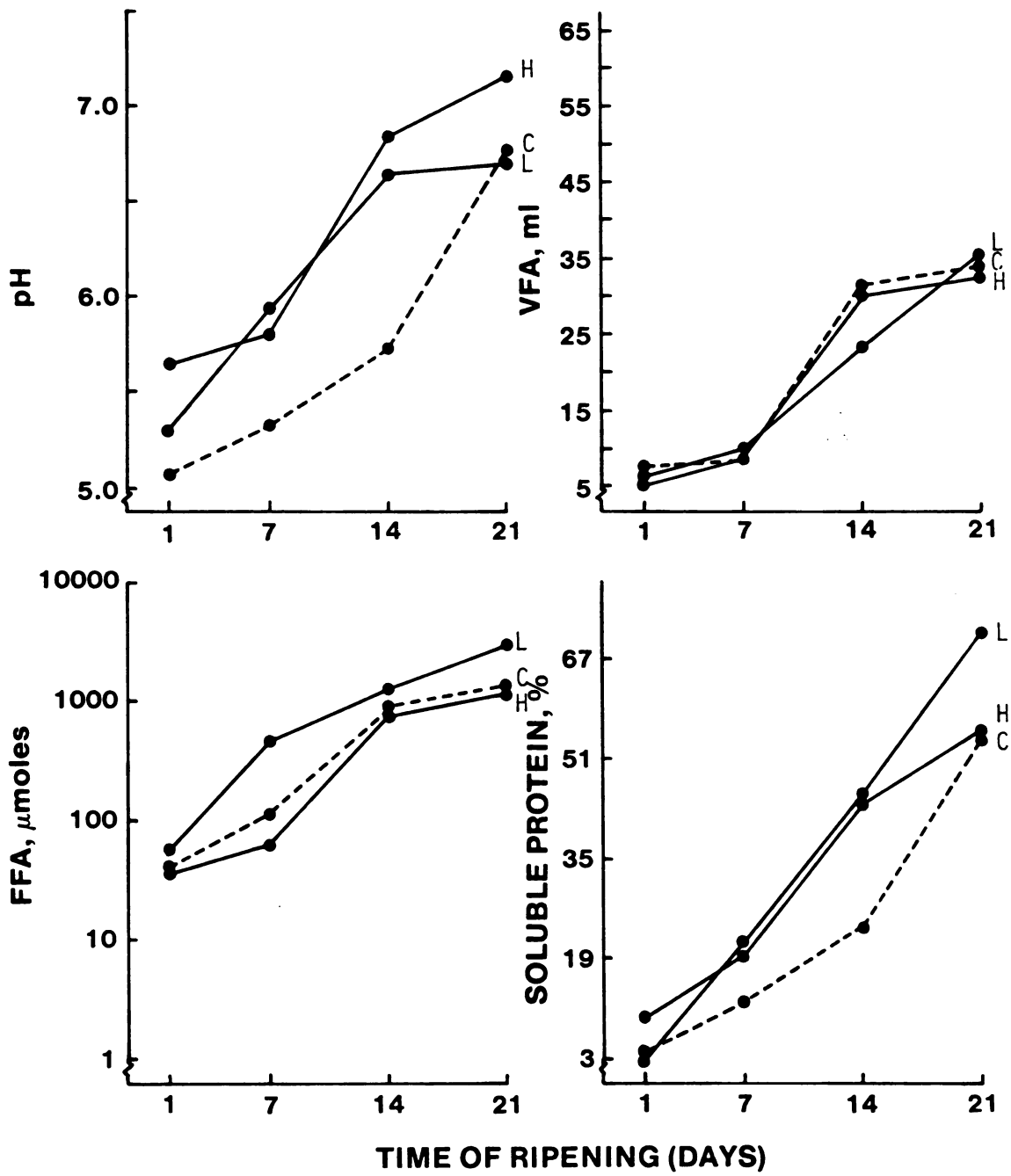

pH
7.0
6.0
5.0
H
C
L
VFA, ml
65
55
45
35
25
15
5
L
C
H
FFA, μmoles
10000
1000
100
10
1
L
C
H
SOLUBLE PROTEIN, %
67
51
35
19
3
L
H
C
1
7
14
21
TIME OF RIPENING (DAYS)

Table 9. Chemical composition of fresh cheese with high fat content in dry matter (FDM).

Batch	A	B	C	Mean
pH[4]	5.75	5.58	5.61	5.64
Moisture	57.06	55.74	55.35	56.05
Total Solids	42.94	44.26	44.65	43.95
Fat	22.37	24.15	22.70	23.07
Fat in Dry Matter	52.10	54.60	50.80	52.50
Volatile Fatty Acids[1]	4.61	4.34	7.33	5.43
Free Fatty Acids[2]	34.70	35.25	33.42	34.46
Total Protein (TP)[3]	14.96	16.09	15.84	15.63
Soluble Protein (SP)	1.28	1.18	1.48	1.31
(SP/TP)x(100)[3]	8.56	7.33	9.34	8.40
Salt (NaCl)	2.36	1.91	2.09	2.12
Salt/Moisture	3.97	3.31	3.64	3.64

[1] Expressed as ml N/10 acid per 100 g cheese.

[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

[4] All others, except pH, expressed as percentage.

All data are the average of duplicate tests.

Table 10. Effect of high fat content in dry matter (FDM) on pH, volatile fatty acids, free fatty acids and soluble protein contents of cheese during ripening.

Batch	Time of Ripening (Days) 1	7	14	21
		pH		
A	5.75	5.73	6.88	7.31
B	5.58	5.76	6.98	7.15
C	5.61	5.92	6.65	7.06
Mean	5.64	5.80	6.84	7.17
		Volatile Fatty Acids[1]		
A	4.61	10.31	32.58	28.79
B	4.34	6.51	33.65	36.90
C	7.33	7.60	27.13	33.12
Mean	5.43	8.14	31.12	32.94
		Free Fatty Acids[2]		
A	34.70	58.89	874.44	1161.83
B	35.25	58.56	625.69	1448.28
C	33.42	59.22	772.10	1109.85
Mean	34.46	58.89	757.41	1239.90
		Soluble Protein[3]		
A	8.56	20.45	47.53	52.81
B	7.33	17.78	36.11	59.54
C	9.34	19.32	46.72	52.97
Mean	8.40	19.18	43.45	55.11

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]µmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

and lactose are sources of many flavor precursors for Cheddar cheese, milk fat is perhaps more important because cheese made from skimmilk does not develop a typical flavor.

The results are shown in Tables 8 and 10, and plotted in Figure 3; the chemical composition of cheeses with low FDM and high FDM can be seen in Tables 7 and 9, respectively. The cheese pH does not seem to be substantially affected by variations in cheese fat. After 21 days of ripening, cheese with low FDM and high FDM showed a cheese pH of 6.72 and 7.17, respectively. There was a net stimulation of free fatty acids production in the cheese made with low FDM, which presented 2,849.1 μmoles/g cheese fat, as compared to 1266.90 and 1,239.9 μmoles/g cheese fat in control and high FDM cheeses, respectively. The difference was found to be significant at 5% level. As it can be seen in Tables 1, 7 and 9, low FDM cheese showed a significantly higher moisture content (62.41%) as compared to control cheese (49.10%) and high FDM cheese (56.05%). This may explain the higher amount of FFA produced. It is well known that cheeses with higher moisture content ripen faster (Alais, 1974; Veisseyre, 1975) and that cheese with lower fat content generally has a higher moisture content. As discussed earlier, cheese with low FDM presented a final pH of 6.72 and this could also be related to the higher FFA production. According to Stadhouders and Mulder (1957) and Raadsveld (1953) the fat of cheese with a low pH is hydrolyzed faster than the fat of cheese with high

pH. The same authors pointed out that the hydrolysis of fat in cheese is considerably reduced by pasteurization of cheese milk. This is not in agreement with the results of Soltys et al. (1975) who found that pasteurization of milk had no effect on kind or extent of protein and fat breakdown in the ripening of Camembert cheese. The production of volatile fatty acids appears not to be affected by the amount of fat in the cheese. Control cheese, low FDM and high FDM cheeses showed 33.65, 33.71 and 32.94 ml of N/10 acid per 100 g of cheese. No significant difference was found among these results.

The controbution of a low fat content to the protein breakdown during ripening may be fairly important. Although no significant difference has been found at 5% level, the amount of soluble protein (71.20% of the total protein) in the cheese with low FDM is higher than 53.90% and 55.11% found for the control cheese and high FDM cheese, respectively, after 21 days of ripening. As discussed earlier, low FDM cheese presented a higher moisture content, that could cause the difference found in soluble protein. According to Alais (1974) the casein is broken down faster in low-fat cheeses than in those presenting high fat content, the unsaturated fatty acids produced having an inhibitory effect on proteolytic bacteria.

As seen in the results being discussed, the pH, VFA, FFA and soluble protein increases gradually as a function of

time and ripening. As pH gradually increases, there is an increase in the production of FFA, VFA and soluble protein. This could be explained by the action of proteases and lipases produced by the Penicillium caseicolum whose optimal pH of activity are 6.0-8.5 and 8.0, respectively (Lamberet, 1974; Lenoir and Choisy, 1971). The results discussed here seem to indicate that a low fat content in the loose curd Camembert cheese favor the production of free fatty acids and protein breakdown during ripening.

Effect of Salt Concentration on the Ripening of Loose Curd Camembert Cheese

The concentration of salt is one of the most important factors controlling the characteristics of a cheese, as it affects its flavor and the development of microorganisms responsible for biochemical changes in the cheese during ripening. This study was undertaken to determine the effect of salt concentration in the ripening of loose curd Camembert cheese. Cheese was dry salted at two levels, averaging 1.97% and 4.60% in the moisture, as compared to 3.38% in control cheese. The salt concentration is presented here as percentage of total salt, as NaCl, dissolved in the cheese moisture (salt/water ratio). It appears that the salt content has a dominating effect on the water activity (a_w) of the cheese, that indicates, in fact, how far the water present is available for the microorganisms (Stadhouders and Langeveld,

1966).

The results are shown in Tables 12 and 14 and plotted in Figure 4. The chemical composition of fresh cheese with low and high salt/water is presented in Tables 11 and 13. Although no significant difference has been found as compared to control cheese results, low salt/water concentration cheese showed a higher pH (7.13 versus 6.56) and soluble protein (68.82 versus 54.43%) when compared to high salt/water concentration cheese. According to Alais (1978) the higher the salt content in the cheese, the lower the proteolysis. Wong (1980) indicates that the salting of Camembert cheese is said to aid in regulating the proper proportions of the organisms which effect the ripening. According to Raadsveld (1953) high salt concentrations in cheese may nullify the increased protein breakdown normally induced by a higher moisture content. The effect of water activity on the development of Penicillium candidum was studied by Stadhouders and Langeveld (1966), by measuring the mold growth at different salt (NaCl) concentrations in the cheese. At zero percent salt, a_w was 0.992 and mold growth, expressed as percentage of maximum growth, was 100%; increasing salt/water concentration to 5%, a_w decreased to 0.975 and mold growth to 80.9%, increasing salt/water concentration to 10% a_w decreased to 0.947 and mold growth to just 36.4%. Hardy (1979) found similar results for Camembert cheese, indicating that water activity is considerably lower at the cheese

Table 11. Chemical composition of fresh cheese presenting low NaCl content (1.97% in the cheese moisture).

Batch	A	B	C	Mean
pH[4]	5.38	5.73	6.05	5.72
Moisture	58.79	60.03	61.58	60.18
Total Solids	41.21	39.97	38.42	39.82
Fat	19.82	18.22	17.67	18.57
Fat in Dry Matter	48.10	45.60	46.00	46.60
Volatile Fatty Acids[1]	5.55	4.33	4.86	4.91
Free Fatty Acids[2]	28.80	24.98	18.27	24.02
Total Protein (TP)	17.02	17.88	16.50	17.13
Soluble Protein (SP)	1.42	1.09	1.09	1.20
(SP/TP)x(100)[3]	8.34	6.10	6.60	7.01
Salt (NaCl)	1.35	1.10	1.18	1.21
Salt/Moisture	2.24	1.80	1.88	1.97

[1] Expressed as ml N/10 acid per 100 g cheese.

[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

[4] All others, except pH, expressed as percentage.

All data are the average of duplicate tests.

Table 12. Effect of low NaCl content (1.97% water) on pH, volatile fatty acids, free fatty acids and soluble protein contents of cheese during ripening.

Batch	Time of Ripening (Days)			
	1	7	14	21
		pH		
A	5.38	6.37	6.55	6.76
B	5.73	5.76	6.32	7.27
C	6.05	5.63	6.28	7.36
Mean	5.72	5.92	6.38	7.13
		Volatile Fatty Acids[1]		
A	5.55	22.40	71.40	48.28
B	4.33	23.30	66.10	47.74
C	4.86	27.12	75.30	53.71
Mean	4.91	24.27	70.93	49.91
		Free Fatty Acids[2]		
A	28.80	1163.40	1271.40	2039.68
B	24.98	1022.85	1358.10	3071.11
C	18.27	1168.59	2067.50	3228.86
Mean	24.02	1118.26	1565.67	2779.90
		Soluble Protein[3]		
A	8.34	25.44	67.04	69.45
B	6.10	34.73	51.12	64.65
C	6.60	37.09	65.21	72.36
Mean	7.01	32.42	61.12	68.82

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]µmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

Figure 4. Changes in pH, volatile fatty acids, free fatty acids and soluble protein during ripening of control cheese (C) and cheese with low (L) and high (H) salt content.

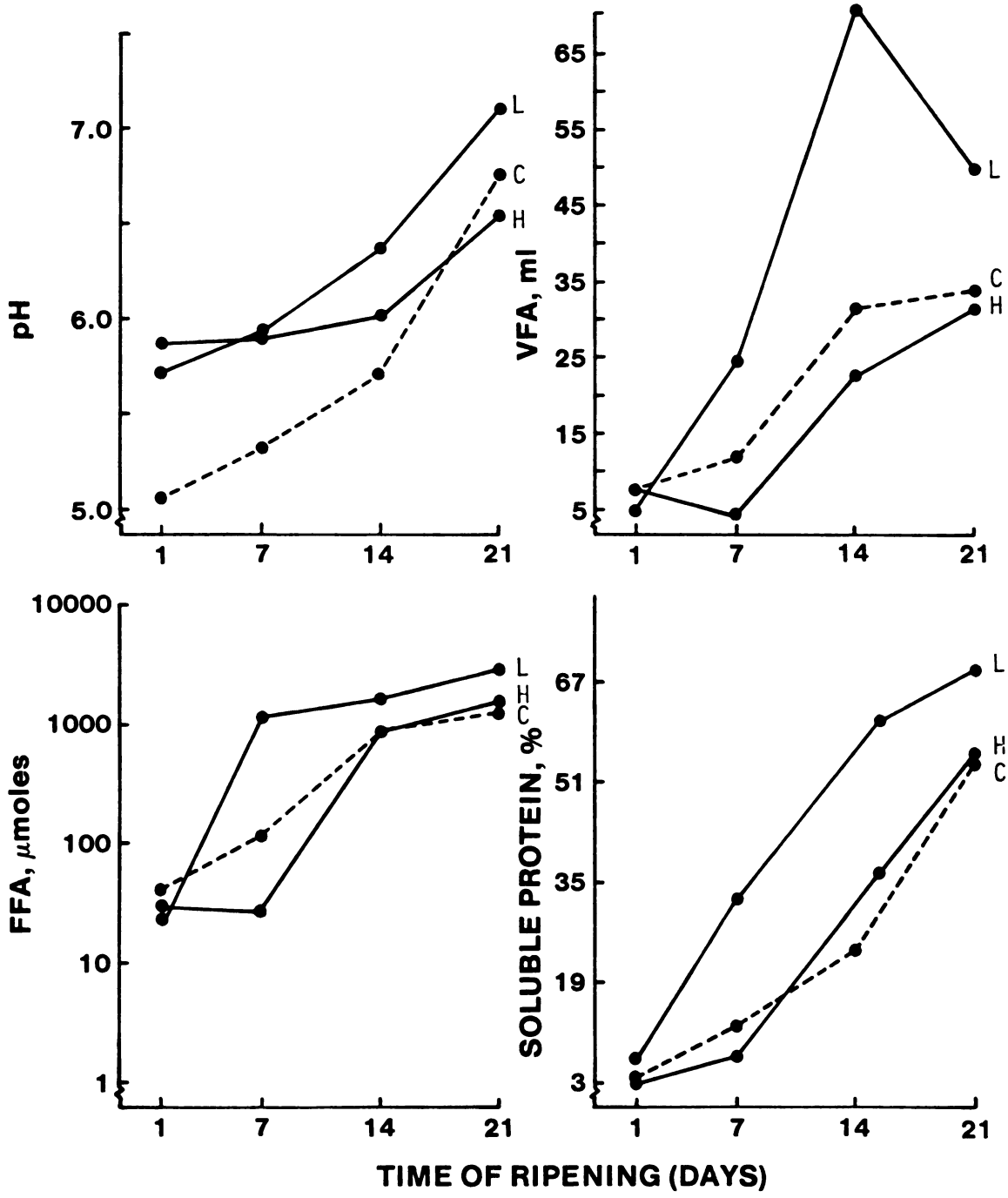

pH
7.0
6.0
5.0
L
C
H
1
7
14
21
VFA, ml
65
55
45
35
25
15
5
L
C
H
FFA, μmoles
10000
1000
100
10
1
L
H
C
SOLUBLE PROTEIN, %
67
51
35
19
3
L
H
C
TIME OF RIPENING (DAYS)

Table 13. Chemical composition of fresh cheese presenting high NaCl content (4.60% in the cheese moisture).

Batch	A	B	C	Mean
pH[4]	6.10	5.92	5.57	5.86
Moisture	58.45	54.30	53.52	55.43
Total Solids	41.55	45.70	46.48	44.58
Fat	17.58	20.90	20.34	19.61
Fat in Dry Matter	42.30	45.70	43.80	43.90
Volatile Fatty Acids[1]	9.78	8.15	5.43	7.79
Free Fatty Acids[2]	22.51	29.16	36.54	29.40
Total Protein (TP)	18.35	18.83	18.53	18.57
Soluble Protein (SP)	0.89	0.40	0.50	0.60
(SP/TP)x(100)[3]	4.83	2.12	2.70	3.22
Salt (NaCl)	2.65	2.72	2.63	2.67
Salt/Moisture	4.34	4.77	4.68	4.60

[1] Expressed as ml N/10 acid per 100 g cheese.

[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

[4] All others, except pH, expressed as percentage.

All data are the average of duplicate tests.

Table 14. Effect of high NaCl content (4.60%/water) on pH, volatile fatty acids, free fatty acids and soluble protein contents of cheese during ripening.

Batch	Time of Ripening (Days) 1	7	14	21
		pH		
A	6.10	6.10	5.98	6.45
B	5.92	5.79	6.11	6.23
C	5.57	5.78	5.99	6.99
Mean	5.86	5.89	6.03	6.56
		Volatile Fatty Acids[1]		
A	9.78	4.34	21.72	31.67
B	8.15	2.72	27.70	32.54
C	5.43	5.16	17.92	29.57
Mean	7.79	4.07	22.45	31.26
		Free Fatty Acids[2]		
A	22.51	28.91	876.62	1669.43
B	29.16	27.50	891.35	1399.63
C	36.54	24.98	770.25	1330.70
Mean	29.4	27.13	846.07	1466.59
		Soluble Protein[3]		
A	4.83	7.14	36.67	58.04
B	2.12	6.11	34.57	52.36
C	2.70	7.99	37.83	52.89
Mean	3.22	7.08	36.32	54.43

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]µmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

surface, where the concentration of salt is usually higher. According to Davis (1965) the germination of spores of Penicillium camemberti may be retarded by from 7.5 to 10% salt and the growth of mycelium ultimately stopped.

From the results of volatile fatty acids and free fatty acids shown in Tables 12 and 14, and plotted in Figure 4, one can conclude that high salt concentrations did not appear to influence substantially in the formation of these compounds. In the low salt/water concentration cheese (1.97%) production of free fatty acids increased sharply in 7 days to 1118.6 µmoles, and went gradually up to 2779.9 µmoles FFA per g cheese fat, as compared to 1266.9 µmoles/g of cheese fat in 21 days, in control cheese, showing a significant difference at 5% level. The high salt/water concentration cheese (4.60%) seemed to slow down the FFA production, showing 1466.59 µmoles/g of cheese fat after 21 days, with no significant difference as compared to control cheese. The production of volatile fatty acids (VFA) showed similar results to that of FFA, indicating little inhibition in presence of higher salt concentration. In the cheese with low salt/water concentration, there was a sharp increase up to 70.93 ml (N/10 acid per 100 g of cheese) after 14 days, followed by a decrease to 49.91 ml after 21 days of ripening. A significant difference at 5% level was observed, as compared to 33.65 ml reported for the control loose curd Camembert cheese. According to Foster et al. (1957) an initial

increase in fatty acids, followed by a decrease, may be due to the utilization of these fatty acids by some organisms in the cheese. Cheese with high salt/water content (4.60%) presented 31.26 ml N/10 acid per 100 g of cheese after 21 days of ripening, and when compared to control cheese, no significant difference was found at 5% level. The results indicate that high salt concentrations seem to affect the protein more than the fat breakdown in the loose curd Camembert cheese. According to Alais (1974) NaCl inhibits much more the proteases than the lipases, and in cheese highly salted, lipolysis goes on, whereas proteolysis is stopped. Raadsveld (1953) found that neither the moisture content, nor the salt concentration in the water phase of Edam cheese had any influence upon fat degradation. These results are not in agreement with those found by Godinho and Fox (1981a) who indicated that lipolysis in Blue cheese was delayed by higher salt concentration. According to Davies et al. (1937) salt in cheese may influence the solubility of certain proteins or protein degradation compounds and the proportion of bound and free water.

Effect of the Addition of a Commercial Lipase on the Ripening of Loose Curd Camembert Cheese

This study was undertaken to determine the effects of adding a commercial veal oral lipase to the milk, on the ripening characteristics of the loose curd Camembert cheese.

Table 15. Chemical composition of fresh cheese made with addition of veal oral lipase.

Batch	A	B	C	Mean
pH[4]	5.62	5.47	5.72	5.60
Moisture	57.89	56.70	56.50	57.03
Total Solids	42.11	43.30	43.50	42.97
Fat	18.78	18.11	17.91	18.27
Fat in Dry Matter	44.60	41.82	41.20	42.52
Volatile Fatty Acids[1]	5.43	6.51	5.43	5.79
Free Fatty Acids[2]	43.26	43.13	43.75	43.38
Total Protein (TP)	19.30	17.97	18.67	18.65
Soluble Protein (SP)	1.09	1.39	1.28	1.25
(SP/TP)x(100)[3]	5.65	7.74	6.86	6.70
Salt (NaCl)	2.19	1.86	2.09	2.05
Salt/Moisture	3.65	3.18	3.57	3.47

[1] Expressed as ml N/10 acid per 100 g cheese.

[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

[4] All others, except pH, expressed as percentage.

All data are the average of duplicate tests.

One ounce of the veal lipase per 1000 pounds of milk, was added to the milk right before setting. It was intended to encourage the liberation of the volatile and total free fatty acids. The results are shown in Table 16 and plotted in Figure 5, the chemical composition of the fresh cheese is presented in Table 15. The cheese pH increased gradually from 5.18 to 6.22 between 7 and 21 days of ripenings. During the first week, the pH dropped from 5.60 to 5.18, due probably to fermentation of lactose left in the fresh curd. When compared to results of pH in control cheese, as shown in Table 2, no significant difference was found at 5% level. It was observed that the amount of soluble protein increased steadily from 6.70 to 71.00% in 21 days of ripening, a result very similar to that found for the cheese with low fat content in the dry matter. This value could be considered atypical, since the fresh cheese composition presented in Table 15 shows a normal moisture, fat and salt content, none of which would contribute to significant changes in the protein breakdown.

Although no significant difference has been found at 5% level, the amount of free fatty acids produced after 21 days of ripening (1,815,60 μmoles/g cheese fat) was higher than that found for the control cheese in the same period (1266.90 μmoles/g of cheese fat). The same trend was observed concerning the production of volatile fatty acids: cheese made with addition of veal lipase presented 42.90 ml of N/10 acid

Table 16. Effect of the use of veal oral lipase on pH, volatile fatty acids, free fatty acids and soluble protein contents of cheese during ripening.

Batch	Time of Ripening (Days)			
	1	7	14	21
		pH		
A	5.62	5.10	6.05	6.01
B	5.47	5.15	6.18	6.66
C	5.72	5.28	5.98	5.98
Mean	5.60	5.18	6.07	6.22
		Volatile Fatty Acids[1]		
A	5.43	7.61	62.06	46.17
B	6.51	8.69	68.98	34.74
C	5.43	8.15	69.51	47.80
Mean	5.79	8.15	66.85	42.90
		Free Fatty Acids[2]		
A	43.26	54.59	1286.62	1686.49
B	43.13	51.76	1388.61	2079.95
C	43.75	58.45	1178.60	1680.23
Mean	43.38	54.93	1284.61	1815.60
		Soluble Protein[3]		
A	5.65	13.26	33.21	72.10
B	7.74	16.47	29.16	73.01
C	6.86	13.77	37.49	68.02
Mean	6.70	14.50	33.35	71.00

[1]Expressed as ml N/10 acid per 100 g cheese.

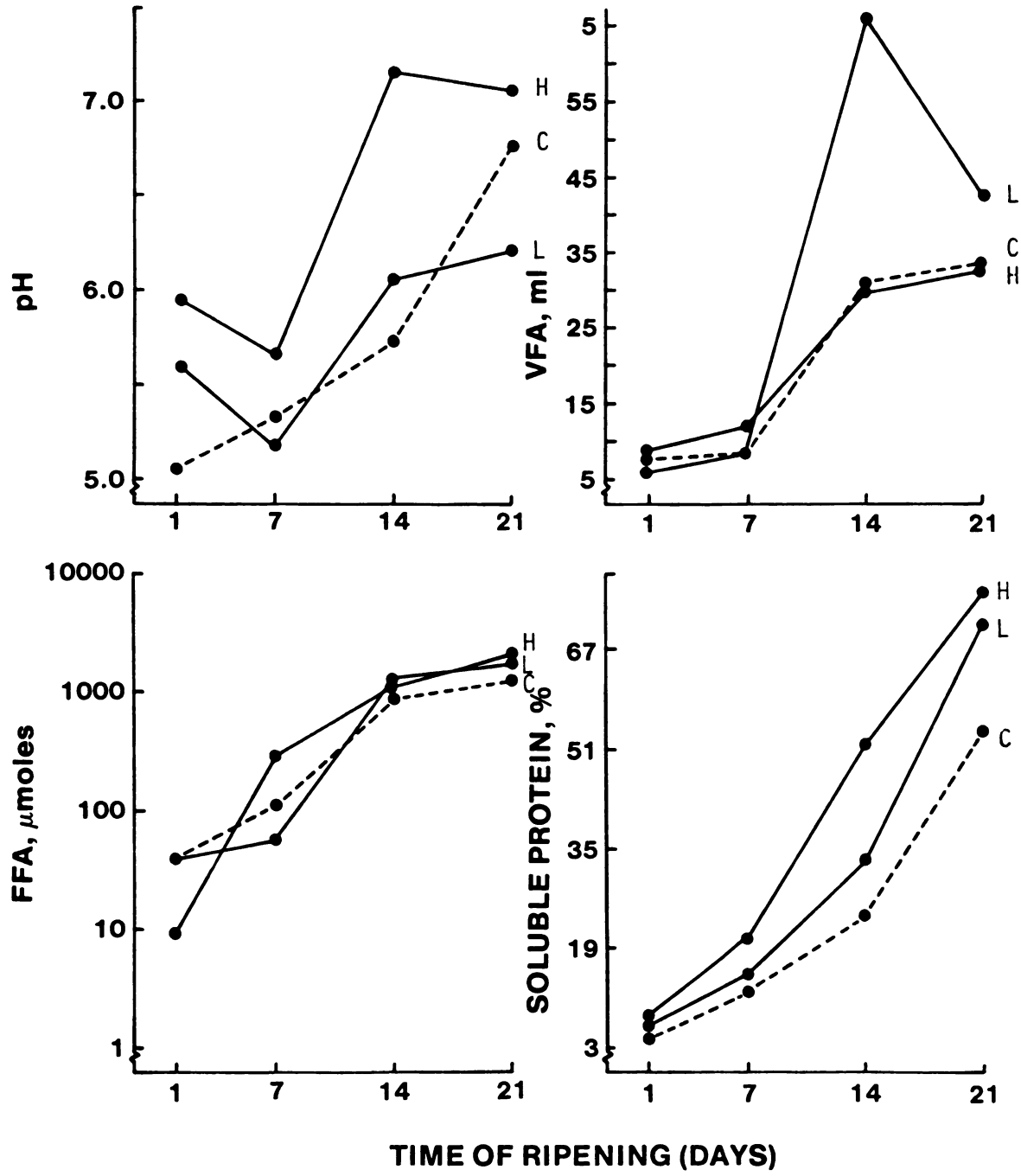
[2]μmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

per 100 g cheese as compared to 33.65 ml observed in control cheese. No significant difference was found. As it was observed earlier in the cheese made with low salt/water concentration (see Table 12), the amount of volatile fatty acids dropped between 14 and 21 days, probably for the same reasons pointed out.

The results found concerning the increase in volatile and total free fatty acids seem to be in agreement with those found by some authors. Kornacki et al. (1979) studied the action of lipolytic preparations of Penicillium roqueforti and Penicillium candidum on a substrate of milkfat and demonstrated that they had a extreme specificity to produce lower fatty acids, as they released from milkfat up to ten times as much those acids as other preparations did. Coulter and Combs (1939) obtained results indicating that the ripening of Blue cheese may be accelerated by the addition of steapsin to the milk or to the curd. Approximately the same degree of flavor development was secured in five months in cheese to which commercial steapsin was added as in 12-month ripened normal cheese. An esterase from Mucor miehei was found to exhibit the type of lipolytic activity needed to produce Romano and Fontina cheeses of excellent quality (Huang and Dooley, 1976). Richardson and Nelson (1968) reported a method for evaluating flavor producing enzymes for cheese manufacture and their data indicated that lamb gastric extracts in combination with lamb pregastric esterase resulted

Figure 5. Changes in pH, volatile fatty acids, free fatty acids and soluble protein during ripening of control cheese (C) and cheese made from homogenized milk (H) and with addition of veal oral lipase (L).


pH
7.0
6.0
5.0
H
C
L
VFA, ml
5
55
45
35
25
15
5
L
C
H
FFA, μmoles
10000
1000
100
10
1
H
L
C
SOLUBLE PROTEIN, %
67
51
35
19
3
H
L
C
1
7
14
21
TIME OF RIPENING (DAYS)

in more desirable "Provolone-like" flavors.

Effect of Milk Homogenization on the Ripening of Loose Curd Camembert Cheese

The homogenization of milk for the manufacture of loose curd Camembert cheese would be a logical procedure in attempting to bring about a comparatively rapid formation of fatty acids, since the process would enormously increase the surface area of the fat globules and thus facilitate the action of lipolytic enzymes. According to Campbell and Marshall (1975) fat globules of milk range in diameter from about 0.1 to 16 μm and approximately 80 to 90 percent are from 2 to 6 μm, after homogenization the globules are disrupted so 98 percent or more are 2 μm or less in diameter. For this study, milk with 3.5% fat, at 130°F (54°C) was homogenized at 1800 psi in the first stage and 500 psi in the second stage. Milk was pasteurized prior to homogenization. Ideally milk should be pasteurized before being homogenized to destroy lipase and avoid rancidity (Deeth and Fitz-Gerald, 1976).

The results are shown in Table 18 and plotted in Figure 5, the chemical composition of fresh cheese is presented in Table 17. Some changes were observed in the curd during manufacture. Setting time was 20% longer than usually observed in previous experiments and the curd was softer and weaker. Humbert et al. (1980) observed that curds from homogenized milk show less whey exudation as compared to

curds from non-homogenized milk. According to Wong (1980) the curd-softening effect may be caused by increased adsorption of casein on the greater area of the newly formed fat surfaces.

There was a slight but progressive increase in the cheese pH from 7 to 21 days of ripening. This elevation of pH was followed by an increase in the amount of soluble protein that reached 76.40% after 21 days of ripening, along with an observed pH of 7.07. Although none of these results has shown significant difference at 5% level, they are apparently higher when compared to cheese control results (see Table 2). It is not unusual that a long ripened conventional Camembert cheese present high levels of protein breakdown. Hammer (1944) reported finding more than 80 percent of water soluble protein in a 4-week old Camembert cheese. Campbell and Marshall (1975) state that in some surface-ripened cheeses such as Camembert and Limburger, practically all protein is converted to water-soluble compounds. In the present case, it must be pointed out that the cheese contained a high moisture content (60.84%) along with a fairly low salt/water concentration (3.26%) as it can be seen in Table 17. The effect of moisture and salt content in the protein breakdown have been discussed earlier. One could yet conjecture on the effects of high pressures of homogenization on the casein structure. According to Wong (1980) homogenized milk represents a denaturated form of its nonhomogenized

Table 17. Chemical composition of fresh cheese made from homogenized milk.

Batch	A	B	Mean
pH[4]	5.88	6.02	5.95
Moisture	60.36	61.32	60.84
Total Solids	39.64	38.68	39.16
Fat	19.42	17.49	18.45
Fat in Dry Matter	49.00	45.22	47.13
Volatile Fatty Acids[1]	8.58	8.04	8.31
Free Fatty Acids[2]	10.98	7.24	9.11
Total Protein (TP)	17.07	17.73	17.40
Soluble Protein (SP)	1.37	1.37	1.37
(SP/TP)x(100)[3]	8.03	7.73	7.87
Salt (NaCl)	2.0	2.10	2.05
Salt/Moisture	3.21	3.31	3.26

[1] Expressed as ml N/10 acid per 100 g cheese.

[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

[4] All others, except pH, expressed as percentage.

All data are the average of duplicate tests.

Table 18. Effect of milk homogenization on pH, volatile fatty acids, free fatty acids and soluble protein contents of cheese during ripening.

Batch	Time of Ripening (Days)			
	1	7	14	21
		pH		
A	5.88	5.65	7.18	7.05
B	6.02	5.68	7.18	7.08
Mean	5.95	5.67	7.18	7.07
		Volatile Fatty Acids[1]		
A	8.58	10.72	28.95	33.27
B	8.04	13.40	31.62	34.27
Mean	8.31	12.06	30.38	33.77
		Free Fatty Acids[2]		
A	10.98	267.30	1118.04	1747.20
B	7.24	296.10	1112.20	2036.43
Mean	9.11	281.70	1115.20	1892.00
		Soluble Protein[3]		
A	8.03	21.26	58.06	73.17
B	7.73	19.47	45.91	79.60
Mean	7.87	20.40	51.90	76.40

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]μmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

part. Campbell and Marshall (1975) state that the physical condition of protein is modified by homogenization thus coagulation by heat and acid occur more readily.

The homogenization of milk did not seem to affect substantially the breakdown of short chain fatty acids. Volatile acidity increased from 8.31 to 33.77 ml of N/10 acid per 100 g of cheese, in 21 days of ripening, a result very similar to that found in control cheese. However, homogenization of milk seems to stimulate the production of free fatty acids. In 7 days of ripening the cheese showed 281.70 μmoles of FFA, which increased to 1892.00 μmoles of FFA/g of cheese fat in 21 days. No significant difference was detected at 5% level, as compared to control cheese. These results seem to indicate that homogenization of milk could induce reasonably the formation of free fatty acids and thus accentuate the cheese flavor. Lane and Hammer (1938) found that in Blue cheese made from homogenized milk, hydrolysis of fat was definitely speeded up. According to Wong (1980) homogenization of the milk for Blue cheese causes considerable improvement in its ripening and flavor development due to increased lipolysis, that results in a marked increase in liberation of fatty acids and formation of methyl ketones. Harte (1974) indicates that homogenization of the cheese milk enlarges the fat surface thus allowing increased lipolysis by milk and mold lipase systems, in the manufacture of Blue cheese.

Effect of Dipping the Curd into Molds in the Manufacture of Loose Curd Camembert Cheese

This project was undertaken to standardize a procedure for making a Camembert-like cheese that could produce the same flavor produced by a conventional Camembert cheese, but in a shorter period of time. To achieve this goal, several modifications were introduced in the original technique, as has been described in this work. One of these modifications was the ripening of the cheese in a loose curd form, instead using the traditional 240 g wheel format. To know exactly how much influence the cheese format would have on its ripening characteristics, an assay was done by utilizing the curd from the Suggested Method of Manufacture (to be discussed ahead) and dipping this curd into round molds, right after draining the whey in the vat. The molds were turned frequently for 30 minutes, dry salted and 4 hours later taken to the same curing room used for the ripening of the loose curd Camembert cheese. The chemical changes during 21 days of ripening were followed and are presented in Table 21 and plotted in Figure 6. The fresh chemical composition is, of course, the same of the loose curd from the suggested Method of Manufacture and is presented in Table 19.

It may be seen that the cheese dimensions and form affect indeed the characteristics of the loose curd Camembert cheese. The pH dropped sharply between 1 and 7 days of ripening,

indicating that besides draining out slower, the lactic acid in the curd is neutralized or consumed slower by the spores of Penicillium caseicolum. Between 7 and 21 days of ripening, there has been some neutralization of the curd, the pH raising up from 4.84 to 5.96, but still considerably lower than the pH of 6.77 observed for control cheese (see Table 2) in the same period. The same trend was observed concerning the protein breakdown. The amount of soluble protein increased from 7.60% in the first day of ripening to 39.40%, 21 days later showing clearly a decrease in the protein breakdown as compared to 53.90% found for the control cheese in the same period. Penicillium caseicolum grows at the cheese surface and produces proteases that migrate towards the cheese center and hydrolyze the proteins gradually, bringing about increases in water soluble protein and thus raising the pH. The thicker the cheese, the slower the proteolytic action in the cheese as a whole. This could explain the differences observed in the curd ripened in the traditional form as compared to the loose and Camembert cheese. Kosikowski (1978) indicates that Camembert cheese requires a critical diameter to thickness ratio since a too thick cheese ripens faster externally and becomes overripe there before the internal section is affected.

The same effect was observed in the production of free fatty acids and volatile fatty acids. Both increased gradually during 21 days of ripening, but to a much lesser

extent than in the control cheese. Free fatty acids increased up to 359.10 μmoles per g of cheese fat as compared to 1266.90 μmoles in control cheese, after 21 days of ripening. In the same period, volatile fatty acids increased up to 14.70 ml as compared to 33.65 in the control cheese. Significant differences were found, at 5% level, for all data presented, as compared to those of control cheese.

From the results presented one can conclude that the cheese form and thickness affect the rate of fat hydrolysis and protein breakdown in Camembert cheese, and that the loose curd form stimulates the production of water soluble protein, volatile fatty acids and free fatty acids, thus enhancing body and texture as well as flavor in the cheese. Stadhouders and Mulder (1957) state that microorganisms growing on the surface of the cheese have a marked influence on the fat hydrolysis and it shows that the dimensions and the form of the cheese can have an influence on production of flavor.

The Suggested Method for the Production of Loose Curd Camembert Cheese

Based upon the information obtained from the preceeding trials the procedure was modified accordingly to test different parameters all together and find a final optimum method for the manufacture of a loose curd Camembert cheese which could ripen faster than the conventional cheese. The

Table 19. Chemical composition of fresh cheese made by the suggested method of manufacture.

Batch	A	B	Mean
pH[4]	5.65	5.73	5.69
Moisture	60.47	59.65	60.06
Total Solids	39.53	40.35	39.94
Fat	13.99	13.28	13.64
Fat in Dry Matter	35.40	32.91	34.15
Volatile Fatty Acids[1]	12.33	12.87	12.60
Free Fatty Acids[2]	55.49	51.95	57.72
Total Protein (TP)	22.30	22.40	22.34
Soluble Protein (SP)	2.18	1.21	1.70
(SP/TP)x(100)[3]	9.76	5.40	7.60
Salt (NaCl)	0.62	0.62	0.62
Salt/Moisture	1.01	1.03	1.02

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]μmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

[4]All others, except pH, expressed as percentage.

All data are the average of duplicate tests.

following are the modifications introduced in the technique:

a) The milkfat was standardized to 1.0% to obtain a cheese with low fat content in dry matter.

b) Milk pH before setting was 6.7 and the milk was not ripened with starter.

c) One ounce of commercial veal oral lipase per 1000 lbs of milk was added right before setting.

d) After pasteurization milk was homogenized at 1800 psi (1st stage) and 500 psi (2nd stage), at 130^{o}F (54^{o}C).

e) Stirring time in vat was cut down to 15 minutes to obtain a cheese with higher moisture content.

f) To have a cheese with lower salt content; just 1% of NaCl was added to the fresh curd. The cheese was ripened in the loose curd form and under the same conditions of temperature and humidity previously described.

The chemical composition of the fresh cheese is shown in Table 19. One can see that the moisture content of the cheese (60.06%) was considerably high, affecting favorably the rate of proteolysis and lipolysis in the cheese during ripening. The content of fat in dry matter was also distinctly low (34.15%) as well as the ratio of salt/water (1.02%). The latter, as discussed earlier, seems to have a clear effect in the rate of protein breakdown of the cheese.

The changes in pH, volatile fatty acids, free fatty acids and water soluble protein during 21 days of ripening are presented in Table 20 and plotted in Figure 6. The pH

Table 20. Changes in pH, volatile fatty acids, free fatty acids and soluble protein during ripening of cheese made by the suggested method of manufacture.

Batch	Time of Ripening (Days)			
	1	7	14	21
			pH	
A	5.65	5.45	6.49	6.51
B	5.73	5.53	6.48	6.71
Mean	5.69	5.49	6.48	6.61
			Volatile Fatty Acids[1]	
A	12.33	8.04	25.19	46.10
B	12.87	6.97	25.72	43.40
Mean	12.60	7.51	25.46	44.80
			Free Fatty Acids[2]	
A	55.49	134.22	1256.90	2289.50
B	51.95	128.47	1163.70	2288.60
Mean	53.72	131.35	1210.30	2289.10
			Soluble Protein[3]	
A	9.76	19.91	46.64	68.40
B	5.40	20.54	47.14	71.60
Mean	7.60	20.23	46.90	70.00

[1]Expressed as ml N/10 acid per 100 g cheese.

[2]µmol FFA per g fat in cheese.

[3]Expressed as percentage of total protein.

Table 21. Effect of hooping the curd on pH, volatile fatty acids, free fatty acids, and soluble protein contents of cheese during ripening.

Batch	Time of Ripening (Days) 1	7	14	21
		pH		
A	5.65	4.76	5.08	5.95
B	5.73	4.92	5.09	5.97
Mean	5.69	4.84	5.08	5.96
		Volatile Fatty Acids[1]		
A	12.33	10.18	12.33	13.90
B	12.87	8.04	10.19	15.50
Mean	12.60	9.11	11.26	14.70
		Free Fatty Acids[2]		
A	55.49	91.52	173.46	380.90
B	51.95	70.73	140.32	337.20
Mean	53.72	81.13	156.89	359.10
		Soluble Protein[3]		
A	9.76	21.34	30.04	40.20
B	5.40	21.96	29.87	38.50
Mean	7.60	21.65	29.90	39.40

[1] Expressed as ml N/10 acid per 100 g cheese.

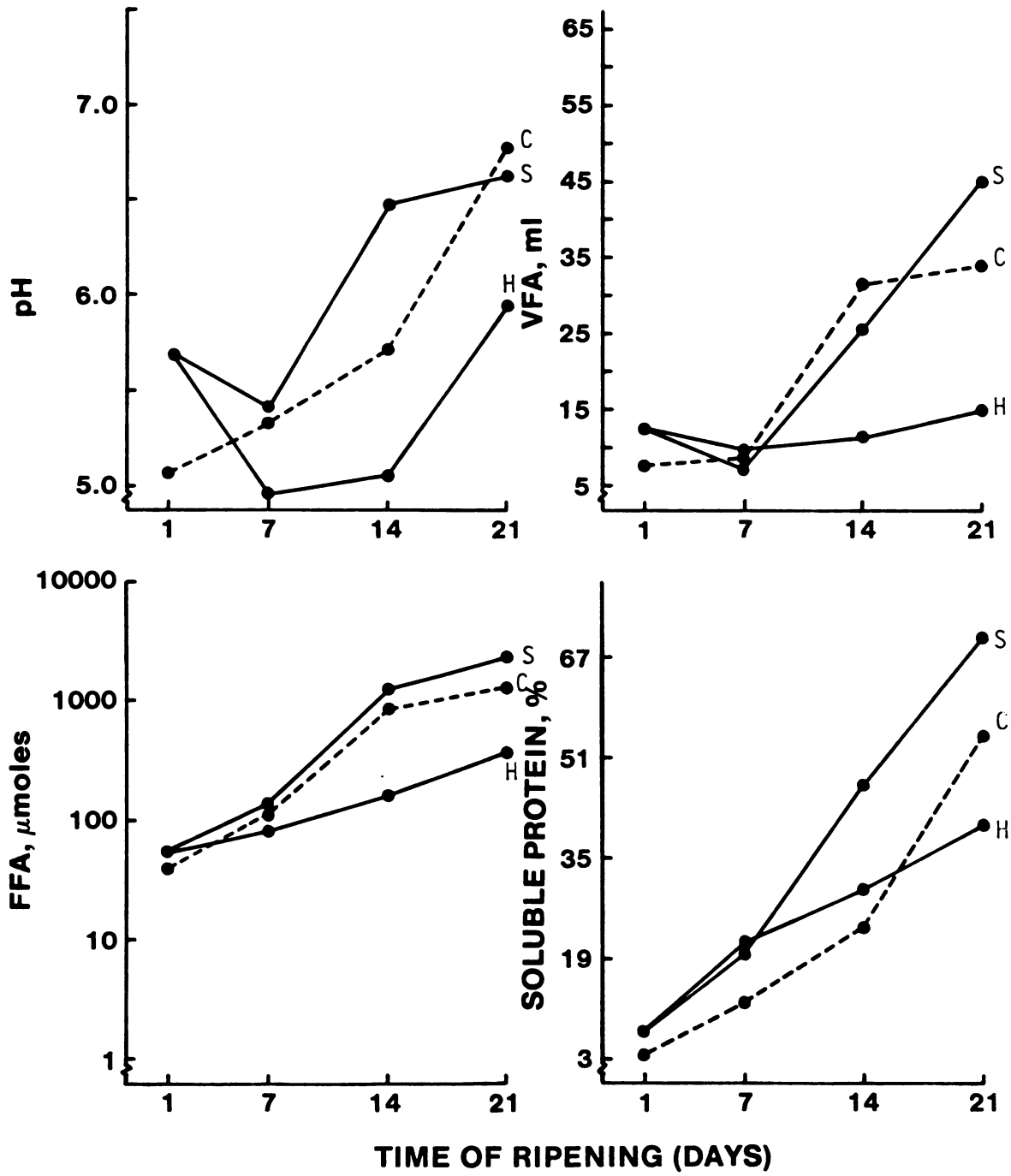
[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

decreased in the first 7 days of ripening, from 5.69 to 5.49, and then raised gradually up to 6.61 after 21 days of ripening. The raise in pH corresponded to an increase in water soluble protein, from 7.60% after 1 day to 70.00% 21 days later. These results showed no significant difference at 5% level as compared to control cheese (see Table 2) but are important as they demonstrated that by the Suggested Procedure about the same level of protein breakdown was reached as for the cheeses made from homogenized milk (Table 18), with addition of lipase (Table 16), with low fat content (Table 8) and low salt/water concentration (Table 11).

The production of volatile fatty acids and free fatty acids seem to be enhanced by the Suggested Method of manufacture. Both increased gradually during ripening. Free fatty acids production reached 2,289.10 μmoles per g cheese fat after 21 days and volatile fatty acids increased up to 44.80 ml of N/10 acid for 100 g of cheese, in the same period. No significant difference was found for the result of volatile acidity, but the production of free fatty acids was significantly higher, at 5% level, as compared to the respective results of control cheese (see Table 2). Stimulation of free fatty acids and volatile fatty acids production may be the contribution of homogenizing the milk and adding of the veal lipase, as well as the result of having a cheese with a lower fat and higher moisture contents, presenting consequently a lower rate of salt in the moisture. All these

Figure 6. Changes in pH, volatile fatty acids, free fatty acids and soluble protein during ripening of control cheese (C), loose curd (S) and hooped curd (H) made by the Suggested Method of Manufacture.


pH
7.0
6.0
5.0
C
S
H
VFA, ml
65
55
45
35
25
15
5
S
C
H
1
7
14
21
FFA, μmoles
10000
1000
100
10
1
S
C
H
SOLUBLE PROTEIN, %
67
51
35
19
3
S
C
H
TIME OF RIPENING (DAYS)

factors have been discussed earlier and they indeed seem to influence the ripening characteristics of the cheese.

The Composition of Conventional Camembert Cheese as Compared to the Loose Curd Procedure

Three different brands of Camembert cheeses were purchased from a commercial store and had their chemical composition determined, as shown in Table 22. Assuming a code of 100 days for the commercial cheese, brand 1 was aged 110 days and brand 2 aged 86 days. The age of brand 3 was not known, since it was originated from France and the label did not present any indication that could lead to the cheese age. This cheese seemed to be heat treated after 12 days of ripening before sending shipped from France, this is a usual procedure in that country aimed at the inactivation, total or partial, of enzymes that could deteriorate the cheese after long periods of storage.

There seems to be a relationship between the elevation of pH and the formation of water soluble protein in the cheese during ripening. Brand 1 showed a pH of 7.19 and 58.84% of soluble protein; Brand 2 had a pH of 6.44 and 30.71% of soluble protein. Brand 3 showed a pH of 6.11 and just 18.15% of protein. It is also interesting to point out that Brand 1 had the highest moisture content, the lowest fat in dry matter content and salt/water concentration, besides being more ripened than the other brands. These

Table 22. Chemical composition of three commercial ripened Camembert cheese*.

	Brand		
	1	2	3
pH[4]	7.19	6.44	6.11
Moisture	53.46	48.10	49.70
Total Solids	46.54	51.90	50.30
Fat	20.12	23.90	22.90
Fat in Dry Matter	43.23	46.05	45.53
Volatile Fatty Acids[1]	15.00	18.90	11.26
Total Protein (TP)	19.80	21.00	22.20
Soluble Protein	11.65	6.45	4.03
(SP/TP)x(100)[3]	58.84	30.71	18.15
Free Fatty Acids[2]	337.50	305.21	66.93
Salt (NaCl)	1.90	1.82	1.95
Salt/Moisture	3.43	3.64	3.78

*Brand 1 was aged 110 days; brand 2, 86 days; brand 3 the ripening was unknown.

[1] Expressed as ml N/10 acid per 100 g cheese.

[2] µmol FFA per g fat in cheese.

[3] Expressed as percentage of total protein.

[4] All other data, except pH, expressed as percentage.

All data are the average of duplicate tests.

results show that the procedure for making the loose curd Camembert cheese seems indeed to stimulate the protein breakdown during ripening. The cheese from Suggested Method, after just 21 days of ripening, presented 70.00% of soluble protein, as compared to 58.84% found in the 110 day old Brand 1 Camembert cheese. These results are in agreement with those of other authors, for conventional Camembert cheeses. Jacquet and Lenoir (1954) found that a fresh Camembert (2 days) cheese presents 8.7% of its total nitrogen as soluble nitrogen, a ripened one (31 days) 27.5% and an over-ripened cheese (more than 2 months), 50%. According to Lenoir (1963b) a typically ripened Camembert cheese would present from 31 to 34% of its total nitrogen as soluble nitrogen. Similar results were found by Jacquet and Thevenot (1961) who indicate that a 31 day old Camembert contains about 30% soluble nitrogen, 20% being protease nitrogen. Kikuchi (1966) found a relationship between the rise of pH and the protein breakdown in Camembert cheese. Cheese (20 days old) showed a pH of 6.3 and 25% of soluble nitrogen. After 30 days of ripening, the pH increased to 7.7 and soluble nitrogen to 42% and finally, after 45 days of ripening pH was 7.9 and soluble nitrogen 48% (expressed as percentage of total nitrogen).

The formation of volatile fatty acids in all 3 brands was definitely lower than in the Suggested Method loose curd Camembert cheese (Table 20) which showed 44.80 ml of N/10

acid per 100 g of cheese after 21 days of ripening whereas Brand 1 presented 15.00 ml, Brand 2, 18.90 ml and Brand 3, 11.26 ml. Brand 3 being presumably heat treated, with consequent inactivation of cheese lipases, the results seem reasonable. However, results from Brand 1 and 2 may be atypical for Camembert cheese. Smiley et al. (1946) and Dahlberg and Kosikowski (1947) indicated that a ripened Camembert cheese contained 39.9 ml N/10 acid solution per 100 g of cheese as volatile fatty acids.

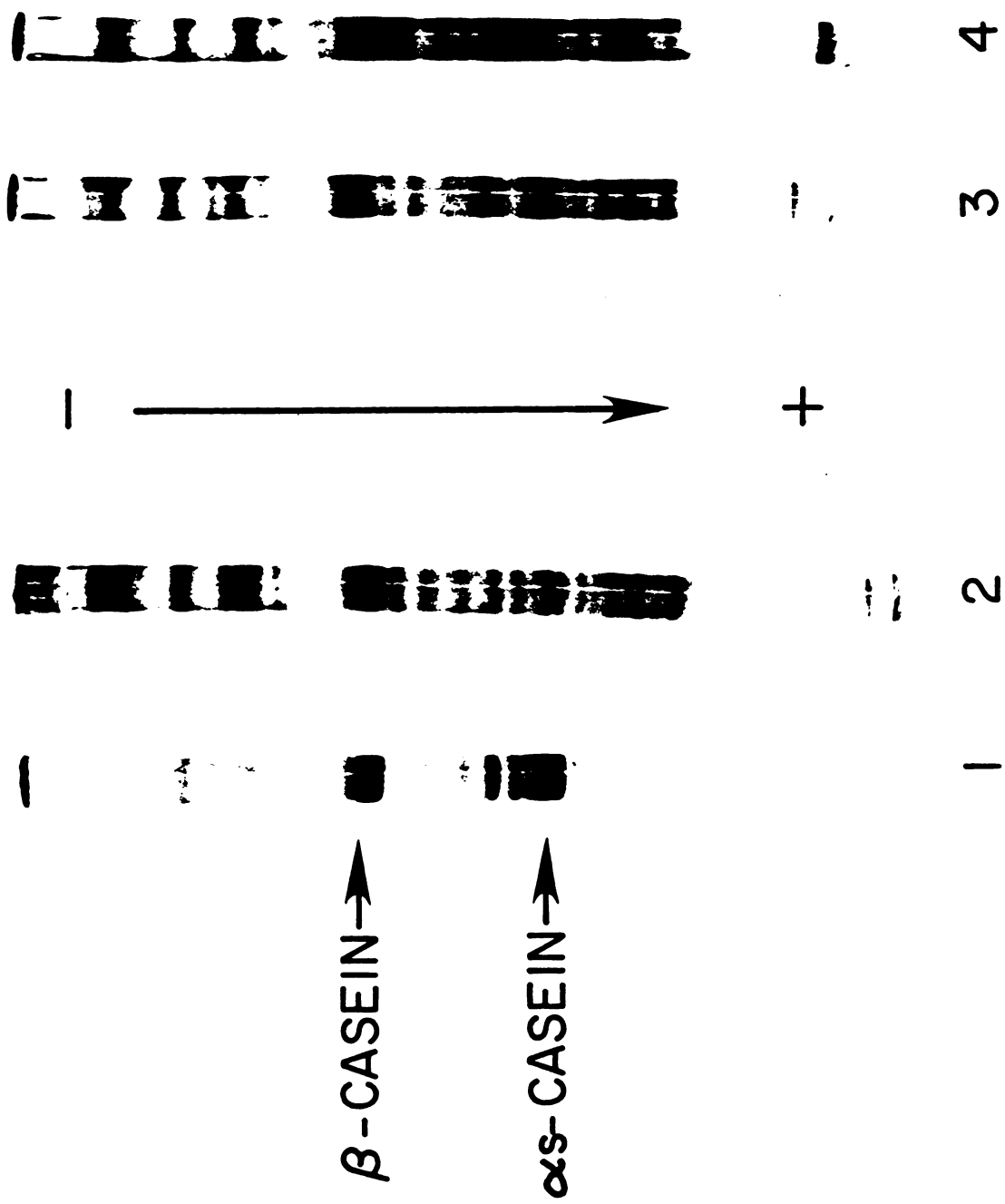
The production of free fatty acids in Brand 1 (337.50 μmoles), Brand 2 (305.21 μmoles) and Brand 3 (66.93 μmoles) was also significantly lower than that observed in the Suggested Method loose curd Camembert cheese (2289.1 moles FFA per g of cheese fat) after 21 days of ripening, as was shown in Tables 20 and 22. These results seem to indicate that the much higher surface area available for mold growth and the reduced thickness of the loose curd Camembert cheese contribute decisively for the increase in fat hydrolysis, as well as in protein breakdown. The results found for the three commercial Camembert cheese are quite similar to those found for the cheese made in the traditional wheel form, by using curd from the Suggested Method of Manufacture (see Table 21), which shows once more the effectiveness of ripening the cheese in a loose curd form, thus widening the surface for mold growth and activity.

Electrophoretical Study of α_{S1} and β-casein Breakdown During Ripening of the Loose Curd Camembert Cheese

Polyacrylamide gel electrophoreses (PAGE) was used as a suitable method for the estimation of α_{S1}- and β-casein breakdown during ripening of the loose curd Camembert cheese. Disc-PAGE was performed according to technique adapted from Ornstein (1964) and Davis (1964). Gels were scanned at a wavelength of 550 nm and the relative areas of the individual protein peaks were recorded. The concentration of each protein component was derived from the densitograms. As long as the optical density remained constant no change in the concentration was observed and the protein was considered to be unattacked. The percentage of breakdown in α_{S1}- and β-casein were calculated from the decrease in the concentration as compared to the original value. Protein loads on the gels were 50 μg for fresh cheeses (1 day) and 200 μg for ripened cheeses (21 days).

Electrophoretic patterns of fresh and ripened samples are shown in Figure 7. The photographic technique employed here did not permit discernment of all protein bands which actually appear as numerous diffused zones. The two most important bands are signalled and α_{S1}-casein, presenting higher electrophoretic mobility than β-casein, is visible in the front part of gel 1, which represents a fresh cheese sample. Gel 2 shows the result of the protein breakdown during 21 days of ripening. Several components of β- and

Figure 7. Discontinuous polyacrylamide gel electrophoretic patterns of fresh (1) and 21 day old loose-curd control Camembert cheese (2), 21 day old hooped cheese from Suggested Method (3) and 60 day old conventional Camembert cheese (4). Proteins loads on the gels were 50 μg (1) and 200 μg (2, 3, and 4). The major bands (β- and α_{S1}-caseins) are shown.


4
3
2
1
+
β-CASEIN
αs-CASEIN

α_{S1}-casein degradation, with higher or lower electrophoretic mobility, were formed. The densitometric patterns of both gels 1 and 2 are shown in Figure 8, under numbers 1 and 2, respectively. It can be seen in gel number 1 that the α_{S1}-casein fraction is degraded early in the ripening. Two peaks of lower electrophoretic mobility show up next to the α_{S1}-casein peak, being probably results of its degradation by rennet enzymes. Ledford et al. (1966) studied residual casein fractions in Cheddar cheese and found that a change in the caseins was evident following overnight pressing, a band of slightly greater mobility than α_{S1}-casein appearing in that period. According to Desmazeaud and Gripon (1977) the utilization of aseptic curds demonstrated that rennet has a strong and early action on α_{S1}-casein.

The electrophoretic patterns of a 21 day old hooped cheese (curd from the Suggested Procedure, as previously described) and a 60 day old commercial Camembert cheese are also shown in Figure 7, under numbers 3 and 4, respectively. The respective densitograms are presented in Figure 9, under numbers 3 and 4. These results will be discussed later on.

To evaluate the extent of breakdown in α_{S1}- and β-casein during 21 days of ripening, the results of the densitograms for each parameter discussed herein were summarized in Table 23. From the results of the concentrations in fresh

cheese, it is shown that α_{S1}-casein represented the major portion of the densitograms. This casein represented an average of 48.64 ± 7.6% of the total bands, as compared to 42.67 ± 5.9% for β-casein. There seems to be no significant difference in the concentration of each casein, from a parameter to another, in fresh cheese. But the difference between α_{S1}- and β-casein breakdown after 21 days of ripening is evident. For curds ripened in a loose form, in most of cases, more than 70% of α_{S1}-casein had disappeared, showing clearly that β-casein is more resistant to degradation than α_{S1}-casein. The strong attack of rennet and microbial enzymes on α_{S1}-casein has been demonstrated by different authors. Gripon et al. (1975) studied the action of rennet on caseins and found that α_{S1}-casein was extensively degraded and had nearly disappeared after 20 days of ripening of a soft cheese, β-casein being more resistant, with 2/3 of this casein remaining unaltered after 40 days. The protein breakdown in soft cheese was also studied by Jong (1976) who found that whereas α_{S1}-casein had almost completely vanished after 30 days, at least 70% of the β-casein remained unattacked. Results shown in Table 23 demonstrate that in the ripening of the loose curd Camembert cheese an average of 79.15% of α_{S1}-casein is degraded to minor compounds, as compared to only 41.30% of breakdown in β-casein. According to Ledford et al. (1966) most of the β-casein remains intact during the ripening of Camembert

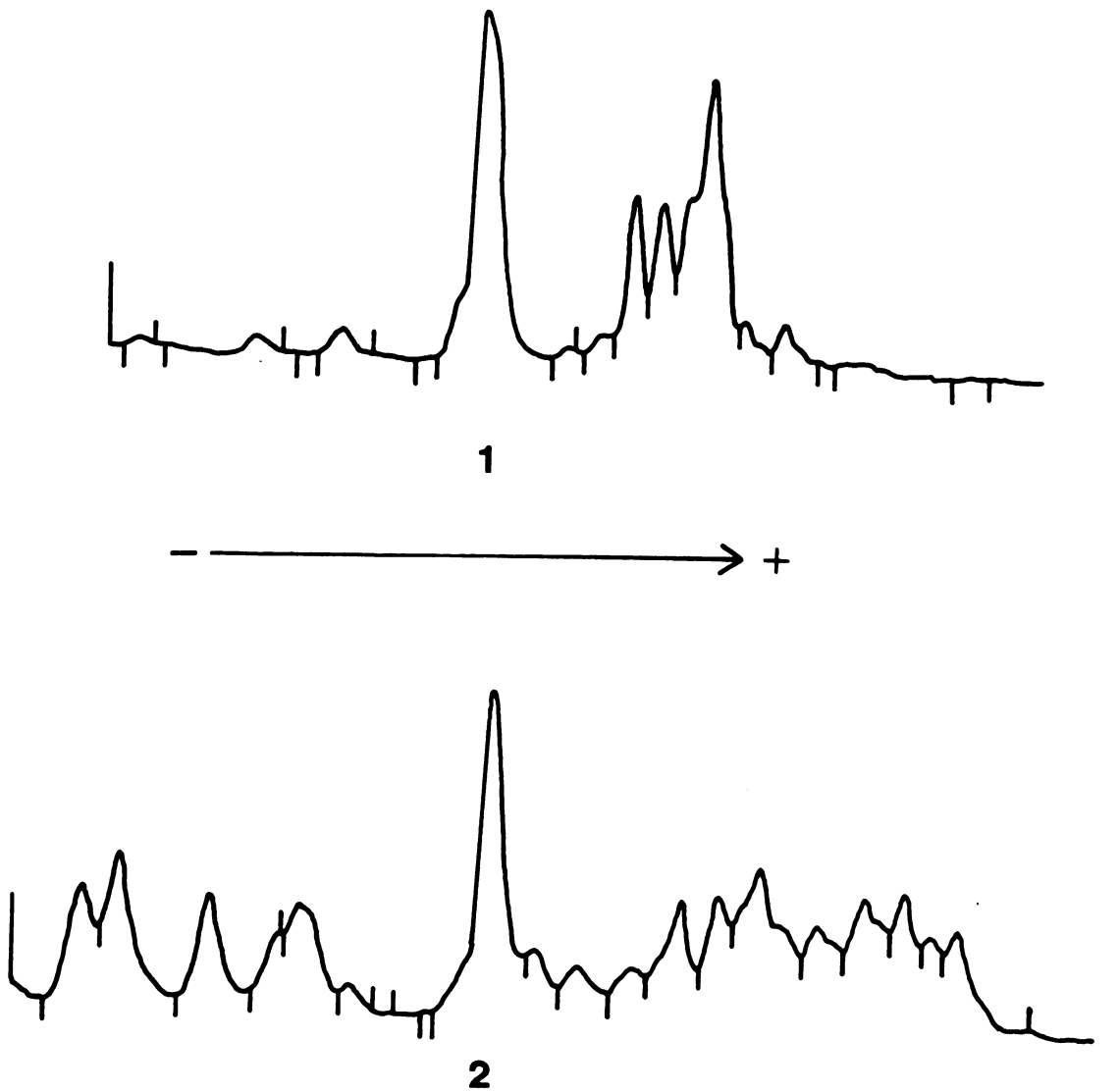


Figure 8. Densitometric patterns of discontinuous polyacrylamide gel electrophoresis of fresh (1) and 21 day old loose-curd control Camembert cheese (2). (For identification of bands, results were compared with the electropherogram of casein freshly prepared from skimmilk by HCl precipitation.)

cheese. Gripon et al. (1977) found that Penicillium proteases account for the appearance of various peptides of high or low molecular weights, but do not seem responsible for the presence of free amino-acids. These results are not in agreement with those found by Desmazeaud et al. (1976) who described that Penicillium releases great amount of amino-acids and low molecular weight peptides and degraded simultaneously α_{S1}- and β-caseins. The resistance of β-casein to hydrolysis by cheese enzymes has been described by many authors (Lindqvist and Storgards, 1962; Phelan et al., 1973; Visser and Groot-Mostert, 1977). It appears that in cheese made from milk with pH 6.43 more α_{S1}-casein (79.47%) was attacked than in cheese made from milk pH 6.16 (50.91%), suggesting an effect of pH of milk in the extent of breakdown. Further study is necessary to substantiate this speculation. According to Lindqvist and Storgards (1962) the entire cheese process usually takes place within a pH range of 5.0-6.0 and thus a slight divergence in the pH of a ripening cheese necessarily entails great changes in the casein degradation. The results of Table 23 seem to indicate that a low salt concentration could stimulate the degradation in both α_{S1}- and β-caseins. In cheese with higher salt concentration 71.21% of α_{S1}-casein and 42.30% of β-casein was broken down as compared to 83.39% and 50.30%, respectively, in the cheese with lower salt concentration. Kikuchi and Takafuji studied

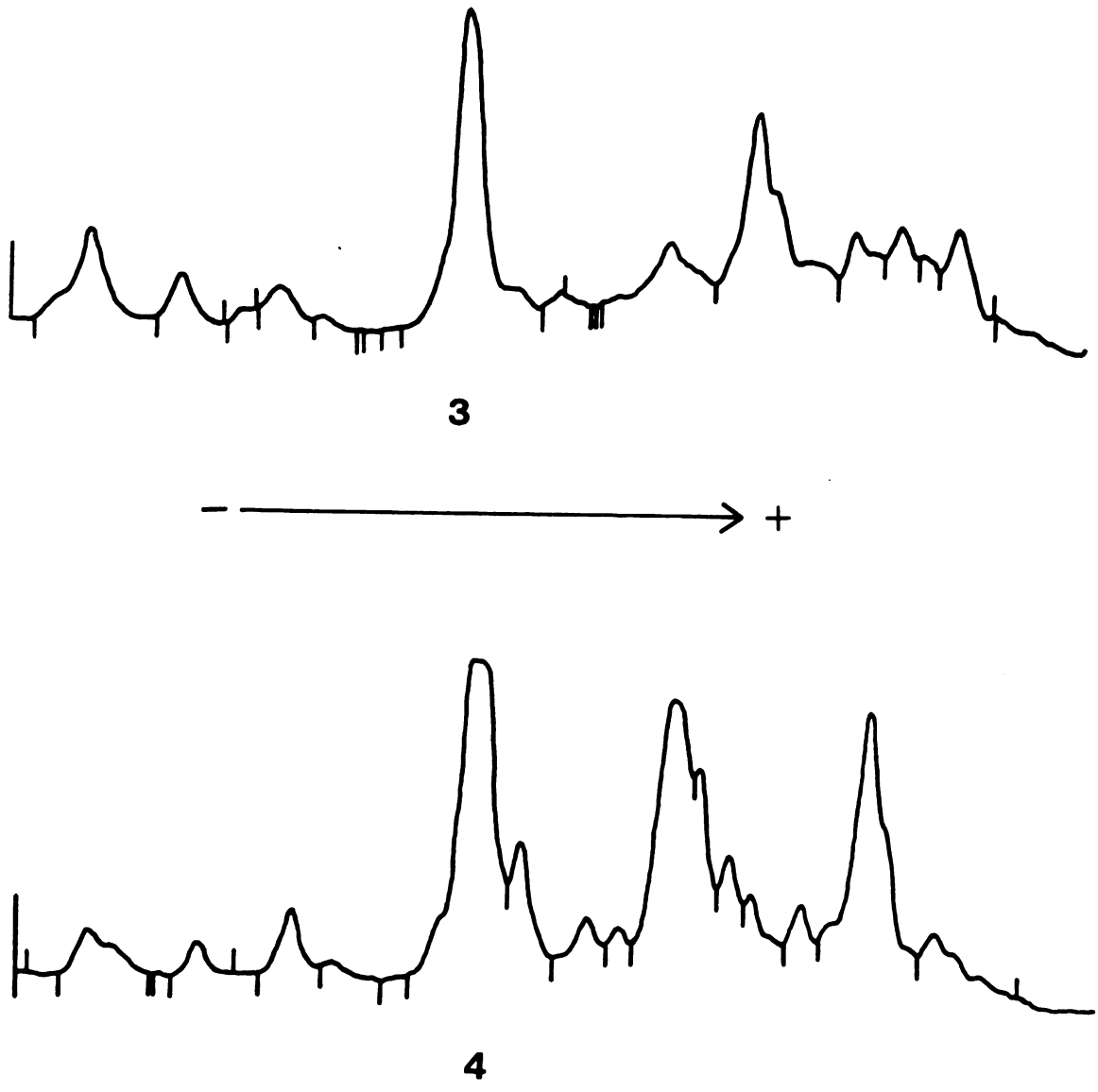


Figure 9. Densitometric patterns of discontinuous polyacrylamide gel electrophoresis of 21 day old hooped Camembert cheese (3) from Suggested Method and 60 day old conventional Camembert cheese (4).

Table 23. Results from densitograms showing concentration of β- and α_{S1}-caseins in fresh and ripened loose-curd Camembert cheese and percentage of breakdown after 21 days of ripening as related to the ratio SP/TP.*

Parameters	Fresh Cheese (1 Day)		Ripened Cheese (21 Days)		Percentage of Breakdown		Ratio SP/TP (1)
	β-casein	α_{S1}-casein	β-casein	α_{S1}-casein	β-casein	α_{S1}-casein	
Control	36.16	62.32	13.48	11.54	62.70	81.48	53.85
Milk pH 6.43	43.71	51.15	30.20	10.50	30.90	79.47	-
Milk pH 6.16	37.49	52.70	17.51	25.87	53.30	50.91	50.74
High Salt Cont.	55.19	35.40	31.85	10.19	42.30	71.21	54.43
Low Salt Cont.	47.39	51.46	23.55	8.55	50.30	83.39	68.82
High Fat Cont.	43.20	44.00	26.72	4.77	38.15	89.16	55.11
Low Fat Cont.	43.90	44.57	35.97	9.79	18.10	78.03	71.20
Adding Lipase	44.83	44.45	-	-	-	-	-
Homog. Milk	37.87	55.95	22.25	4.32	41.25	92.28	76.40
Hooping Effect	36.97	44.39	38.86	31.60	-	28.81	39.40
Suggested Meth.	36.97	44.39	24.18	6.01	34.60	86.46	70.00

*Concentration of proteins derived from the densitograms and presented as the percentage area of the total densitogram area.

(1) Soluble protein, expressed as percentage of total protein.

the action of NaCl on the protease of Penicillium casei-colum and found that as NaCl increased the protease activity decreased. In a soft cheese (Meshanger type) the degradation of α_{S1}-casein was stimulated by NaCl concentrations (in the moisture) up to about 4% and retarded by higher salt contents, the breakdown of β-casein being maximal in the absence of NaCl (Noomen, 1978). Proteolysis of β-casein by rennin and pepsin is inhibited in presence of high salt concentrations (Jong and Groot-Mostert, 1977; Fox and Walley, 1971).

Apparently, there is a relationship between the amount of soluble protein found in the loose curd Camembert cheese and the extent of breakdown of α_{S1}-casein during ripening. A Regression Analysis according to Bhattacharyya and Johnson (1977) was conducted for the results of soluble protein and the percentage of α_{S1}-casein breakdown shown in Table 23 and a correlation of 0.79 was found. However, further determinations are necessary to substantiate this speculation.

By comparing the densitometric patterns of fresh and ripened loose curd Camembert cheese shown in Figure 8, one can see that both α_{S1}- and β-casein are attacked by rennet, bacteria and mold enzymes during ripening and split into a number of breakdown products with different mobilities. In the β-casein region several compounds of lower mobility were found, after 21 days of ripening. In the α_{S1}-casein

region more compounds of lower and higher electrophoretical mobility were found, due to the greater degradation undergone by this casein. In the case of homogenized milk the degradation on α_{S1}-casein reached up to 92.28% of its original value. In some cases, the extent of breakdown was so high that it could not be clearly evaluated from the many bands diffused in the gel.

When the cheese was prepared by the Suggested Method of Manufacture, part of the fresh curd was put in hoops and ripened under the same conditions as the loose curd Camembert cheese. The objective was to study the effect of the cheese form on its characteristics, as previously described. In Figure 9, the densitometric pattern of disc-PAGE of this cheese after 21 days of ripening is shown under number 3. From the total area of bands in the densitogram, 31.60% represent α_{S1}-casein and 38.86% represent β-casein, as compared to just 6.01% and 24.18% respectively, in the cheese made by the Suggested Method of Manufacture (see Table 23). These results indicate that by ripening the cheese in a loose curd form more α_{S1}- and β-casein are degraded. In the same Figure 9, under number 4, the densitometric pattern of disc-PAGE of a 60 day old commercial Camembert cheese is shown. The pattern is different from that found for a 21 day old loose curd Camembert cheese (Figure 8, number 2), showing formation of fewer products of protein breakdown. In this pattern α_{S1}-casein represents

23.55% of the total area of all bands, as compared to 6.01% found for the cheese made by the Suggested Method of Manufacture. It seems to indicate that despite being much less ripened, the loose curd Camembert cheese undergoes α_{S1}- and β-casein breakdown to a greater extent than observed in the commercial Camembert cheese.

Organoleptic Evaluation of Loose Curd Camembert Cheese

The development of flavor in the loose curd Camembert cheese was assessed by organoleptic evaluation of the product at 12 days of ripening. A panel of 4 to 5 experienced judges were asked to score the cheese samples from 1 to 7, according to the Hedonic Scale shown in Figure 1. Judges were asked to evaluate flavor, general acceptability and overall preference of the samples and a conventional Camembert cheese aged at least 60 days was used as the control. The significance of any difference among results, at 5% level, was determined by Analysis of Variance, according to the American Society for Testing and Materials (1977).

The results of evaluation of flavor intensity are shown in Table 24. A significant difference was found only between the score of the control Camembert cheese (6.0) and that of the conventional cheese (3.3). For all other parameters, the flavor intensity was statistically the same

Table 24. Comparison of flavor scores of loose-curd and conventional Camembert cheese.

Parameters	Sample (1)		Conventional (2)		Observed Difference (3)
	Score	Intensity	Score	Intensity	
Control	6.0	Med. strong	3.3	Slt. mild	S
Milk pH 6.43	5.3	Slt. strong	6.0	Mod. strong	NS
Milk pH 6.16	4.6	Neither strong/mild	5.2	Slt. strong	NS
High Salt Cont.	5.5	Slt. strong	3.8	Slt. mild	NS
Low Salt Cont.	6.0	Mod. strong	4.3	Neither strong/mild	NS
High Fat Cont.	4.5	Neither strong/mild	4.0	Neither strong/mild	NS
Low Fat Cont.	5.7	Slt. strong	5.3	Slt. strong	NS
Adding Lipase	5.2	Slt. strong	3.4	Slt. mild	NS
Homog. Milk	5.7	Slt. strong	5.0	Slt. strong	NS
Suggested Method	6.3	Mod. strong	4.0	Neither strong/mild	NS

The scores represent the average of 4 to 5 experienced judges.

(1) Loose-curd Camembert cheese aged 12 days.

(2) Commercial Camembert cheese aged at least 60 days.

(3) Significance at 5% level: S = Significant; NS = Not significant.

between experimental samples and the conventional cheese. The lowest score was attributed to the cheese with high fat content in the dry matter (4.5) whose flavor was considered "neither strong nor mild". The highest score was attributed to the cheese made by the Suggested Method of Manufacture (6.3) whose flavor intensity was considered "moderately strong". It is important to point out that the cheese made by the Suggested Method of Manufacture received the highest grade, since the technique adopted was the result of application of all modifications previously experienced with some degree of success, as related to flavor improvement in the cheese. Since the loose curd Camembert cheese is to be used in the manufacture of other food products requiring a strong Camembert flavor, special attention was paid to the evaluation and results of this characteristic in the samples.

Table 25 shows the results of the general acceptability of the loose curd Camembert cheese. A significant difference was found only between the score attributed to the cheese made with milk pH 6.43 (4.8) as compared to 2.3 attributed to the control conventional Camembert cheese. All samples were considered either "slightly acceptable" or "neither acceptable nor unacceptable", with exception of sample of the cheese made from homogenized milk whose score (3.0 was the lowest and the sample considered "slightly unacceptable". This cheese contained soluble

Table 25. Comparison of general acceptability scores of loose-curd and conventional Camembert cheese.

Parameters	Sample (1) Score	Sample (1) Intensity	Conventional (2) Score	Conventional (2) Intensity	Observed Difference (3)
Control	5.0	Slt. acceptable	3.5	Slt. acceptable	NS
Milk pH 6.43	4.8	Neither accep./unaccep.	2.3	Mod. unaccep.	S
Milk pH 6.16	4.8	Neither accep./unaccep.	5.6	Slt. acceptable	NS
High Salt Cont.	4.0	Neither accep./unaccep.	5.5	Slt. acceptable	NS
Low Salt Cont.	5.0	Slt. acceptable	5.8	Slt. acceptable	NS
High Fat Cont.	5.5	Slt. acceptable	5.5	Slt. acceptable	NS
Low Fat Cont.	4.3	Neither accep./unaccep.	5.7	Slt. acceptable	NS
Adding Lipase	4.4	Neither accep./unaccep.	5.2	Slt. acceptable	NS
Homog. Milk	3.0	Slt. unaccep.	5.3	Slt. acceptable	NS
Suggested Method	4.8	Neither accep./unaccep.	5.8	Slt. acceptable	NS

The scores represent the average of 4 to 5 experienced judges.

(1) Loose-curd Camembert cheese aged 12 days.

(2) Commercial Camembert cheese aged at least 60 days.

(3) Significance at 5% level: S = Significant; NS = Not significant.

protein equal to 76.40% (Table 18), considered the highest. It is interesting to notice that the highest score (5.5) was attributed to the cheese with high fat content, which contained just 55.11% of soluble protein (Table 10). It appears that the content of soluble protein affects the evaluation of the cheeses, probably due to the fact that the higher the degree of proteolysis in a cheese similar to Camembert, the softer its body is. According to Dahlberg and Kosikowski (1947) cheese ripening is followed by increased volatile fatty acids and increased soluble nitrogen, both chemical changes are used to indicate the degree of ripening as associated with the development of flavor and increased soluble nitrogen with the development of a mellow, waxy body.

Table 26 shows the results of overall preference. The only significant difference at 5% level detected was between the scores of cheese made with milk pH 6.43 and the conventional Camembert cheese. The cheese with high salt concentration received the lowest score (4.0) and the highest score was attributed to the cheese with high fat content. In all parameters evaluated, the preference of the judges for the samples was expressed either as "like slightly" or as "neither like nor dislike".

In this organoleptic assessment the flavor scores has been taken as the most important point, as related to the evaluation of the achievements of the modifications

Table 26. Comparison of overall preference scores of loose-curd and conventional Camembert cheese.

Parameters	Sample (1)		Conventional (2)		Observed Difference (3)
	Score	Intensity	Score	Intensity	
Control	4.8	Neith. like/dislike	3.8	Dislike slightly	NS
Milk pH 6.43	5.0	Like slightly	2.5	Dislike moderately	S
Milk pH 6.16	4.8	Neith. like/dislike	5.4	Like slightly	NS
High Salt Cont.	4.0	Nieth. like/dislike	5.0	Like slightly	NS
Low Salt Cont.	4.3	Neith. like/dislike	5.8	Like slightly	NS
High Fat Cont.	5.5	Like slightly	5.5	Like slightly	NS
Low Fat Cont.	4.3	Neith. like/dislike	4.3	Neith. like/dislike	NS
Adding Lipase	4.2	Neith. like/dislike	4.6	Neith. like/dislike	NS
Homog. Milk	4.7	Neith. like/dislike	4.7	Neith. like/dislike	NS
Suggested Methods	4.8	Neith. like/dislike	5.4	Like slightly	NS

The scores represent the average of 4 to 5 experienced judges.

(1) Loose-curd Camembert cheese aged 12 days.

(2) Commercial Camembert cheese aged at least 60 days.

(3) Significance at 5% level: S = Significant; NS = Not significant.

introduced in the cheese technique, to speed up the ripening time and flavor formation. If taken into account that the loose curd Camembert cheese ripened for just 12 days was being compared to a at least 60 day old Camembert cheese, any non-significant difference in the flavor scores, must be interpreted as an indication that a similar flavor intensity was obtained in a much shorter period of time in the loose curd cheese, showing the efficiency of this technique.

Some comments made by the panel were: "Too salty"; "Bleu cheese flavor" (cheese with high fat content); "Creamy body helped acceptability" (cheese with milk pH 6.16); "I don't like the texture" (cheese with low fat content); "Flavor is very piccant" (cheese with addition of lipase); "Slightly bitter" (cheese from Suggested Method of Manufacture); "This sample has smoother body and texture with pleasant taste and flavor" (cheese from Suggested Method).

SUMMARY AND CONCLUSIONS

Twenty seven batches of loose curd Camembert cheese were prepared and changes in pH, volatile fatty acids, free fatty acids and soluble protein were determined at 7 day intervals during the 21 day ripening period. Polyacrylamide gel electrophoresis was used to determine the extent of degradation of α_{S1}- and β-caseins during ripening. A panel of 4 to 5 experienced judges evaluated the flavor intensity, overall preference and general acceptability of 12-day old samples as compared to a 60 day or more old conventional Camembert cheese. The experiments were performed under different conditions to evaluate the influence of several parameters such as milk pH, cheese salt concentration, cheese fat content, cheese form, adding of lipase and use of homogenized milk on the final characteristics of the loose curd Camembert cheese. A Suggested Method of Manufacture was adopted by optimization of various parameters investigated. The following were the findings:

1. The cheese pH, volatile fatty acids, free fatty acids and soluble protein increased as a function of time and ripening. The elevation in cheese pH seems to be related with the increase of protein breakdown along the

ripening period.

2. The lowering of milk pH to 6.43 or 6.16, by ripening the milk with starter before setting, does not appear to affect substantially the characteristics of the cheese. It appears that milk must be ripened more intensively with starter, to bring about significant changes in the ripening process.

3. Making the cheese with a high content of fat in dry matter appears not to influence substantially the ripening characteristics. However, cheese with a low content of fat in the dry matter showed a significant increase in free fatty acids production and protein breakdown. The same cheese presented also a higher moisture content which may account for increase in fat and protein breakdown.

4. The effect of salt concentration was very clear in the loose curd Camembert cheese. The protein breakdown was inhibited by increase in salt concentration. Consequently the cheese pH at the end of the ripening period was relatively low. The high salt concentration did not appear to influence substantially the formation of volatile and free fatty acids. However, low salt concentrations stimulated the production of free fatty acids and volatile fatty acids.

5. The addition of veal oral lipase to the milk before setting seems to increase slightly the amount of

free fatty acids produced. Volatile fatty acids and protein breakdown showed substantial increase when the lipase was used.

6. The homogenization of milk increased setting time by 20% and resulted in relatively higher moisture content in the cheese. The cheese pH was raised substantially during ripening, along with a definite increase in the water soluble protein content. Homogenization seems to induce lipid hydrolysis in the cheese.

7. The higher the moisture content of the cheese, the faster it ripens. It is well defined that protein breakdown is stimulated at high levels of moisture in the cheese. This effect is still substantially accentuated if the cheese contains a low salt concentration.

8. The cheese form and thickness affect drastically the pace of the ripening process. Fat hydrolysis and protein breakdown were substantially slowed down in the curd hooped in the traditional wheel format, as compared to the curd cured in the loose form.

9. In the Suggested Method of Manufacture, the loose curd Camembert cheese was prepared from homogenized milk containing oral lipase. The milk was not ripened with starter. In general the cheese had a high moisture content, a low content of fat in dry matter and a very low salt concentration as compared to normal Camembert cheese. These modifications resulted in a high level of protein breakdown,

and in a substantial formation of free fatty acids and volatile fatty acids.

10. The determination of the chemical composition of three ripened commercial Camembert cheeses showed that the loose curd Camembert cheese could produce much more free fatty acids and volatile fatty acids, as well as have a stronger protein breakdown, in a shorter period of time as compared to conventionally made cheeses.

11. The electrophoretic determinations in fresh and ripened cheese showed that α_{S1}-casein and β-casein represent an average of 48.64 and 42.67% respectively, of all bands detected. During ripening, α_{S1}-casein is degraded to a much greater extent than β-casein. An average of 79.15% of α_{S1}-casein had disappeared after 21 days of ripening, as compared to just 41.30% of β-casein, in the same period of time.

12. From the organoleptic evaluation of the loose curd Camembert cheese it has been demonstrated that a cheese with similar flavor intensity could be produced after 12 days of ripening as compared to a commercial Camembert cheese ripened for at least 60 days. No significant difference was detected either in the overall preference and general acceptability of the samples.

APPENDIX

Appendix Table A1. List of chemicals used in this study.

Chemical	Reference Number	Company
Acetic acid Glacial	3121	Mallinckrodt
Acetone	2440	Mallinckrodt
Acrylamide monomer	161-0100	Bio-Rad
Ammonium hydroxide	2440	Mallinckrodt
Ammonium persulfate	161-0700	Bio-Rad
Boric acid	2549	Mallinckrodt
Bromophenol blue	332	National Aniline Div.
Butyl-alcohol	2990	Mallinckrodt
Calcium chloride	1-1332	Baker
Chloroform	4440	Mallinckrodt
Coomassie brilliant blue	161-0400	Bio-Rad
Ethanol	-	Aaper Alcohol Chemical
Ethyl-ether	0844	Mallinckrodt
Ferric ammonium sulfate	1-75	Fisher
Glycine	161-0718	Bio-Rad
Hydrochloric acid	2612	Mallinckrodt
Magnesium sulfate	1-2506	Baker
2-Mercaptoethanol	161-0710	Bio-Rad
Mercuric oxide	M-174	Fisher
Methanol	3024	Mallinckrodt
Methylene blue	922	National Aniline Div.
Methyl red	2696	Baker

Appendix Table A1. (cont'd)

Nitric acid	2704	Mallinckrodt
N,N-Methylenebisacrylamide	161-0200	Bio-Rad
N,N,N',N'-tetramethylethylenediamine (TEMED)	161-0800	Bio-Rad
Petroleum ether	PX-0424	MCB
Phenolphthalein	6600	Mallinckrodt
Phenol red indicator	-	U.S. Biochemical Corp.
Photo-Flo 200	-	Eastman Kodak
Potassium hydroxide	6984	Mallinckrodt
Potassium permanganate	7068	Mallinckrodt
Potassium phosphate dibasic	7092	Mallinckrodt
Potassium phosphate monobasic	7100	Mallinckrodt
Potassium sulfate	7140	Mallinckrodt
Potassium thiocianate	3326	Baker
2-Propanol	11003-5	Aldrich
Silicilic acid	2847	Mallinckrodt
Silver nitrate	S-181	Fisher
Sodium acetate	1-3470	Baker
Sodium chloride	7581	Mallinckrodt
Sodium hydroxide	7708	Mallinckrodt
Sodium thiosulfate	-	Fisher
Sucrose	8360	Mallinckrodt
Sulfuric acid	2468	Mallinckrodt

Appendix Table A1. (cont'd)

Tris (Hydroxi-methyl) amino methane	161-0716	Bio-Rad
Urea	8648	Mallinckrodt

Appendix Table A2. List of instruments used in this study.

Instrument	Company
Autoclave (Type 20)	Wilmost Castle Company, Rochester, NY
Balance Mettler H30	Mettler Instrument Co., Highstown, NJ
Balance (Mettler top-load Type 120)	Mettler Instrument Co., Highstown, NJ
Digestor Microkjeldahl FF699	Lab. Con Co., Kansas City, MO
Distillator (Micro-kjeldahl)	Fisher Scientific, Pittsburg, PA
Electrophoresis cell (Model 150-A)	Bio-Rad Laboratories, Richmond, CA
Electrophoretic destainer (Model 1200-A)	Bio-Rad Laboratories, Richmond, CA
Gel scanner Model 2520	Gilford Instrument Laboratories, Inc., Oberlin, OH
Integrator Model 3380-5	Hewlett Packard, Avondale, PA
Mojonnier Apparatus	Mojonnier Bros., Co., Chicago, IL
pH-Meter CHEMTRIX 60-A	Chemtrix Inc., Killboro, OR
Photometer Model 252	Gilford Instrument Laboratories, Inc., Oberlin, OH
Powerstat 116-B	The Superior Electric Company, Bristol, CO
Power Supply Model 500	Bio-Rad Laboratories, Richmond, CA
Spectrophotometer Model 2400	Beckman Instruments, Inc., Fullerton, CA
Vacuum Oven Model 19	GCA/Precision Scientific Co., IL
Vacuum Pump Welch (Model 1402)	Sargent Welch Scientific Co., IL

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