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# Cheese Flavor Development in Unfiltered Whole Milk Concentrates

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# CHEESE FLAVOR DEVELOPMENT IN ULTRAFILTERED WHOLE MILK CONCENTRATES

by

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David Long-Ying Hwang

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ABSTRACT

Cheese Flavor Development in Ultrafiltered

Whole Milk Concentrates

by

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The development of cheese flavor in ultrafiltered whole milk retentates was investigated. Acidified (pH 5.7) pasteurized whole milk was concentrated to 21% fat, 17% protein and 41% total solids, and then divided into six lots. Each lot was subdivided into three groups of two samples each. Each group was inoculated with one of three lactic cultures -- Streptococcus lactis $C_6$, commercial mixed concentrated Marschall's MD294S or CCI299S. One sample in each group was treated with rennet and the other sample left rennet free. All samples were incubated at 30 C until the pH reached 5.2-5.1. Each of the fermented retentates was further divided into 12 samples. Six of them were incubated at 22 C and the other six at 30 C. After two weeks incubation, the samples were evaluated for flavor quality, body quality and flavor intensity. A similar analysis was conducted after two weeks for a total incubation period of four weeks.

The effects of culture, rennet and incubation temperature on product quality were determined. Rennet and temperature were the only

factors with significant impact on flavor intensity or body and flavor quality. No significant effects were attributed to the lactic cultures, although bitterness was more frequently found in retentates fermented with culture $C_6$.

Samples containing rennet and ripened at 30 C developed the highest levels of soluble nitrogen (23-25% of total nitrogen) but had the poorest flavor intensity, flavor quality and body quality. Samples without rennet and ripened at 22 C had the lowest levels of soluble nitrogen (13-18% of total nitrogen), and the most satisfactory organoleptic scores.

(67 pages)

# INTRODUCTION

The development of flavor during the aging of Cheddar and other cheese varieties is a lengthy and costly process (16) (45). Numerous attempts have been made to accelerate cheese ripening, but with limited success (22) (63) (64).

The demand for aged highly flavored natural cheese for direct consumption has decreased markedly in the United States in recent years, yet there remains a considerable demand for such cheese as a flavoring ingredient in snack foods, processed cheese products, crackers and casserole dishes. For such purposes the essential requirement is for cheese flavor, but not necessarily for cheese.

A number of research workers (46) (73) (77) have shown that Cheddar cheese flavor of medium to strong intensity can be produced in curd slurries within a very few weeks. These slurries are generally made from fresh Cheddar cheese curd by pulverizing and blending it with water and salt to increase the moisture to about 60% and the salt content to 3-3.5%. In some instances lipases and/or proteases are added. The slurries are then incubated anaerobically at 20-35 C until the appropriate flavor develops. Some of these products are marketed as enzyme modified cheese (3) (27). The development of cheese slurries as flavor ingredients is promising, and substantially shortens the time required for flavor development. However, it still requires traditional cheese making to produce the original curd.

Ernstrom et al. (24) and Ernstrom (25) reported a method for converting ultrafiltered whole milk into a curd-like material that can be used for the manufacture of processed cheese products. The process involves the production of an untrafiltered-diafiltered whole milk concentrate with approximately 60% moisture and 20% fat. It is then inoculated with a lactic starter culture and incubated until the pH reaches 5.2-5.1. Additional moisture is then removed by evaporation to obtain curd with approximately 36% moisture (24). The curd suffers from the defect of containing very little hydrolyzed protein which gives the processed cheese a rather flinty body with poor melting characteristics. Furthermore, it is necessary to blend the curd with 20% or more aged cheese to provide the necessary flavor in the processed cheese.

The ultrafiltered fermented whole milk concentrate prior to evaporation has close to the same composition and pH as the curd slurries used for making cheese flavor concentrates. If cheese flavor concentrates could be made from fermented whole milk retentates instead of cheese slurries the entire cheese making process could be eliminated for such products. Also, if substantial proteolysis could be induced by incubation of the fermented retentates, it would be possible that the body problems and poor meltability of processed cheese made from ultrafiltered process cheese base might be eliminated.

The purpose of this study was to assess the probability of producing cheese flavor concentrates directly from ultrafiltered fermented whole milk retentates and determine whether substantial proteolysis can be obtained during short term incubation.

# REVIEW OF LITERATURE

## Conventional Cheese Curing

Following the manufacture of cheese, it must be stored for a period of time before it develops significant flavor. Conventional cheese is cured by placing it in a temperature-controlled room for six to twelve months (45). The temperature of the curing room may range from 2 to 10 C (67) (89). During the curing process micro-organisms and enzymes in the cheese act as ripening agents which alter its chemical, physical and organoleptic properties (83).

The curing of cheese is of great economic significance to the dairy industry. Curing time, interest on inventory, and the cost of storage space add substantially to the price of the mature product. The most important physical factors controlling ripening are temperature, and for some varieties, humidity (67) (89). Air conditioning and humidity control equipment are expensive, as is the energy needed to run them. Consequently, the cheese price is correspondingly high (16).

The effect of curing temperature on the quality of cheese has been reported (18) (91). Higher temperatures accelerate ripening, but nearly always lower the quality of the ripened product. Ripening at lower temperatures (5 to 7 C) usually produces higher grading cheese than when ripened at higher temperatures (12 C or above) (91). The temperature range of 8 to 12 C is the economically best temperature to maintain cheese quality during conventional curing (16).

Because of modern film packaging, the humidity of curing rooms is no longer a factor in the curing of Cheddar cheese (87). However, it is of considerable importance for surface ripened cheese varieties such as Brick, Camembert, Brie and Limburger (45).

The moisture content of cheese also affects its quality during ripening. Moisture carries lactose and some of the milk salts in solution. Microorganisms change lactose into acids such as lactic acid. A certain amount of acid formation is necessary for proper cheese making and ripening; excessive amounts of moisture make the cheese taste sour; inadequate amounts may delay ripening. Therefore, the amount of moisture in each particular variety must be properly controlled (85).

Salt in cheese affects flavor, body, texture and keeping quality. The body of unsalted cheese breaks down rapidly, and the flavor is not normal. Increasing the amount of salt tends to decrease the moisture content of cheese. However, excessive salt makes cheese hard and harsh in body and is associated with delayed curing action (14).

Until recent years, other problems associated with conventional cheese curing included losses of fat and moisture (45). Significant amounts of fat were lost from waxed cheese after curing at 10 C for 2 months (85). When cured at higher temperatures, fat was lost within a week. The amount of moisture lost during curing also increased as the holding temperature increased (91).

Because of the loss of moisture, and thus of weight, the curing of cheese in plastic film wrappers has become popular. The chief advantages of this method are economy, complete protection of the cheese,

prevention of moisture evaporation and prevention of mold growth (17). Flexible wrappers of many types have been used commercially for curing and merchandising Cheddar cheese in packages. Flexible wrappers are relatively impermeable to moisture vapor and oxygen and only slightly permeable to carbon dioxide. Deterioration of cheese from mold growth in flexible wrappers can be prevented by sealing the cheese in an atmosphere of carbon dioxide (85).

## Proteolysis as a measure of cheese curing

One indication of the extent of cheese curing is protein degradation (34) (71). Proteolysis influences the body and flavor characteristics of cheese and may be used to follow the curing process (21) (57) (84). During protein degradation, insoluble proteins decompose into water-soluble compounds, such as peptides, amino acids and ammonia (29). Proteolysis in Cheddar cheese during curing may be measured by polyacrylamide gel-electrophoresis (21) (34) (48) (57), changes in soluble nitrogen at pH 4.4 (53) (80), or amino acid analysis (43). Polyacrylamide gel-electrophoresis is used to determine changes in the individual caseins during ripening. Alpha$_s$-casein is broken down faster than Beta-casein in Cheddar cheese (32) (48) (66); however, Harper et al. (34) observed that the intensity of Cheddar cheese flavor in cheese slurries was directly related to the extent of Beta casein breakdown.

There are three main proteolytic agents in Cheddar cheese: milk coagulants, starter bacteria and their enzymes and non-starter bacteria and their enzymes (57). Proteolysis results in increasing soluble nitrogen during cheese curing, and thus serves as an indicator of the

degree of ripening (14) (32). The soluble nitrogen content increases steadily with the age of cheese as a result of proteolysis (84). Some soluble nitrogen components of cheese may cause bitter flavors (50) (57), while others result in good flavors. The production of bitter flavors is generally attributed to the formation of specific bitter peptides. A high rate of proteolysis also produces a bitter taste in cheese (57) (71). During ripening, amino acids may be liberated gradually by the decomposition of paracasein and polypeptides (5) (89). The degradation of certain amino acids to aldehydes having one less carbon atom than the amino acid (Strecker degradation) has been considered important in the development of Cheddar cheese flavor (41). For example, the Strecker degradation of methionine forms methional, an important flavor compound. However, high concentrations of methional are obnoxious (41).

Various amines are formed during cheese ripening by decarboxylation of amino acids (42) (89). For example, tyramine is derived from tyrosine by bacterial decarboxylase in cultures of Streptococcus faecalis or the usual lactic starters (38) (42). Lactic organisms and S. faecalis together produced more tyramine in cheese than did S. faecalis starter alone (42). Although tyramine is not considered a Cheddar flavor compound, increasing tyramine content is sometimes associated with increased cheese flavor intensity. Thus, the tyramine content serves as an indicator of bacterial activity, and in turn, flavor intensity in cheese (15).

Histidine also may be bacterially decarboxylated to histamine. It has been reported that histamine-producing microorganisms do not

decarboxylate tyrosine and tyramine producers do not decarboxylate histidine (33).

Attempts to accelerate conventional cheese curing

For desirable full flavor to develop in Cheddar cheese, curing from six to twelve months is usually required. Therefore, shortened procedures for accelerated ripening of Cheddar cheese have been widely studied (22) (29) (63).

Addition of cell free extracts of lactobacilli to cheese curd caused a significant increase in the rate of ripening (22). Lactobacilli, as well as the starter bacteria release proteolytic enzymes which may hydrolyze casein and other proteins during curing (22). The organisms also release lipolytic enzymes which liberate free fatty acids and enhance cheese flavor development. However, excessive numbers of even a desirable bacterial strain may cause many undesirable changes and a poor flavor (62).

Peterson and Sjostrom (63) demonstrated that ripening of Swedish semi-hard cheese could be accelerated by adding additional lactic starter bacteria to the curd. However, an increase in starter population could also be responsible for the production of bitterness due to increased or uncontrolled proteolytic breakdown of casein (49).

The addition of rich sources of proteolytic or lipolytic enzymes also might be used to accelerate ripening (29) (64). The rate of cheese ripening can be accelerated by adding selected species of bacteria and encapsulated enzymes. However, the selection of suitable enzymes that will produce sufficient and desirable changes in cheese is a major problem.

Weaver (88) reported a new procedure involving lactase for the prehydrolysis of lactose in milk prior to conventional Cheddar cheese making. The hydrolyzed lactose Cheddar cheese showed an accelerated rate of protein degradation compared with conventional Cheddar cheese. The progressive proteolysis was responsible for gradual texture improvement; thus, a hydrolyzed lactose Cheddar cheese only three months old was considered equivalent to a conventional Cheddar cheese six to nine months old (88).

## Cheese Slurries and Flavor Development

The activity of enzymes already present in cheese can be affected by storage temperature (67). Wilson et al. (91) reported that rate of flavor development was increased with high storage temperatures and high moisture contents. High moisture levels and high ripening temperatures resulted in mature flavor development over a period of several weeks. This principle was used in the development of Cheddar cheese slurries (19) (46) (73) (74). The cheese slurries were prepared from fresh cheese curd manufactured by the conventional procedure. The curd was pulverized and blended with water and salt to increase the moisture to about 60% and the salt to 3.0-3.5%. The slurries were then incubated anaerobically at 20-35 C until the appropriate flavor developed (25) (46). The slurries incubated at 30 to 35 C developed the desired intense Cheddar cheese flavor after one week, but failed to develop desirable flavor when incubated at 22 C (73). Sodium chloride (NaCl) concentration also affected flavor intensity (73). At high NaCl concentrations, the slurries were considered too salty, and at low NaCl concentrations the slurries developed fermented and Brick cheese-like flavors (77).

The ripening of Swiss cheese curd slurries also has been studied (74). Swiss cheese slurries incubated at 30 C for 5 to 6 days developed a flavor intensity and quality similar to those in one-year or older Swiss cheese. The development of characteristic flavors in Swiss cheese slurries was dependent upon the pH and the formation of active-SH groups (73) (74).

The addition of Cheddar cheese slurries to fresh curd will accelerate the rate of Cheddar cheese ripening, and the ripening time can be shortened by about one and a half months compared to normal cheese (19). The substitution of ripened cheese curd slurries for matured cheese in process cheese manufacture also produced an acceptable product; however, different flavored slurries when blended together produced a more desirable cheese flavor (77).

Enzyme Modified Cheese

Degradative enzymes from a variety of sources, including cheese making bacteria, plants, and animals have been used to accelerate cheese curing (45) (86). Babel and Hammer (4) reported that cheese made with rennet paste (a source of lipase) developed good cheese flavor. In 1950, a U.S. patent for a cheese modifying enzyme preparation was issued to Farnham (26). Since 1974 enzyme modified cheese has been used legally in process cheese, process cheese food, and process cheese spreads (27) whereas non-cheese flavors cannot be used if they resemble cheese flavors (8). Enzyme modified cheese is cheese treated with lipolytic and/or proteolytic enzymes. Lipase breaks down the fat, and protease breaks down the protein to give the finished product a better flavor. The enzyme treatment also gives the cheese better

body and texture (45). Richardson et al. (70) reported that the addition of gastric lipase to milk for the manufacture of cheese produced a good texture and medium to strong flavor intensity. However, blending individual lipases with protease before their addition to cheese blends or cheese slurries, created a strong flavor with bitterness and rancidity (45). Richardson and Nelson (68) also reported that the addition of milk coagulating enzymes to fresh Cheddar curd slurries that were sealed in plastic bags and incubated at 21 C for three days accelerated ripening. Adult bovine rennet also can be used to stimulate cheese ripening (69). Adult bovine rennet is proteolytic at pH 5.2 and could therefore play a role in cheese ripening (69).

## Ultrafiltration in Cheese Making

In conventional cheese making, the whey proteins, lactose and soluble mineral salts are expelled into the whey. The cheese solids remaining in the curd are composed mostly of fat and casein. As a result, about 25% of the protein originally in the milk is lost in the whey when it is drained (80). Kosikowski (45) reported that the greater the recovery of these cheese solids, the greater the yield and the lower the unit cost in cheese manufacturing.

In 1969, Maubois et al. (51) developed a new concept of continuous ultrafiltration (U.F.) for cheese manufacture. As Kosikowski (44) defined it, ultrafiltration is a process in which an emulsion, such as milk, moves continuously across a semipermeable membrane and transfers most of its water, soluble salts, and non-protein nitrogen to the film's outer surface while concentrating fat, protein, and insoluble salts along the inner surface. Hence, ultrafiltration is characterized

by the use of a membrane having a relatively open structure for separating solutes of different molecular weights according to the membrane employed (1) (31). The material on the inner surface of the membrane is the retentate or concentrate. The material that passes through the membrane is the ultrafiltrate or permeate. The solids that compose the retentate can be concentrated from two to six fold (7) (30) (92).

The advantages of using ultrafiltration in cheese manufacture include a 20% increase in yield of some cheese varieties through increased recovery of milk solids (25). However, doing this while maintaining good organoleptic quality is the current challenge (25) (30) (52) (80). The increased concentration made possible by ultrafiltration reduces the rennet requirements by up to 80%, resulting in decreased processing costs for the cheese manufacturer (52) (80). Continuous ultrafiltration under aseptic conditions also reduces labor costs and prevents contamination by microorganisms (31) (65).

Kinds of cheese

After a French patent was issued to Maubois et al. (51) in 1969, a U.S. patent was issued in 1975 (76) for using ultrafiltration for cheese making. Milk concentrates prepared by ultrafiltration have been used for making soft, semi hard and hard cheese (31).

Soft cheeses of the Camembert type have been prepared successfully from liquid pre-cheese (52). Cottage cheese made from ultrafiltered skim milk showed increased yields (56), but no significant difference in flavor, body and texture scores when compared with commercial cheese (10). Cottage cheese curd made into whipped cream Cottage cheese had good color and appearance scores (9). Cream cheese made from ultrafiltered retentates exhibited excellent shelf life and smoothness, and had

greater efficiency than conventional cream cheese in the utilization of milk solids (12). Mozzarella cheese made from ultrafiltered milk had good flavor, body, stretch and melt down properties. The cheese was higher in protein and had greater total solids than commercial Mozzarella cheese (13). Medium fat soft cheese (Loddon Valley soft cheese) made from concentrated milk yielded 41% more cheese than that made by the conventional process (7).

Semi-hard Danish blue cheese was successfully made at a Danish dairy using ultrafiltration. Increases of up to 13.5% in yield were experienced and the rennet consumption decreased by a factor of four while cheese vat capacity was increased by 3.5% (40).

Problems experienced with hard cheeses

Hard cheeses such as Cheddar and Cheshire are the most difficult to make from ultrafiltered retentates (7) (13) (45). Cheddar cheese made from retentates developed flavor more slowly than when made by the conventional process (9) and had crumbly and corky body defects (13). Cheshire cheese made from ultrafiltered milk had good texture and body, but the flavor was lacking in sharpness (7). Both Cheddar and Cheshire cheeses made by ultrafiltration showed no significant increases in yield (7). The high buffer capacity around pH 5.8 and heavy viscosity of the ultrafiltered retentates were also problems (13). Buffering capabilities rose exponentially with increased total solids during ultrafiltration. pH changes in fermented cheese is influenced not only by the lactic acid produced during bacterial fermentation but also by the amount of protein and salts present, i.e. the buffering capacity.

In order to obtain the desired pH in fermented ultrafiltered retentates, the buffer capacity/lactose ratio must be controlled (24).

Ultrafiltration displayed less potential for making Cheddar cheese, than for making soft cheeses (7). Therefore, the successful manufacture of Cheddar cheese by ultrafiltration is a goal of the future for cheese manufacturers.

Ultrafiltration of skim milk vs. whole milk

The value of ultrafiltration to the dairy industry is being increasingly recognized (11) (31) (52). The major application of ultrafiltration to dairy product processing has been in the concentration and fractionation of cheese whey and skim milk (6) (11) (20) (55) (60) (65) (72). The use of whole milk in ultrafiltration also has been studied (24) (30) (92). The potential includes increased recovery of fat, protein and insoluble salts in cheese (24) (25) (30) (61).

The ultrafiltration process, when applied to skim milk, yields an ultrafiltrate or permeate containing mostly lactose, soluble salts, water and non-protein nitrogen; leaving a concentrated retentate of protein and insoluble salts. During the ultrafiltration of skim milk, permeation rates decrease with increasing protein as well as lactose concentrations (61). However, when milk containing different levels of fat was ultrafiltered, it was found that as the fat content increased, the average permeation rate also decreased (79). The highest permeation rate was obtained during ultrafiltration of skim milk and lowest during ultrafiltration of whole milk (61) (79) (92). In whole milk the presence of fat and protein at the membrane surface exerts a greater

hydraulic resistance to the passage of the permeate than is the case for skim milk, which has had the fat removed (79).

Ultrafiltered milk for process cheese

The manufacture of process cheese from ultrafiltered retentates was recently studied by Kumar and Kosikowski (47). They demonstrated that process cheese could be made from ultrafiltered skim milk retentates combined with plastic cream. The required concentration of protein was achieved by the addition of freeze dried skim milk retentates to the liquid concentrate. Ernstrom et al. (24) and Ernstrom (25) also reported that ultrafiltered whole milk retentates can be used for the manufacture of process cheese. The ultrafiltered whole milk concentrate was composed of approximately 20% fat and 60% moisture. It was then inoculated with lactic culture and incubated at 30 C until the pH reached 5.2-5.1. Additional moisture was then removed by evaporation to obtain a curd with approximately 36% moisture (24). The process cheese base resulted in the recovery of nearly 100% of the fat and 98-99% of the protein. However, the product was tacky, lacked a fibrous structure and had poor melting characteristics (24) (25). Therefore, they suggested that combining 80% of ultrafiltered processed cheese base with 20% aged Cheddar cheese might result in an improved process cheese.

Effect of acid vs. sweet milk on permeation and composition

Ernstrom et al. (24) and Ernstrom (25) demonstrated the effect of pH on the permeation rate during the ultrafiltration of whole milk.

Sweet milk was higher to start with during the initial concentration stage of ultrafiltration, and the permeation rate decreased comparably for both acidified (pH 5.7) and unacidified (sweet) milk. However, as the process continued, the sweet milk maintained a permeation rate higher than the acidified milk. During diafiltration, permeation rates increased in both sweet and acid milk as the lactose concentration decreased. More diafiltration water was required with acid milk than with sweet milk to reduce the lactose concentration to a level such that the desired final pH would be reached after fermentation (24) (25). The amount of diafiltration water required was 38.5% of the original sweet milk and 75% with the pH 5.7 acid milk (24).

The gross compositions of the final UF concentrates from acid and sweet milks also were different. The product from sweet milk was composed of 21.4% fat, 59.4% moisture and 0.98% lactose. The total permeate removed (including the diafiltration step) was 120% of the original acid milk weight. In addition, there was a loss of both calcium and phosphorus during ultrafiltration of acid milk (24) (25).

The sweet milk was preferred because of the higher permeation rate during ultrafiltration, lower diafiltration requirements and retention of larger quantities of calcium and phosphorus.

Diafiltration for pH control

After concentration of whole milk by ultrafiltration, in which protein and fat are concentrated to an optimum value, water is added to the concentrate and the process is continued. This process is called diafiltration (31). In diafiltration, more lactose and salts are eliminated, along with the added water. The volume of water added is equal

to that of the permeate removed, allowing the retentate to be maintained at a constant volume and thus reducing the concentration of lactose (61). By diafiltration, the correct ratio of buffer capacity to lactose may be established so that the subsequent fermentation of the product will produce the desired pH of 5.1-5.2.

## Effect of Age, Proteolysis and pH of Natural Cheese on the Body of Process Cheese

By Federal definition (8), pasteurized process cheese is a dairy product resulting from the mixing and heating of several lots of natural cheese with suitable emulsifying agents into a homogeneous plastic mass followed by air cooling (45). A good process cheese has a smooth compact body, is devoid of fermentative gas holes, and has a uniform color (45). The body of process cheese is a criterion of it's quality. Process cheese body is influenced by the age, proteolysis and pH of the natural cheese (58) (83) (84).

The age of cheese at processing has a direct influence on the body of the finished product. If the natural cheese is too young, the process cheese exhibits a rubbery texture. If the cheese is too old, the process cheese has a soft, grainy texture (78). The acidity of the cheese used for processing also has been recognized as a factor influencing the body of process cheese (58). Natural cheese with a high pH (sweet) contributed an undesirably firm, woody body at every age. This firmness persisted in the process cheese (58). In addition, natural cheese with a high pH exhibited unsatisfactory melting properties during processing. Natural cheese with a pH range of 5.6 to 5.1 was preferred for the manufacture of process cheese with good body (78).

Natural cheese with a high pH can be improved for processing by curing it for 60 days. The melting properties also are improved after 60 days of curing (83). Thus, acidity and age of the natural cheese at the time of processing are important factors associated with changes in body characteristics of process cheese (58).

Proteolysis is another factor influencing the body of process cheese (84). Proteolysis during aging causes the body of natural cheese to lose its firm, tough, curdy properties and develop a smooth waxy consistency. These changes improve it's processing qualities (58) (84).

## METHODS AND PROCEDURES

### Milk Supply and Treatment

Whole raw milk was obtained from the Dairy Products Laboratory, Utah State Univeristy. About 150 to 170 pounds of milk in ten-gallon cans was pasteurized at 62.8 C for 30 minutes (LTLT) in a water bath. After pasteurization, the milk was cooled to 10 C, then removed to a refrigerated cooler overnight to bring the temperature to 2 C before acidification.

Acidification of whole milk was according to the procedure of Ernstrom (25). Cooled pasteurized whole milk was acidified to pH 5.7 with reagent grade lactic acid (J.T. Baker Chemical Co., Phillisburg, N.J.).

### Ultrafiltration Equipment

The ultrafiltration (UF) membrane system was the PCI "$B_1$" tubular module with series flow end caps, (Patterson Candy International, London, England). The module was equipped with T6/B noncellulosic tubular membranes designed for the rejection of 80% of 70,000 molecular weight dextrans. The module contained 18 tubes, each one holding a paper-coated membrane that was 244 cm long and 1 cm in internal diameter. The module enclosed a total membrane surface area of 1.7 square meters. The unit was operated at inlet and outlet pressures of 621 and 206 Kpa (90 and 30 psi) respectively.

## Ultrafiltration Procedures

The acidified milk was warmed to 50 C and poured into the ultrafiltration tank. During ultrafiltration, the retentate temperature was kept at 50 C. As the milk flowed through the tubes, the permeate passed through the membranes, while the retentate was recirculated until the solids reached the desired concentration. The permeate was collected and permeation rates measured. A schematic of the ultrafiltration procedure is shown in Figure 1.

### First ultrafiltration

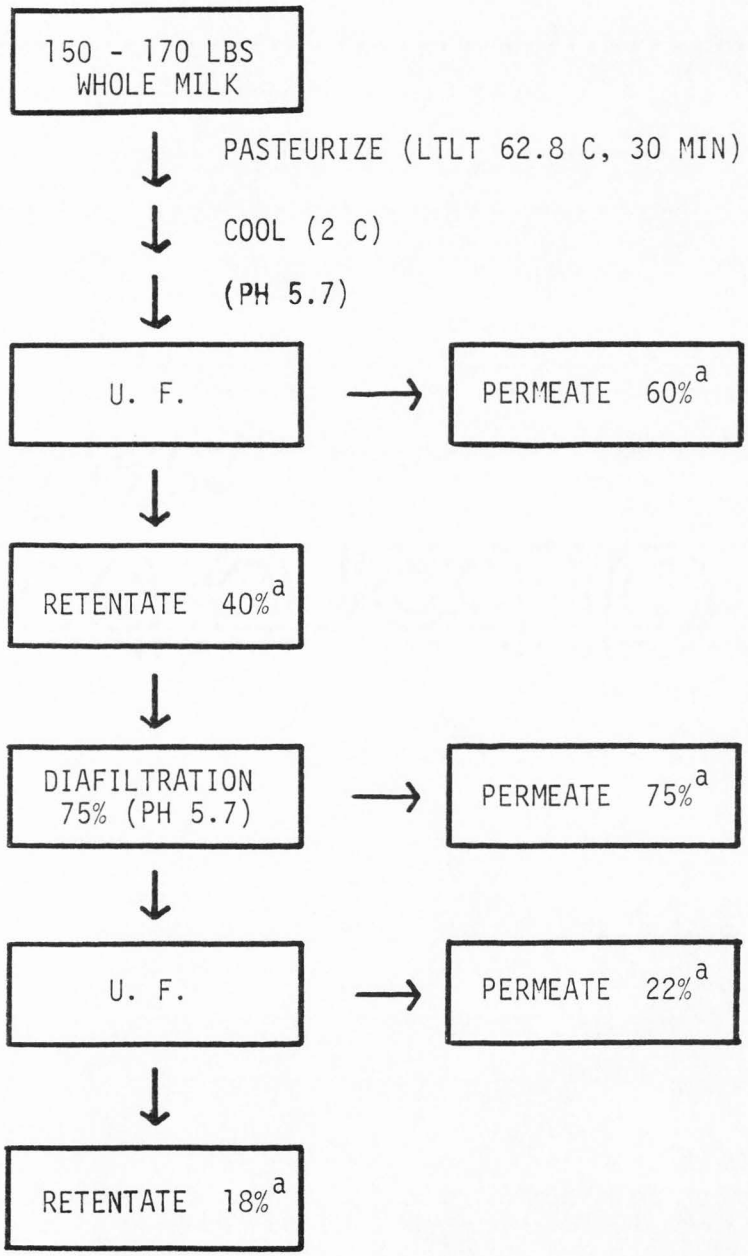
The whole milk was continuously ultrafiltered until the retentate was reduced to 40% of the weight of the original milk. This point was determined by measuring the permeate until 60% of the original milk weight was removed.

### Diafiltration

Water equal to 75% of the original milk weight was measured into a tank, adjusted to 50 C and acidified to pH 5.7 with lactic acid. The water was introduced into the retentate at the same rate the permeate was removed. In this way the retentate was maintained at a constant volume during diafiltration (61). These conditions for diafiltration were used to establish an appropriate ratio of lactose to buffer capacity so that complete fermentation of the lactose would leave the product with a pH of 5.1-5.2 (24).

### Final ultrafiltration

Following diafiltration, ultrafiltration was continued until the retentate was reduced to 18% of the weight of the original milk. The total permeate removed during the entire process was equal to 157% of the original milk weight. At the end of the process, the retentate was pumped in-




a - Percent of original milk weight.

Figure 1. Schematic of ultrafiltration process.

to a stainless steel milk can for fermentation. This entire process was replicated eight times.

Cleaning and sanitation of UF membranes

After the UF process was completed, the system was immediately flushed with clean water to remove all excess residue. Following the water rinse, a .04% solution of an enzyme detergent (Osmonic Ultrazyme, Osmonic Inc. 1540 Industrial Road, Hopkins, Minnesota), was circulated through the system for 45 minutes at 50 C. The detergent solution was then drained and the system rinsed with clean water. Finally, the module was filled with a .02% sodium azide solution to prevent bacterial growth while not in use.

Before running the next experiment, the sodium azide solution was drained and flushed with clean water. A sodium hypochlorite solution (200 ppm) was then added to sanitize the tank and the entire UF system.

Bacterial Cultures and Fermentation Procedures

Streptococcus lactis $C_6$ was obtained from the Dairy Research Laboratory, Commonwealth Scientific and Industrial Research Organization (CSIRO) Highett, Victoria, Australia. "Superstart" mixed concentrated cultures MD294S (MD) and CCI299S (CCI) were obtained from Marschall Division, Miles laboratories, Elkhart, Indiana. S. lactis $C_6$ was carried in sterile milk and transferred weekly. Following transfer, the inoculated tubes were held at 4 C until the day before use at which time they were incubated at 22 C for 16 h. The commercial mixed cultures were held at -20 C and inoculated directly into the retentate. The ultrafiltered retentate. The ultrafiltered retentate was divided into six lots, as shown in Figure 2. These lots were subdivided into three groups of two samples each. Ten milliliters of S. lactis $C_6$ and .25 g of the frozen concentrated cultures were inoculated into each kilogram of UF retentate

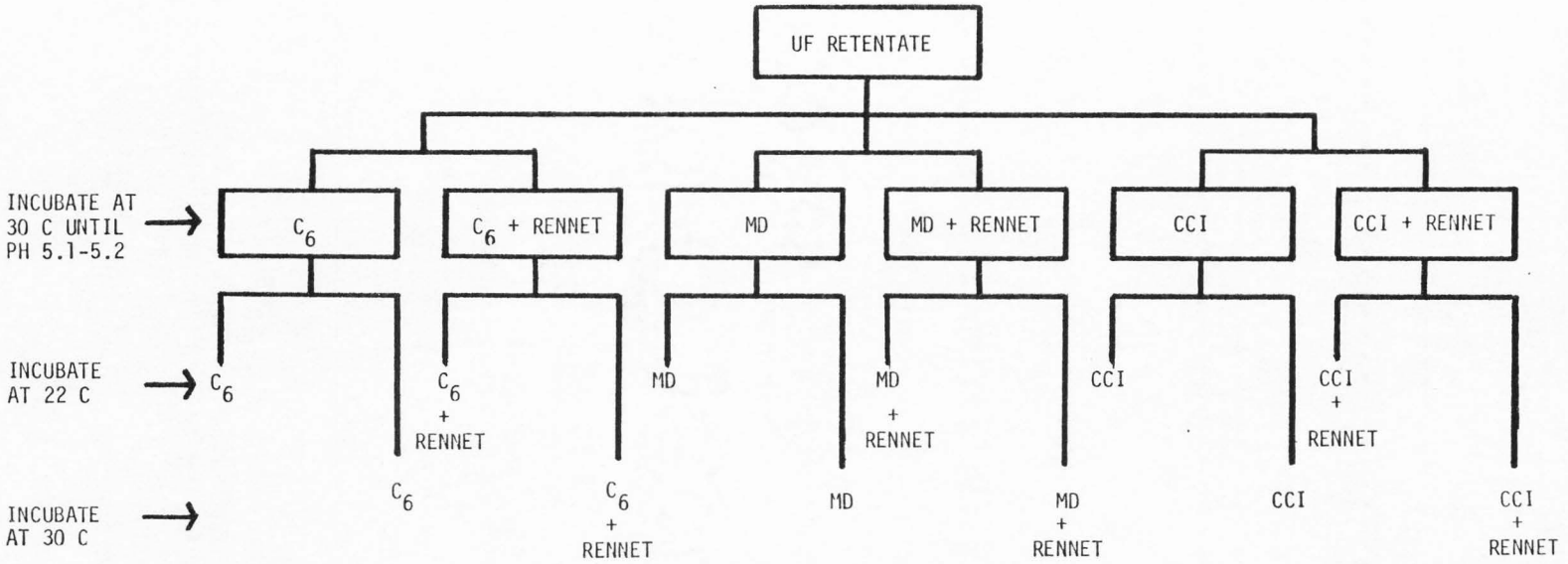



Figure 2. Preparation of samples from ultrafiltered retentates.

(Fig. 2) (24). One sample in each group was treated with 1.2 ml (1:100 dilution) calf rennet per kilogram of ultrafiltered retentate. This was based on the amount of rennet normally retained in Cheddar cheese (36). The other sample was left rennet free (Fig. 2). All samples were incubated at 30 C until the fermented retentate reached a final pH of 5.1-5.2.

## Description of Treatments

Two 250 g samples from each of the six fermented retentates were placed in 15.18 x 25.3 cm plastic pouches (90). Salt (NaCl) was added to a concentration of 4.5% of the moisture content of the retentate (24) (25). The salt and fermented retentates were mixed well then sealed under vacuum by a "VACU FRESH" vacuum sealer (Webomatic, Meat Packers and Butchers Supply Co., Model no. I-25, Vacuum Packaging System, 2820 E. Washington Blvd. Los Angeles, California).

## Sensory Evaluation

Fermented ultrafiltered samples were evaluated by five judges after two and four weeks incubation. The five panel members were trained by a flavor critique.

The samples were removed from storage and tempered to 22 C for 1h before sampling by panel members. Fermented ultrafiltered samples were judged for flavor intensity, body and flavor quality using the scoring system shown in Figure 3. Flavor intensity scores ranged from 1 - no cheese flavor to 9 - intense cheese flavor. Flavor and body quality scores ranged from 1 - unsaleable to 9 - superior. Judges also were asked to indicate specific flavor and body criticisms, that were most evident in each sample. These were mealy, smooth, putrid,

Cheese Slurry Scores

Name: _____ Date: _____

| Sample | Flavor Intensity | Body | | Flavor | |
|---|---|---|---|---|---|
| | | Scores | Description | Scores | Description |
| | | | | | |
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| | | | | | |
| | | | | | |

Flavor Intensity

1. No Cheese Flavor
2. ----
3. Slight Cheese Flavor
4. ----
5. Moderate Cheese Flavor
6. ----
7. High Cheese Flavor
8. ----
9. Intense Cheese Flavor

Flavor & Body Score

1. Unsaleable
2. ----
3. Objectionable
4. ----
5. Satisfactory
6. ----
7. Excellent
8. ----
9. Superior

Figure 3. Grading forms for flavor intensity, body quality and flavor quality of fermented ultrafiltered retentates.

acid, and bitter. Not all the judges indicated specific criticisms for every sample.

## Statistical Procedures

An analysis of variance (ANOVA) was performed for the 3 dependent variables, i.e. flavor intensity, body quality and flavor quality. Included were 8 replications, 3 culture strains, presence or absence of rennet and two incubation temperatures. Three two-way interactions (CxR, CxT, RxT) and one three-way interaction (CxRxT) also were included in this model. The computation was performed by a factorial analysis of variance (FCTCVR) of the statistical package (STATPAC) developed by Hurst (39). Standard deviations, correlation coefficients, and least significant differences were calculated (59).

A Chi-square analysis was made of the relationship between the treatments and numbers of specific flavor and body criticisms or comments reported by the judges. Each Chi-square analysis was based on the total numbers of criticisms or comments reported. ( $X^2 = \frac{(|Z-Y|-1/2)^2}{Y}$ ). Where Z = observed value; Y = expected value.

Significance was detected by comparing Chi-square values with standard Chi-square tables.

## Total Solids

Total solids was measured in the whole milk and in the final ultrafiltered retentates. About 2.5 g of milk and 1 g of retentate were accurately weighed into an aluminum dish and placed over a steam bath for about 10-15 minutes until all free moisture was evaporated. It was then heated in an air oven at 100 C for 16 h following a modified procedure of Sutherland (77).

## Milk Fat and Protein

The whole milk was analyzed for fat and protein on a Milk-O-Scan 300 (A/S N. Foss Electric, Denmark; Sold by Foss American Inc. Fishkill, N.J.).

The fat content of the UF retentate was measured by the Mojonnier procedure (2). Two grams of sample were used for the determination.

## Protein in Retentate

Total protein in the retentate was measured by diluting about 7 g of sample with distilled water and making to 100 ml in a volumetric flask. One milliliter of solution was removed and analyzed by a modification of the semimicro Kjeldahl procedure of Hiller et al. (35).

### Digestion

A half gram of anhydrous sodium sulfate ($Na_2SO_4$) and .5 ml of mercuric sulfate solution (dilute 12 ml concentrated $H_2SO_4$ to 100 ml with $H_2O$ and dissolve 10 grams red mercuric oxide) was added to a Kjeldahl digestion flask containing 1 ml of sample, then 1 ml of concentrated $H_2SO_4$ was added. The flask was boiled gently on a digestion rack until the water was boiled off; then the heat was increased so that the solution boiled constantly with slight motion. When entirely clear, the contents was cooled and the sides of the flask washed down with approximately 3 ml of water. Gentle boiling was continued for 30 min.

### Distillation

Two 125 ml Erlenmeyer flasks, each containing 15 ml of saturated boric acid and four drops of Tashiro's indicator (.25 g methylene blue, .375 g methyl red and 300 ml 95% ethanol) were placed under the con-

densers with their tips under the surface of the solution. Samples were quantitatively transferred to the steam distillation unit. Four distilled water washes of about 3 to 4 ml each were used to effectively complete the transfer. Five milliliters of sodium hydroxide-sodium thiosulfate solution (dissolve 60 g NaOH and 5 g $Na_2S_2O_3.5\ H_2O$ and dilute to 100 ml) were added. All stopcocks were closed and the condenser water turned on and the steam generator started. As soon as the first drop of distillate entered the receiving flask the distillation was allowed to continue for 5 min. The flask was lowered until the tip of the delivery tube was above the liquid level in the flask, and distillation continued for 2 min more. The tip of the delivery tube was rinsed with distilled water from a wash bottle, the flask removed and replaced with 50 ml distilled water rinse solution. The steam generator was turned off and the material in the distillation chamber was drawn out followed by the rinse solution. The stopcocks were then opened to drain the spent sample and rinse solutions.

Titration

The sample was titrated with .0302 N hydrochloric acid using a five milliliter micro-burette graduated in .02 ml. Titration was continued until the color changed from green to the first faint gray color. A blank titer was subtracted from the sample titer and the percent nitrogen calculated. Percent protein was reported as N x 6.38.

Soluble Nitrogen Analysis

Soluble nitrogen in the fermented retentate was determined by preparing a sodium citrate slurry solution according to the method of Mogensen (53) as modified by Vakaleris and Price (82). A 15 g sample

of retentate was mixed with 40 ml of .5M solution of sodium citrate. After precipitation with 10 ml of 1.410 N hydrochloric acid, the mixture was centrifuged for five minutes and the supernatant filtered through Whatman No. 42 filter paper. A clear sodium citrate hydrochloric acid filtrate was thus obtained which contained the soluble nitrogen protion of the sample. Three milliliters of filtrate were used for the nitrogen determination using the semimicro Kjeldahl procedure previously described for total protein analysis.

Soluble nitrogen was calculated as a percentage of total nitrogen.

# RESULTS

## UF Retentates from Sweet Milk

Preliminary experiments were conducted on fermented ultrafiltered sweet milk. After two weeks incubation at 22 and 30 C, all samples were extremely bitter. The bitterness was attributed to the high concentration of calcium phosphate salts retained in the caseinate micelles and subsequently solubilized during acid fermentation. When whole milk was preacidified to pH 5.7 much of the calcium phosphate was solubilized and eliminated during ultrafiltration (24). The flavors resulting from fermented retentates prepared from preacidified milk were satisfactory. Based on these results all subsequent experiments were carried out on preacidified (pH 5.7) whole milk.

## Permeation Rates during Ultrafiltration

Tubular ultrafiltration (UF) membranes were used to concentrate whole milk by a factor of 5.6:1. Permeation rates were influenced by the concentration and temperature of the retentate. Table 1 shows that water at 50 C had a permeation rate of 1500 ml/min. However, when acidified whole milk (pH 5.7) was used, the initial permeation was 210 ml/min. Permeation rates decreased rapidly during the initial concentration stage. When the retentate was concentrated to 40% of the weight of the original milk, the permeation rate decreased to 140 ml/min. The higher the concentration of fat and protein, the lower the rate of permeation. When diafiltration ended, the permeation rates had increased to 190 ml/min. This increase in permeation resulted from a

Table 1. Average permeation rates during ultrafiltration and diafiltration of whole milk at pH 5.7 compared to water (50 C) (Eight replications)

| Sample | Permeation Rate ---ml/min--- | | |
|---|---|---|---|
| | $\bar{X}$ | | S.D. |
| Water | 1500 | ± | 145 |
| Initial whole milk | 210 | ± | 21 |
| Concentrated to 40%[a] | 140 | ± | 22 |
| End of diafiltration | 190 | ± | 14 |
| Concentrated to 18%[a] | 65 | ± | 10 |

[a]% of original milk weight

decrease in the lactose concentration (24). This observation supported the finding of Peri et al. (61), who reported that permeation rates were inhibited by increasing concentrations of lactose as well as protein. Diafiltration reduced the lactose content in relation to the buffer capacity of the retentate so that complete fermentation of the residual lactose would yield a final product with a desired pH (24) (25). During the final ultrafiltration stage, whole milk was concentrated to 18% of the original milk weight. At this concentration, the permeation rate had decreased to 65 ml/min. The increased viscosity of the retentate at high protein and fat concentrations became the limiting factor of the process, and resulted in rapidly declining permeation rates (28) (79) (92).

Composition of Whole Milk and Ultrafiltered Retentates

The mean composition of eight replicates of whole milk and their corresponding ultrafiltered retentates is presented in Table 2 along with standard deviations from the mean. The concentration factor of the milk by weight was a little over five fold. The fat was concentrated six fold and the protein 5.3 fold. The quantity of soluble nitrogen present in the ultrafiltered retentates was independent of protein concentration. Much soluble nitrogen was lost in the permeate during UF concentration of the whole milk (61). With the increase in solids and reduction in moisture to 60%, the ultrafiltered retentates acquired the body characteristics of a soft viscous sour cream.

Production of Soluble Nitrogen in Fermented Whole Milk Retentates

Ultrafiltered retentates containing three different lactic cultures with and without rennet were incubated at 30 C until the pH

Table 2. Mean composition of eight replicates of whole milk and ultrafiltered retentates.

| Ingredients | Whole milk ----%----- | | | UF retentates -----%------- | | | Concentration Factor |
|---|---|---|---|---|---|---|---|
| | $\bar{X}$ | | S.D. | $\bar{X}$ | | S.D. | |
| Fat | 3.5 | ± | .2 | 21 | ± | 1 | 6 |
| Protein | 3.2 | ± | .2 | 17 | ± | 1 | 5.3 |
| Soluble nitrogen | .046 | ± | .004 | .053 | ± | .003 | --- |
| Total solids | 12.3 | ± | .3 | 41 | ± | 2 | 3.4 |
| Moisture | 87.7 | ± | .3 | 59 | ± | 2 | -1.5 |

reached 5.1-5.2. The fermented retentates were then placed into Mylar bags under vacuum and incubated at both 22 and 30 C for four weeks. Soluble and total nitrogen in the fermented retentates were determined after four weeks incubation (Table 3). The soluble nitrogen was expressed as a percentage of the total nitrogen. There was a significant increase in soluble nitrogen in the fermented retentates during four weeks storage. The fermented retentates with rennet incubated at 30 C produced the highest amount of soluble nitrogen (23-25%); whereas, those without rennet incubated at 22 C produced the least soluble nitrogen (13-18%). Fermented retentates containing rennet that were incubated at 22 C produced quantities of soluble nitrogen (19-20%) similar to those without rennet and incubated at 30 C (19-21%). The rennet acted as a protease during the ripening of the retentates, which resulted in protein being converted to soluble nitrogen compounds, such as peptides, amino acids and ammonia (29). These findings correspond to published results on cheese ripening where the extent of proteolysis also was increased with increasing ripening temperature (67) (89). Research workers have associated some proteolytic activity in cheese with rennet (71) (75) (89). The level of rennet used in cheese making affects the rate of proteolytic breakdown during curing (23) (71). Vakaleris et al. (84) reported that when soluble nitrogen developed to approximately 12-15% of total nitrogen at 60 days of age good quality cheese was produced. Too much proteolysis on the other hand resulted in inferior cheese.

The moisture content of cheese has a marked influence on the rate of proteolysis during curing (83) (84). An attempt therefore was made

Table 3. Soluble nitrogen as a percent of total nitrogen in fermented (*S. lactis* $C_6$; Marschall MD 294S; Marschall CCI-299S) ultrafiltered whole milk retentates after four weeks at 22 and 30 C.

| Temperature (C) | Rennet | Cultures | | | | | |
|---|---|---|---|---|---|---|---|
| | | $C_6$ | | MD | | CCI | |
| | | % | | | | | |
| | | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. | $\bar{X}$ | S.D. |
| 22 | Yes | 19 | ± 2 | 20 | ± 2 | 20 | ± 2 |
| | No | 13 | ± 2 | 18 | ± 2 | 14 | ± 2 |
| 30 | Yes | 23 | ± 3 | 25 | ± 3 | 24 | ± 3 |
| | No | 19 | ± 4 | 21 | ± 4 | 20 | ± 4 |

Initial soluble nitrogen of ultrafiltered retentates was 1.85% of total nitrogen.

to determine whether the moisture content of the fermented UF retentates was related to soluble nitrogen development. The correlation coefficients presented in Table 4 suggest that there was very little if any relationship between moisture and soluble nitrogen. The moisture content in the retentates was substantially higher than in Cheddar cheese, and thus small variations in moisture at this high level probably were not important. Also other factors such as rennet and temperature far overshadowed the effect of moisture.

Flavor Development in Fermented Ultrafiltered Retentates

Fermented ultrafiltered retentates were evaluated by a taste panel after two and four weeks incubation. The scores and descriptions given by panel members were then subjected to analysis of variance (ANOVA), and least significance difference (LSD). The effects of the cultures, rennet and incubation temperatures were of particular interest. The effects on cheese flavor intensity are shown in Table 5. After two weeks incubation, a significantly higher flavor intensity (1% level) was found in rennet-free samples than in samples containing rennet, but after four weeks, this difference was no longer evident. After four weeks a significantly higher cheese flavor intensity was found in samples incubated at 22 than at 30 C. This was probably due to the presence of strong non-cheese off flavors in samples incubated at 30 C that masked cheese flavor. There were no significant differences in flavor intensity that could be attributed to the cultures at either two or four weeks. After four weeks incubation, flavor intensity was higher than at two weeks in all samples.

Table 4. The correlation coefficients of soluble nitrogen/total nitrogen and moisture in fermented ultrafiltered retentates.

| Temperature (C) | Rennet | Cultures | | |
|---|---|---|---|---|
| | | $C_6$ | MD | CCI |
| | | ----------(r)---------- | | |
| 22 | Yes | -.220 | -.192 | .175 |
| | No | -.386 | -.022 | .073 |
| 30 | Yes | .209 | .226 | .167 |
| | No | .132 | .027 | .205 |

Table 5. Mean flavor intensity of fermented ultrafiltered retentates after 2 and 4 weeks at 22 and 30 C.

| Age | Culture | | | | Rennet | | | Temperature | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $C_6$ | MD | CCI | | Yes | No | | 22 C | 30 C | |
| | -------Scores------- | | | LSD | ----Scores---- | | LSD | ---Scores--- | | LSD |
| Two Weeks | 3.70 | 3.54 | 3.52 | .41 | 3.29 | 3.88** | .44 | 3.53 | 3.65 | .33 |
| Four Weeks | 4.32 | 4.56 | 4.18 | .52 | 4.26 | 4.44 | .43 | 4.59* | 4.11 | .43 |

* LSD (Least Significant Difference) comparisons between variables within each other factor are at the 5% level ( /2=.0025)

**LSD (Least Significant Difference) comparisons between variables within each other factor are at the 1% level ( /2=.005)

Quality scores of the retentates at two and four weeks of age are given in Table 6. Cultures had no effect on flavor or body quality at two or four weeks of age. The addition of rennet had a highly significant effect on reducing the body quality. All rennet treated samples were extremely mealy. At two weeks of age, the flavor quality of rennet treated samples was poorer than the non-treated samples, but at four weeks, the difference was not significant. Flavor quality after two weeks incubation at 22 C was not different than when incubated at 30 C. However, flavor quality after four weeks was significantly better at 22 C. Body quality was better in all samples at two and four weeks when incubated at 22 C instead of 30 C.

A Chi-square analysis of the number of mealy and smooth body comments resulting from the effect of culture, temperature and rennet after two and four weeks incubation was determined.

Culture had no effect on the number of criticisms at two (Tables 7-8) or four (Tables 9-10) weeks of age. Samples incubated at 22 C had a significantly smoother body (Table 11) at two weeks of age than those incubated at 30 C. After four weeks samples were smoothest at 22 C and most mealy at 30 C (Tables 13-14). The addition of rennet had a highly significant effect on reducing body quality. All rennet treated samples were extremely mealy and non-treated samples were mostly smooth after both two (Tables 15-16) and four (Tables 17-18) weeks incubation.

The effects of culture, temperature and rennet on the number of specific flavor criticisms recorded by the judges were also determined.

Table 6. Mean quality scores of fermented ultrafiltered retentates after 2 and 4 weeks at 22 and 30 C.

| | Culture | | | | Rennet | | | Temperature | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $C_6$ | MD | CCI | | Yes | No | | 22 C | 30 C | |
| Two Weeks | -------Scores------- | | | LSD | ----Scores---- | | LSD | ----Scores---- | | LSD |
| Flavor | 4.34 | 4.45 | 4.27 | .41 | 4.17 | 4.53* | .33 | 4.35 | 4.35 | .33 |
| Body | 4.78 | 4.57 | 4.42 | .43 | 3.27 | 5.92** | .46 | 4.93** | 4.26 | .46 |
| Four Weeks | | | | | | | | | | |
| Flavor | 4.29 | 4.81 | 4.53 | .54 | 4.59 | 4.50 | .44 | 4.88** | 4.22 | .58 |
| Body | 4.73 | 4.50 | 4.46 | .53 | 3.39 | 5.73** | .57 | 5.12** | 4.00 | .57 |

* LSD (Least Significant Difference) comparisons between variables within each other factor are at the 5% level ( /2=.0025)

**LSD (Least Significant Difference) comparisons between variables within each other factor are at the 1% level ( /2=.005)

Table 7. Effect of culture ($C_6$, MD and CCI) on the number of smooth comments after two weeks.

| Total Criticisms | Comments of $C_6$ | Comments of MD | Comments of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 125 | 52 | 38 | 35 | 3.47 |

Table 8. Effect of culture ($C_6$, MD and CCI) on the number of mealy criticisms after two weeks.

| Total Criticisms | Criticisms of $C_6$ | Criticisms of MD | Criticisms of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 265 | 79 | 92 | 94 | 1.29 |

Table 9. Effect of culture ($C_6$, MD and CCI) on the number of smooth comments after four weeks.

| Total Criticisms | Comments of $C_6$ | Comments of MD | Comments of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 150 | 51 | 50 | 49 | .02 |

Table 10. Effect of culture ($C_6$, MD and CCI) on the number of mealy criticisms after four weeks.

| Total Criticisms | Criticisms of $C_6$ | Criticisms of MD | Criticisms of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 250 | 81 | 82 | 87 | .17 |

Table 11. Effect of temperature (22 vs 30 C) on the number of smooth comments after two weeks.

| Total Criticisms | Comments at 22 C | Comments at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 125 | 75 | 50 | 4.61* |

Table 12. Effect of temperature (22 vs 30 C) on the number of mealy criticisms after four weeks.

| Total Criticisms | Criticisms at 22 C | Criticisms at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 265 | 120 | 145 | 2.17 |

* Significant difference at the 5% level (P=.05)

Table 13. Effect of temperature (22 vs 30 C) on the number of smooth comments after four weeks.

| Total Criticisms | Comments at 22 C | Comments at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 146 | 97 | 49 | 15.13** |

Table 14. Effect of temperature (22 vs 30 C) on the number of mealy criticisms after four weeks.

| Total Criticisms | Criticisms at 22 C | Criticisms at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 250 | 101 | 149 | 8.84** |

Table 15. Effect of rennet on the number of smooth comments after two weeks.

| Total Criticisms | Comments w/ rennet | Comments w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 125 | 2 | 123 | 115.20** |

** Significant difference at the 1% level (P=.01)

Table 16. Effect of rennet on the number of mealy criticisms after two weeks.

| Total Criticisms | Criticisms w/ rennet | Criticisms w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 265 | 201 | 64 | 69.79** |

Table 17. Effect of rennet on the number of smooth comments after four weeks.

| Total Criticisms | Comments w/ rennet | Comments w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 146 | 10 | 136 | 107.02** |

Table 18. Effect of rennet on the number of mealy criticisms after four weeks.

| Total Criticisms | Criticisms w/ rennet | Criticisms w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 250 | 194 | 56 | 75.07 |

** Significant difference at the 1% level (P=.01)

Acid flavor was observed more frequently in samples inoculated with culture $C_6$ after two weeks incubation (Table 19) but this culture had no effect on the number of bitter and putrid flavors detected by the judges (Tables 20-21). After four weeks incubation, samples inoculated with culture $C_6$ had a significantly greater number of bitter flavor criticisms (Table 23), but the acid and putrid flavor differences were not significant (Tables 22, 24). Incubation temperature had the most important effect on the number of flavor criticisms. Samples incubated at 22 C had a significantly high number of acid flavors, however, the bitter and putrid flavors were more numerous in samples incubated at 30 C after both two (Tables 25-27) and four-(Tables 28-30) week incubation periods. Rennet had no significant effect on flavor quality criticisms after two weeks incubation (Tables 31-33), however, the non-treated samples were more putrid after four weeks incubation (Table 36). Rennet also had no effect on the number of acid and bitter flavors detected by the judges (Tables 34-35).

The acid flavors produced in the samples were no doubt caused by the lactic fermentation (73). Too high temperatures can create pronounced bitterness, rancidity or putrid flavors in cheese (71).

Table 19. Effect of culture ($C_6$, MD and CCI) on the number of acid criticisms after two weeks.

| Total Criticisms | Criticisms of $C_6$ | Criticisms of MD | Criticisms of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 130 | 53 | 30 | 47 | 5.96* |

Table 20. Effect of culture ($C_6$, MD and CCI) on the number of bitter criticisms after two weeks.

| Total Criticisms | Criticisms of $C_6$ | Criticisms of MD | Criticisms of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 11 | 5 | 4 | 2 | .57 |

Table 21. Effect of culture ($C_6$, MD and CCI) on the number of putrid criticisms after two weeks.

| Total Criticisms | Criticisms of $C_6$ | Criticisms of MD | Criticisms of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 40 | 15 | 14 | 11 | .37 |

* Significant difference at the 5% level (P=.05)

Table 22. Effect of culture ($C_6$, MD and CCI) on the number of acid criticisms after four weeks.

| Total Criticisms | Criticisms of $C_6$ | Criticisms of MD | Criticisms of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 93 | 23 | 38 | 32 | 3.18 |

Table 23. Effect of culture ($C_6$, MD and CCI) on the number of bitter criticisms after four weeks.

| Total Criticisms | Criticisms of $C_6$ | Criticisms of MD | Criticisms of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 27 | 20 | 2 | 5 | 18.30** |

Table 24. Effect of culture ($C_6$, MD and CCI) on the number of putrid criticisms after four weeks.

| Total Criticisms | Criticisms of $C_6$ | Criticisms of MD | Criticisms of CCI | Chi-square $X^2$ |
|---|---|---|---|---|
| 51 | 22 | 13 | 16 | 1.92 |

** Significant difference at the 1% level (P=.01)

Table 25. Effect of temperature (22 vs 30 C) on the number of acid criticisms after two weeks.

| Total Criticisms | Criticisms at 22 C | Criticisms at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 130 | 104 | 26 | 45.6** |

Table 26. Effect of temperature (22 vs 30 C) on the number of bitter criticisms after two weeks.

| Total Criticisms | Criticisms at 22 C | Criticisms at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 12 | 2 | 10 | 4.08* |

Table 27. Effect of temperature (22 vs 30 C) on the number of putrid criticisms after two weeks.

| Total Criticisms | Criticisms at 22 C | Criticisms at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 40 | 6 | 34 | 18.22** |

* Significant differenct at the 5% level (P=.05)

** Significant difference at the 1% level (P=.01)

Table 28. Effect of temperature (22 vs 30 C) on the number of acid criticisms after four weeks.

| Total Criticisms | Criticisms at 22 C | Criticisms at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 93 | 79 | 14 | 44.04** |

Table 29. Effect of temperature (22 vs 30 C) on the number of bitter criticisms after four weeks.

| Total Criticisms | Criticisms at 22 C | Criticisms at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 27 | 6 | 21 | 7.26** |

Table 30. Effect of temperature (22 vs 30C) on the number of putrid criticisms after four weeks.

| Total Criticisms | Criticisms at 22 C | Criticisms at 30 C | Chi-square $X^2$ |
|---|---|---|---|
| 51 | 4 | 47 | 34.58** |

** Significant difference at the 1% level (P=.01)

Table 31. Effect of rennet on the number of acid criticisms after two weeks.

| Total Criticisms | Criticisms w/ rennet | Criticisms w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 130 | 69 | 61 | .37 |

Table 32. Effect of rennet on the number of bitter criticisms after two weeks.

| Total Criticisms | Criticisms w/ rennet | Criticisms w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 12 | 6 | 6 | 0 |

Table 33. Effect of rennet on the number of putrid criticisms after two weeks.

| Total Criticisms | Criticisms w/ rennet | Criticisms w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 40 | 19 | 21 | .02 |

Table 34. Effect of rennet on the number of acid criticisms after four weeks.

| Total Criticisms | Criticisms w/ rennet | Criticisms w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 93 | 52 | 41 | 1.07 |

Table 35. Effect of rennet on the number of bitter criticisms after four weeks.

| Total Criticisms | Criticisms w/ rennet | Criticisms w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 27 | 14 | 13 | 0 |

Table 36. Effect of rennet on the number of putrid criticisms after four weeks.

| Total Criticisms | Criticisms w/ rennet | Criticisms w/o rennet | Chi-square $X^2$ |
|---|---|---|---|
| 51 | 12 | 39 | 13.25** |

** Significant difference at the 1% level (P=.01)

# DISCUSSION

Pasteurized whole milk was concentrated by a factor of 5.6:1 using a tubular ultrafiltration membrane system. The ultrafiltered retentates were composed approximately of 21% fat, 17% protein, .053% soluble nitrogen, 41% total solids and 59% moisture.

Incubating the fermented retentates anaerobically at 22 and 30 C for two and four week periods produced varied flavor intensities and body and flavor qualities in samples with and without rennet. Renneted samples ripened at 30 C produced the highest level of soluble nitrogen (23-25% of total nitrogen), but the quality was judged inferior to non-renneted samples. The inferior quality was due to mealiness at all ages and putrid flavors after four weeks at 30 C. Samples without rennet and ripened at 22 C produced the lowest levels of soluble nitrogen (13-18% of total nitrogen) and resulted in the most satisfactory quality scores. Fermented retentates containing rennet and incubated at 22 C contained soluble nitrogen (19-20% of total nitrogen) similar to those without rennet and incubated at 30 C (19-21% of total nitrogen).

Three treatments were starter culture, rennet and temperature. No significant differences in body quality could be attributed to the culture used in the experiment. Samples containing strain $C_6$ were criticized more frequently for being acid than those containing strains MD and CCI after two week incubation. Strain $C_6$ also produced more bitter flavors than the other two strains after four weeks of age.

However neither the acid nor bitter criticisms of strain $C_6$ had a significant effect on flavor quality scores.

Rennet and temperature were the most important factors that affected the body quality of fermented ultrafiltered retentates. Rennet treated samples were extremely mealy. Conversely, the body of samples without rennet were smooth. Similarly, samples were smoothest when incubated at 22 C and were mealy when incubated at 30 C.

Rennet had no significant effect on flavor quality, but incubation temperature was an important factor. Acid flavors were observed more frequently in samples incubated at 22 C than at 30 C; however, samples were significantly more bitter and putrid at 30 C than at 22 C for both two and four week incubation periods. The rapid breakdown of protein to soluble compounds due to proteolytic activity at 30 C produced an inferior product that was mealy, bitter and putrid.

Chi square analyses relating specific flavor and body criticisms to the various treatments cannot be considered completely reliable. Taste panel judges did not always indicate a criticism or comment when scoring the samples. All analyses given in tables 7 through 36 were based on the number of judges comments that were rendered. There may or may not have been some significance to judges not indicating specific criticisms on all samples.

This work necessarily must be considered of a preliminary nature. It has shown that the soluble nitrogen content of fermented ultrafiltered retentates can be rapidly increased during 2-4 weeks incubation at 22 C and 30 C. However flavor quality at 30 C makes that curing temperature inadvisable. Short-term incubation of fermented retentates with or

without added proteases might improve the meltability and soften the body of process cheese made from these retentates (25).

Additional work is needed to demonstrate the feasability of using fermented UF retentates for the production of cheese flavor concentrates. Some of the samples seemed to give encouraging results, but it was apparent that all of the factors affecting flavor quality were not under control in these studies. The effect of salt concentration needs more evaluation as does other factors affecting flavor variations produced by a single starter. The addition of gastric lipase (68) and the advantage of adult bovine rennet as accelerators of cheese flavor development (69) are suggested for further studies.

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# APPENDIX

Table 37. Analysis of variance of flavor intensity in fermented ultrafiltered retentates after two weeks.

| Source | Df | S.S. | M.S. | F |
|---|---|---|---|---|
| Total | 383 | 900.9896 | 2.3524 | --- |
| Replication | 7 | 101.1146 | 14.4449 | 5.1881** |
| Culture | 2 | 2.5364 | 1.2682 | 0.4555 |
| Rennet | 1 | 33.8437 | 33.8437 | 12.1556** |
| Temperature | 1 | 1.2604 | 1.2604 | 0.4527 |
| Culture x Rennet | 2 | 0.2968 | 0.1484 | 0.0533 |
| Culture x Temperature | 2 | 4.6302 | 2.3151 | 0.8315 |
| Rennet x Temperature | 1 | 6.5104 | 6.5104 | 2.3383 |
| Culture x Rennet x Temperature | 2 | 2.4114 | 1.2057 | 0.4330 |
| Error | 77 | 214.3854 | 2.7842 | --- |
| Judges | 288 | 534.0000 | 1.5841 | --- |

** Significant at 1% level

Table 38. Analysis of variance of flavor intensity in fermented ultrafiltered retentates after four weeks.

| Source | Df | S.S. | M.S. | F |
|---|---|---|---|---|
| Total | 479 | 1513.4980 | 3.1579 | --- |
| Replications | 7 | 181.9812 | 25.9973 | 5.6951** |
| Culture | 2 | 12.2792 | 6.1396 | 1.3449 |
| Rennet | 1 | 3.8521 | 3.8521 | 0.8439 |
| Temperature | 1 | 27.5521 | 27.5521 | 6.0358* |
| Culture x Rennet | 2 | 3.3042 | 1.6521 | 0.3619 |
| Culture x Temperature | 2 | 5.0542 | 2.5271 | 0.5536 |
| Rennet x Temperature | 1 | 1.3021 | 1.3021 | 0.2852 |
| Culture x Rennet x Temperature | 2 | 3.8792 | 1.9369 | 0.4249 |
| Error | 77 | 351.4938 | 4.5648 | --- |
| Judges | 384 | 590.8000 | 1.5384 | 0.3370 |

* Significant at 5% level

** Significant at 1% level

Table 39. Analysis of variance of body scores in fermented ultrafiltered retentates after two weeks.

| Source | Df | S.S. | M.S. | F |
|---|---|---|---|---|
| Total | 383 | 1394.8100 | 3.6418 | --- |
| Replication | 7 | 65.4557 | 9.3508 | 3.0744** |
| Culture | 2 | 8.3489 | 4.1745 | 1.3725 |
| Rennet | 1 | 674.6901 | 674.6901 | 221.8280** |
| Temperature | 1 | 43.3359 | 43.3359 | 14.2482** |
| Culture x Rennet | 2 | 1.8177 | 0.9088 | 0.2988 |
| Culture x Temperature | 2 | 0.4219 | 0.2109 | 0.0693 |
| Rennet x Temperature | 1 | 3.1901 | 3.1901 | 1.0488 |
| Culture x Rennet x Temperature | 2 | 0.0989 | 0.0494 | 0.0162 |
| Error | 77 | 234.2005 | 3.0415 | --- |
| Judges | 288 | 363.2500 | 1.2612 | 0.4147 |

** Significant at 1% level

Table 40. Analysis of variance of body scores in fermented ultra-filtered retentates after four weeks.

| Source | Df | S.S. | M.S. | F |
|---|---|---|---|---|
| Total | 479 | 1932.1250 | 4.0337 | --- |
| Replications | 7 | 144.6583 | 20.6655 | 4.3430** |
| Culture | 2 | 6.9875 | 3.4938 | 0.7342 |
| Rennet | 1 | 658.0083 | 658.0083 | 138.2864** |
| Temperature | 1 | 151.8750 | 151.8750 | 31.9179** |
| Culture x Rennet | 2 | 10.7042 | 5.3521 | 1.1248 |
| Culture x Temperature | 2 | 0.3875 | 0.1938 | 0.0407 |
| Rennet x Temperature | 1 | 0.4083 | 0.4083 | 0.0858 |
| Culture x Rennet x Temperature | 2 | 1.9042 | 0.9521 | 0.2000 |
| Error | 77 | 366.3917 | 4.7583 | --- |
| Judges | 384 | 590.8000 | 1.5385 | 0.3233 |

** Significant at 1% level

Table 41. Analysis of variance of flavor scores in fermented ultrafiltered retentates after two weeks.

| Source | Df | S.S. | M.S. | F |
|---|---|---|---|---|
| Total | 383 | 669.5391 | 1.7481 | --- |
| Replications | 7 | 21.1015 | 3.0145 | 1.0968 |
| Culture | 2 | 1.9375 | 0.9687 | 0.3524 |
| Rennet | 1 | 12.3984 | 12.3984 | 4.5114* |
| Temperature | 1 | 0.0026 | 0.0026 | 0.0009 |
| Culture x Rennet | 2 | 0.7500 | 0.3750 | 1.3645 |
| Culture x Temperature | 2 | 4.1458 | 2.0729 | 0.7542 |
| Rennet x Temperature | 1 | 0.7526 | 0.7526 | 0.2738 |
| Culture x Rennet x Temperature | 2 | 3.0833 | 1.5416 | 0.5609 |
| Error | 77 | 211.6172 | 2.7482 | --- |
| Judges | 288 | 413.7500 | 1.4366 | 0.5227 |

* Significant at 5% level

Table 42. Analysis of variance of flavor scores in fermented ultrafiltered retentates after four weeks.

| Source | Df | S.S. | M.S. | F |
|---|---|---|---|---|
| Total | 479 | 1588.9920 | 3.3173 | --- |
| Replications | 7 | 263.4919 | 29.0702 | 5.8826** |
| Culture | 2 | 21.5792 | 10.7896 | 2.1834 |
| Rennet | 1 | 1.0083 | 1.0083 | 0.2040 |
| Temperature | 1 | 52.0083 | 52.0083 | 10.5243** |
| Culture x Rennet | 2 | 7.6042 | 3.8020 | 0.7694 |
| Culture x Temperature | 2 | 0.5292 | 0.2646 | 0.0535 |
| Rennet x Temperature | 1 | 11.4083 | 11.4083 | 2.3086 |
| Culture x Rennet x Temperature | 2 | 2.4542 | 1.2271 | 0.2483 |
| Error | 77 | 380.5083 | 4.9417 | --- |
| Judges | 384 | 908.4000 | 2.3656 | 0.4787 |

** Significant at 1% level