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Natural Cheese from Prefermented Whole Milk Retentate

Charles Gordon Brown
Utah State University

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NATURAL CHEESE FROM PREFERMENTED
WHOLE MILK RETENTATE

by

Charles Gordon Brown

da dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1986
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Charles Gordon Brown
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ABSTRACT

Natural Cheese from Prefermented Whole Milk Retentate

by

Charles Gordon Brown, Doctor of Philosophy

Utah State University, 1986

Major Professor: Dr. Carl Anthon Ernstrom
Department: Nutrition and Food Sciences

A new method for manufacture of natural cheese was developed using 5X ultrafiltered whole milk retentate. The retentate was prefermented to pH 5.0-5.2 before curd formation to simplify the cheese making process. It was demonstrated that the process can be made commercially feasible.

Ultrafiltration and diafiltration of whole milk were done so that the desired level of residual lactose was left in the retentate. Retentate was inoculated with lactic starter culture and incubated (prefermented) until all lactose was converted to lactic acid. The final pH stabilized at about 5.0-5.2; the desired pH of the cheese curd. Incubation at 25 °C allowed the retentate to remain liquid during prefermentation and easily pumped through pipes. After prefermentation, retentate was passed through a mechanical curd former where rennet was injected and mixed. The retentate-rennet mixture coagulated as it traveled upward through a cylinder. The curd emerged from the curd former and was cut into cubes by a grid of knives. The curd
cubes were treated for removal of moisture by cooking in liquid and evaporation under vacuum. After moisture removal, curd was drained, salted and pressed. Cheddaring and milling were unnecessary.

Experiments were performed to determine proper methods for preparing retentate. Diafiltration level was significantly related to lactose concentration in retentate (p=0.0001) and final pH of fermented retentate (p=0.0001).

Acidified permeate and acidified, deionized water were evaluated as cooking liquids. Cheese made from curd cooked in permeate had acid defects, but curd cooked in water produced cheese with a pH similar to conventionally produced Cheddar cheese. Moisture content of all finished cheese was excessive for Cheddar cheese. Body and texture of cheese made from curd cooked in water was close to conventionally produced Cheddar cheese.

Diafiltration may be used to control final pH of fermented retentate. Prefermentation of retentate prior to cheese making will simplify equipment and shorten manufacturing time. Commercial application of the process is discussed.

(118 pages)
INTRODUCTION

Cheddar cheese originated in England in the 16th century (67). The first commercial Cheddar cheese plant in the United States was built in Rome, New York in 1851. Since then, Cheddar has become a very popular cheese in America, representing 48.8% of total cheese produced in the United States in 1983 (75).

It is not surprising then, that much research has been conducted to elucidate chemical and physical changes of milk constituents during their conversion into Cheddar cheese. Such research helps to improve manufacturing methods, yield, and quality. Improvements in starter cultures used in Cheddar cheese manufacture include development of phage-resistant strains and better systems for propagation of bacteria (22,39,57,59,66). Research into alternative enzyme coagulants has reduced dependence on a dwindling supply of calf rennet (38,46,58,69,72).

Improvements in manufacturing methods have generally followed two pathways. One pathway involves mechanization of traditional processes and has resulted in such systems as the Bell Siro Cheese making system from Australia (17,30), the Cheddarmaster system from New Zealand (61,63), the Stoelting cheddaring machine (1,73), the Double "O" Vat built by Damrow Company (18), vertical vats for setting, cutting, and cooking (61), towers for cheddaring and/or pressing (60,62,64), and numerous other mechanical devices (7,34,53,54,67,70). Another pathway has been concerned with studying the chemical and physical changes that occur when milk is converted into Cheddar cheese, and trying to find better ways to accomplish these changes. This has led to the development of the stirred curd process,
which reduces labor costs by eliminating the cheddaring step (35). Attempts to produce cheese by direct acidification also have been successful (6).

Along the second pathway, the development of ultrafiltration (UF) techniques for cheese making has shown great promise (2,3,5,11,14,19,36,37,52). Reductions in labor, energy, enzyme requirements, manufacturing time, and work space may be realized by utilization of UF techniques. By removing water through UF, milk may be converted directly to the solids content of certain high moisture cheeses (2,3,5,11,43). Soft cheese may be made from the concentrated retentate, and whey proteins, which normally would be lost in the whey, are incorporated into the cheese. The result is increased yield. Research has shown that it is more difficult to produce acceptable hard cheeses, such as Cheddar and mozzarella by UF methods, and achieve an increased yield (11,15,37,65).

Equipment designed for Cheddar cheese manufacture by traditional methods provides basically for batch processing. It also is rather large, since its design must accommodate cheese making and fermentation simultaneously.

The objective of the present investigation was to develop a process based on UF technology for making low-moisture natural cheese. The process should be adaptable for continuous operation. Whole milk, concentrated five-fold by UF, was fermented prior to cheese making (prefermented) to simplify the manufacturing process and equipment and shorten manufacturing time.
LITERATURE REVIEW

Ultrafiltration (UF) is a process whereby small molecular weight compounds such as water and salts are separated from large molecular weight compounds such as protein through a molecular sieve. In 1969, Maubois et al. were granted a patent for use of UF technology in cheese making (44). The process involves passage of whole or skim milk across a porous membrane. Removal of water, lactose, salts, and a small amount of organic and nitrogenous material is effected by application of pressure. Milk is thus fractionated into two parts; filtered milk called retentate, and filtrate, commonly called permeate. Retentate has a high fat and protein content and may be used to make cheese either directly or in combination with milk or cream. Permeate is essentially a waste material containing water, lactose, salts, vitamins, and organic and nitrogenous compounds of low molecular weight. Practically complete retention of fat and protein is achieved during concentration of milk by UF. Water, lactose, and salts pass through the membrane easily (19,23,24,28).

Peri et al. used this principle of membrane dynamics to describe a method of adjusting lactose levels in retentate (55). Called diafiltration, the process involves bleeding deionized water into the UF holding tank at the same rate permeate is removed. The effect is a "washing out" of lactose to levels that are consistent with those of the desired product. Diafiltration is carried out at constant volume in order to maintain a proper balance between low retentate viscosity and high lactose concentration in the water phase. Flux rate (liters of permeate passing through each square meter of membrane surface per hour) decreases with increasing concentration. Thus, retentate is concentrated as much as possible during
the first step of UF while maintaining an adequate flux rate. Diafiltration is then initiated in the feed and bleed mode, which insures that lactose concentration in the retentate, and therefore in the permeate, is as high as possible. Conditions are balanced in this way to maximize the efficiency of lactose removal.

An equation was evolved for prediction of the amount of diafiltration water required to achieve a given lactose level in retentate. The equation relates to constant volume diafiltration (55):

\[ U = \left[ \frac{M - v}{(1 - R)} \right] \ln \frac{l_1}{l_2} \]

Where
- \( U \) = volume of water to be added in liters
- \( M \) = mass of water in the feed before diafiltration
- \( R \) = retention factor for lactose (ideally this factor would be zero; in practice, the value is approximately 0.1)
- \( l_1 \) = mass of lactose in feed prior to diafiltration
- \( l_2 \) = mass of lactose in feed following diafiltration

Application of the above equation is useful in determining lactose concentration in retentate used for cheese making.

Several papers have dealt with the practical limits of UF when used for production of milk retentate. An important factor governing the degree to which milk may be concentrated by UF is development of a proteinaceous deposit on the membrane during operation (21, 25, 77). Sometimes referred to as the "secondary membrane", this deposit is responsible for the drop in flux rate experienced when milk is ultrafiltered (21, 24, 77).

Glover (24) described formation of the secondary membrane as concentration polarization. As soon as milk passes across the UF membrane under pressure, solids collect on the membrane surface and inhibit filtration.
Electron microscopy and enzymic analysis of the deposit formed on reverse osmosis (RO) membranes have revealed a triple gel layer composed primarily of casein (25). Closest to the membrane is a thin (11 nm), electron-dense layer which is probably deposited at the beginning of RO. The second layer is thicker (10 - 15 nm), and electron-lucent. The third layer is thickest (30 um), and most diffuse. Density of the secondary membrane increases with proximity to the membrane.

Yan et al. (77) reported a decrease in flux rate with increasing concentration of whole milk. Limits of UF concentration of whole milk was dependent upon retentate viscosity and development of the secondary membrane. Similarly, Fenton-May et al. (21) reported a decrease in flux rate with increasing protein concentration in retentate. With skim milk concentrated to a protein content five times greater than the original milk, flux rate was 25% of the initial value. It was proposed that high feed velocities during operation would produce shear forces that would inhibit development of the secondary membrane.

Glover reported on the limits that retentate may be concentrated by UF (24). Experimental UF units have successfully concentrated whole milk retentate to a total solids content of 62%, which is the solids content of Cheddar cheese. Flat sheet membrane systems can concentrate milk containing 3.8% fat to a total solids content of 51%. Milk with a fat content of 6.1% may be ultrafiltered to a total solids content of 58% using flat sheet membrane systems.

Because of the limited ability of UF to concentrate milk, cheese produced directly from retentate are generally of a high-moisture variety (2,3,5,11,13,43). Manufacture of hard cheese, such as Cheddar, requires
removal of more moisture from retentate than can be accomplished by UF alone (10,11,26,74).

Desirability of acidification of whole milk prior to UF was demonstrated by Anis (4). Meltability of process cheese made from retentate cheese curd was dependent upon degree of acidification of milk before UF. Process cheese produced from retentate cheese base made from milk acidified to pH 5.8 had lower calcium levels and better meltability than those produced from unacidified or less acidified milks. Sutherland et al. reported that milk acidified to pH 6.2 to 6.4 prior to UF produced retentate with a mineral composition suitable for Cheddar cheese manufacture (74).

Brule and Fauquant (8) investigated effects of acidification of milk and retentate on levels of soluble calcium. The amount of soluble calcium increased with decreasing pH. A drop in temperature also solubilized colloidal calcium. The amount of bound calcium in milk and retentate increased linearly with temperature. When retentate was diluted with water to the water content of normal skim milk, a slow solubilization of calcium followed. Amount of calcium solubilized was temperature dependent. Four to five hours were required for equilibrium to be established between soluble and bound calcium. The authors concluded by stating that physicochemical characteristics of the aqueous phase in milk retentate is responsible for the equilibria of colloidal and solubilized mineral salts.

Ernstrom et al. (19) demonstrated that removal of calcium during UF of whole milk could be enhanced by ultrafiltering at pH 5.7. However, decreased flux rate and frequent membrane fouling were problems associated with UF of acidified milk.
Ultrafiltration and Cheese Making

Development of procedures and equipment for manufacturing cheese from ultrafiltered milk has accelerated in recent years. Since Maubois et al. (44) obtained their patent for production of cheese from ultrafiltered milk, the dairy industry has sought to capitalize on ultrafiltration's potential to increase product yield and decrease production costs. Industrial scale manufacture of soft cheese from ultrafiltered milk is currently practiced in Europe, but is not yet common in the United States (2,3,9). Large scale manufacture of hard cheese such as Cheddar and mozzarella from milk retentate has not been realized due to various difficulties, including the inability to reduce curd moisture content without incurring a corresponding loss of solids (26,65).

Hard Cheese

A number of procedures for manufacturing hard cheese from ultrafiltered milk have been developed (10,11,15,26,36,37,74). Most are modifications of conventional cheese making techniques which produced no yield increases. Even so, several recently reported procedures yielded acceptable cheese when compared with conventionally produced product.

Cheddar. Chapman et al. described a procedure for manufacturing Cheddar cheese from 2X concentrated milk retentate (11). Pasteurized whole milk was ultrafiltered to half of the original milk weight, producing retentate with a total solids content between 18 and 20%. After holding the retentate at 50°C for 16 h, it was repasteurized and cooled to 30°C. An inoculum of 2% single strain culture of Streptococcus cremoris was added to the retentate. After 20 min, 40% of the normal quantity of rennet was
added and a firm coagulum resulted which was cut 30 min after addition of
the enzyme. The curds and whey were stirred for 20 min at 30 °C and the
temperature was then raised to 35 °C over 40 min. The curd was milled,
salted, pressed and stored at 13 °C. Five hours elapsed from addition of
starter to milling. Cheese yield was 10% as calculated from original milk
which is similar to cheese yield of conventional Cheddar cheese processes.
After three months curing, the cheese was of good flavor and texture and fat
and moisture levels were within the standards for Cheddar cheese.

Covacevich and Kosikowski described a procedure for successful
manufacture of Cheddar cheese by UF principles (15). Liquid skim milk
retentates, freeze dried skim milk retentates, and plastic cream were
blended in a ratio that would produce a proper total solids and fat content in
the final product. Defects were observed in the flavor of the cheese. The
authors stated that simpler cheese such as mozzarella may be produced
more successfully by UF principles.

Two years later, Kosikowski described a procedure involving
reconstitution of retentates with water to make Cheddar cheese (36). Skim
milk retentates were blended with 40% cream and water to produce cheese-
milk mixtures. The mixtures were poured into small vats resting in warm
water, lactic starter culture was added, and the mixtures were allowed to
ripen for 30 min at 32 °C. Rennet was then added and the curd was cut 30
min after addition of enzyme. The curds were allowed to sit quiescently for
15 min and then stirred continuously for 30 min as the temperature was
raised to 38 °C. The cubed curd was then agitated intermittently for 30 min
and the whey was drained. The curds were then cheddared, milled, salted,
and pressed. Cheese were initially Cheddar-like in flavor, body, and texture.
However, after several months of ripening at 10 °C they exhibited a sweet
flavor, a woody Swiss-like texture, and developed small eyes such as those associated with gouda. Some samples had cracks in the cheese body as well. Although the general flavor quality of the cheese was rated as good to excellent, it was not typically Cheddar. The authors concluded by speculating on the chemical and microbiological reasons for the defects in the cheese and the atypical pH rise during ripening. It was postulated that the rapid rise in pH during ripening was due to a salt balance and buffering system in the cheese that was different from that found in conventionally made Cheddar cheese. It was stated that perhaps a new cheese type should be introduced.

Green et al. published two related articles dealing with Cheddar cheese manufacture from ultrafiltered milk. The first (26) described the cheese making process and effect of concentration factor (1.7, 2, 3, and 4 fold concentration) on chemical parameters and product composition. The second article (29) provided electron micrographs to explain structural and textural characteristics of the cheese.

Cheese making followed the traditional Cheddar procedure except that rennet usage was reduced as concentration of retentate increased. This was done so cutting times for all cheese making trials were close to 40 min.

Rennet clotting time increased with increasing milk concentration. However, the rate and extent of curd firming increased with concentration factor, since a gel is firmer with reduced water content. Therefore, the curd was cut earlier in highly concentrated retentates as it tended to become too firm and difficult to cut later. Electron micrographs of the curd at cutting revealed a lower degree of casein micelle aggregation in more concentrated retentates, possibly due to earlier cutting times for these treatments.
Fat losses from curd into whey during cooking were related to concentration factor, with retentate concentrated four-fold retaining less than half of the original fat in the final curd. Cheese curd made from a four-fold retentate concentration contained only 19.8% fat. However, cheese curd made from a two-fold retentate concentration or less had acceptable fat content. Fat losses were cited as the primary cause of lower yields in cheese made from highly concentrated milks. The authors theorized that excessive fat losses were partially due to less casein micelle aggregation resulting in an inability of curd to entrap fat effectively. Electron micrographs of curd collected during cheese making revealed larger and coarser curd masses and more fat segregation with increasing milk concentration (29).

The proportion of protein retained in the curd during cheese making increased with increasing retentate concentration (26). Cheese made from a 4X concentration contained higher levels of protein (30.5%) and moisture (42.4%) than conventionally produced Cheddar cheese. Cheese made from more highly concentrated retentates contained small pockets of trapped whey at pressing and after five weeks curing. It was presumed that the excess moisture was carried by protein. Although cheese yield was not determined, the higher levels of protein suggest that an increase in yield can be realized if fat retention can be increased.

pH values of the final cheese increased with retentate concentration. With increasing retentate concentration, protein content increased, buffer capacity therefore became greater, and pH drop during cheese making slowed considerably. Final pH of cheese made from a 4X concentration was unacceptably high for Cheddar (5.7).

Sutherland and Jameson (74) investigated effects of two variables on cheese making: extent of diafiltration and milk pH. Fourteen cheese making
trials were carried out. Whole milk was preacidified to various extents at 4 °C with 10% hydrochloric acid, and ultrafiltered 4.8-fold with various degrees of diafiltration. Analysis of retentates revealed a direct relationship between level of diafiltration and residual lactose concentration. Increasing diafiltration resulted in lower lactose levels. Similarly, degree of milk acidification directly affected calcium and phosphorus levels in retentates with retentates derived from highly acidified milks retaining the lowest levels of these salts. Calcium retention was more markedly affected in this regard than phosphorus.

Cheese making followed a procedure similar to conventional Cheddar cheese manufacture. Retentate was inoculated at 32 °C with lactic starter culture and coagulated with a dilute rennet solution. After 40 min, the curd was cut with specially designed harps and allowed to sit quiescently for 20 min at 32 °C. The curds were then hand-stirred at 10 min intervals while the temperature was raised to 38 °C over 60 min. After 90 min in the vat, the curd was drained, dry-stirred three times at 5 min intervals, piled, fused, and cut into blocks. Cheddaring proceeded under weights for 80 min at 38 °C. The curd blocks were then milled, salted at 2% of curd weight and pressed overnight.

During cheese making, fat losses from the curd into the whey were substantial for curd produced from the most highly acidified milks. Fragility of curds increased with increasing preacidification of the original milk. It was theorized that excessive loss of curd fat was due to this fragility. However, the fat content of the final cheese was within acceptable levels with a mean fat in dry matter (FDM) content of 51.4%. Higher FDM values would be expected from cheese made conventionally from the milks used in the experiment (54%), but incorporation of whey proteins into the cheese
probably reduced the values. There was no systematic effect of treatment on protein losses into the whey.

Moisture levels were excessive in the final cheese for many of the experimental treatments. This problem was seen most regularly in cheese made from milk acidified below pH 6. These cheese also had the lowest calcium and phosphorus levels which may influence moisture content.

Lactose levels in retentates were highly correlated to cheese lactose levels immediately following pressing. Cheese made from non-diafiltered retentates exhibited white deposits of calcium lactate on their surfaces, presumably caused by high lactose levels in the retentate. Correction of this defect may have been achieved by appropriate diafiltration of the milk during UF. Cheese made from non-diafiltered retentates also had low pH values after 16 weeks, reflecting conversion of more lactose to lactic acid. Most cheese made from milks ultrafiltered at low pH also showed acid defects. Low pH values for these cheese were probably indirectly the result of removal of more buffering salts and directly the result of simply beginning with a low pH. Results from the study indicate that final cheese pH can be precisely controlled by adjustment of milk pH and extent of diafiltration.

Bush et al. outlined a procedure for manufacture of Colby and brick cheese using a two-fold retentate standardized with cream (10). Moisture levels of both cheese were similar to conventionally made Colby and brick, but excessive fat was lost into the whey.

Kosikowski et al. investigated the possibility of producing acceptable Cheddar cheese from whole milk supplemented to various extents with whole milk retentates (37). The mixtures were made into cheese following a procedure similar to conventional cheese making. With increasing
supplementation, cheese moisture and lactose levels dropped. Fat, total protein, and ash content of cheese increased with increasing supplementation, even though percentage of these components lost into the whey also increased. Retentate supplemented cheese ripened two to four months exhibited better flavor, body, and texture than conventionally produced control cheese.

**Mozzarella.** Covacevich and Kosikowski (15) reported a procedure for successful manufacture of mozzarella cheese using UF techniques. Skim milk was ultrafiltered and retentate composition adjusted to 33.6% total solids by addition of freeze-dried retentate. To the mixture was added plastic cream (69% fat) in such amounts that the resulting blend had a total solids content of 45 to 50%. The cheese-milk mixture was divided into 2.5 kg lots and homogenized. Salt, starter culture, and rennet were added and blended for 3 min and the samples sealed in plastic bags at 32 °C for fermentation. When the samples reached the desired pH, they were stored at 5 °C until further processed. Samples were removed from bags as needed, stretched in hot water and salted with brine as done conventionally.

Cheese was slow to reach the final pH. Development of suitable stretch characteristics also was retarded. Cheese produced from retentates that had been diafiltered displayed good to excellent flavor and body. Melt characteristics of 1 day old cheese was poor, but improved significantly after storage at 5 °C for 4 weeks.

**Cheese Curd for Processing.** Ernstrom et al. (19) invented a process for making cheese curd that may be used as a component of process cheese. Whole milk acidified to pH 5.7 was ultrafiltered until 60% of the original milk weight was removed as permeate. Diafiltration was begun at this point
and proceeded until an appropriate amount of lactose had been removed. An additional 20% of the original milk weight was then removed as permeate. The retentate was inoculated and incubated until fermentation of residual lactose was complete. In this manner, final pH of the fermented retentate was controlled precisely. The fermented retentate was evaporated in a swept surface vacuum evaporator to the desired solids content and used as a component of process cheese. Process cheese and process cheese food produced using 20% aged Cheddar cheese and 80% retentate cheese curd had good flavor. Process cheese exhibited an excessively firm body, but the process cheese food had acceptable body. A 16 to 18% increase in yield was achieved over conventional Cheddar cheese processes.

A similar process for production of cheese curd for processing was reported by Madsen and Bjerre (40). Whole milk was ultrafiltered and diafiltered in the manner described by Ernstrom et al. (19). Retentate was inoculated with lactic bacteria and evaporated at low temperature until the solids content reached 60%. Evaporation at low temperature was necessary to insure survival of bacteria. Manufacturing time was reduced since fermentation proceeded in the packaged cheese rather than during a specific incubation period as described by Ernstrom et al. (19). A disadvantage of the process was the poor efficiency of evaporation at low temperature. A yield increase of 18% over conventional Cheddar cheese processes was claimed. Process cheese made with 30% UF cheese curd was satisfactory in all respects.

Soft Cheese

Maubois and Mocquot reviewed several methods of soft cheese manufacture from ultrafiltered milk (43). Yield increases in production of
high moisture cheese are easily achieved by UF techniques over conventional procedures. Milk may be concentrated directly to the solids content of the cheese being produced. Whey proteins are incorporated into the product rather than drained with the whey. The article provides a description of early laboratory scale UF systems and their use in concentrating milk for production of high moisture cheese such as fresh soft cheese, Camembert, and goat's milk cheese. Bacteriological, biochemical, and physico-chemical criteria for such cheese making are evaluated. It was suggested that UF of skim milk proceed for no longer than 5 h at a UF temperature of 50 to 54 °C to preserve milk quality. UF for longer periods at these temperatures may promote bacterial growth and damage proteins (12,43,77).

Camembert. Maubois and Mocquot (43) developed a procedure for manufacture of Camembert cheese from ultrafiltered whole milk retentate. Retentate used for cheese making contained nitrogenous substances in concentrations 5 to 6 times that of the original milk. Starter was added to the retentate and the mixture was allowed to ripen for a short period. Salt was added (0.5% of the weight of the retentate), and the mixture was renneted when the pH dropped to 5.7. Further processing was unnecessary, since the resulting coagulum was of the proper composition, size, and shape of the final product. A yield increase of 16 to 20% was realized by this method as compared with conventional procedures, since whey proteins normally lost in the whey were incorporated into the product.

Stenne (71) was granted a patent for a mechanized process for producing high moisture cheese such as Camembert. Retentate was renneted and forced through a vertical chamber where the coagulum was cut into blocks. The blocks were then placed in molds. Soluble whey proteins lost in
the whey were replaced with denatured serum proteins added to the milk prior to UF.

**Feta.** Feta is a high moisture cheese easily produced from milk retentate. In Denmark, it is estimated that almost all Feta cheese is produced by UF techniques, with 9 Danish Feta cheese plants in operation at the end of 1980 (3,5). Much of the Feta cheese produced in Denmark is shipped to Iran (2).

A 1980 report described the Danish Feta UF process (2). Whole milk is ultrafiltered and the retentate passed into a vat for adjustment of composition to that of the final cheese. Yoghurt culture and calcium chloride is added and the mixture is renneted. Coagulation is complete after 20 min. The curd is then cut into blocks and placed in hoops for further fermentation and whey drainage. When the pH drops to 5.0, the blocks are dry-salted and placed in tins. A yield increase of 30% over conventional Feta cheese processes was claimed. The plant (2) converts 50,000 gal of milk into Feta cheese every 20 h.

**Cottage Cheese.** Covacevich and Kosikowski reported on the feasibility of producing Cottage cheese from skim milk retentate (14). Retentates with a protein concentration of 15% were processed into Cottage cheese. The final product displayed flavor similar to conventionally produced Cottage cheese, but curd was gelatin-like and possessed poor absorptive qualities when cream dressing was added. The color and general appearance of the cheese was poor.

Mattews, et al. (42) reported on production of Cottage cheese using skim milk retentates. Three separate lots of skim milk (9.0% total solids) were concentrated to total solids contents of 12.2, 12.9, and 13.1% and made
into cottage cheese by a conventional method. Yields of cottage cheese made from the three retentates were similar to yield from the control. Flavor and texture of the final cheese was good although some cheese curd exhibited a tough texture. The authors speculated that simple alterations in the make procedure would correct the defect.

Ocampo and Ernstrom (52) recently showed that heat treating retentate (16% total solids) produced from acidified milk (pH 5.8) resulted in elimination of the translucence and gelatin-like texture of the curd. Retentate was heated to 71 °C for 3 min and cooled in an ice bath. The retentate was then made into Cottage cheese curd by direct acidification. Glucono delta lactone was added and the pH dropped to 4.8 in 75 min. The curd cut easily, cooked well, and had good quality when the cooking temperature reached 53 °C. The final creamed curd was considered excellent in flavor and texture.

Effects of Homogenization on Properties of Cheese Curd
Produced from Retentate and Concentrated Milk

Homogenization of milk before and during UF was investigated by Green et al. in 1983 to determine influence of these factors on properties and structure of Cheddar cheese curd (27). Cheese made from retentates produced from homogenized milk had greater fat and moisture retention but poorer curd fusion than those produced from unhomogenized milk. It was proposed that with lower water content of retentates, there is a greater association between homogenized fat and casein, with fat becoming more a part of the casein network, and less subject to removal during cheese making. Retentate cheese made from homogenized milk exhibited improved texture in some respects when aged.
Maxcy et al. reported on curd tension of renneted concentrates produced from homogenized milk (45). Pasteurized, homogenized milk was concentrated at 57 to 60 °C until the solids-not-fat content (SNF) increased from 8.5 to 16%. There was a five-fold increase in curd tension with the increased SNF content. Similar results were obtained by increasing the SNF content of homogenized milk by addition of low-heat, nonfat dry milk solids (NFDMS). Cheddar cheese, soft-ripened cheese, and pasta filata cheese were made from concentrated homogenized milk and homogenized milk fortified with NFDMS. Upon addition of rennet, gel formation was firm and rapid. Fat loss from curd into whey during cooking was 10 to 20% greater than that found when non-homogenized milk was used for cheese making. It was proposed that the lowering of curd tension in homogenized milk was due primarily to adsorption of casein onto newly formed fat surfaces. Curd tension may have increased in concentrated homogenized milk because remaining casein micelles still available for curd formation were closer together.

Buffer Capacity and Fermentation of Retentate

Several investigations have dealt with the relationship between fermentation and buffer capacity of milk retentates (16,19,31,47,48). Covacevich and Kosikowski reported the effects of total solids content of skim milk retentates on the amount of added lactic acid required to reduce retentate pH to given values (16). The pH range investigated was 4.6 to 6.0. A relationship was found such that buffer capacity increased exponentially with increasing total solids. Skim milk concentrated five-fold by UF exhibited buffer capacity approximately six times greater than milk. This
phenomenon was cited as a reason for difficulties encountered in reduction of pH during ripening of cream cheese made from ultrafiltered retentates (13). Hickey et al. (31) found buffer capacity in five-fold whole milk retentates to be three times that of milk. The increased buffer capacity has been attributed principally to protein and phosphate components of retentate (74). Buffer capacity increases directly with protein concentration of skim milk retentates (48).

Mistry and Kosikowski found two-fold skim milk retentate to have maximum buffer capacity between pH 5.1 and 5.3 (48). Change in pH below 5.2 during fermentation was slow despite production of large amounts of lactic acid. Narasimhan (49) found lactic culture inhibition below pH 5.0 in 20% UF skim milk retentates. Acid solubilization of colloidal calcium phosphate was cited as the cause. A combination of these factors may be responsible for the slow decline in pH in ultrafiltered milk.

On the other hand, Hickey et al. (31) and Mistry and Kosikowski (48) reported an increase in bacterial growth and acid production during fermentation with increasing concentration of milk by UF. Hickey et al. (31) reported greater bacterial growth and acid production during fermentation of five-fold whole milk retentate as compared with whole milk. The process of UF itself was cited as one reason for the increase. Growth factors may be released and concentrated during the process. Metabolic uncoupling of growth from acid production occurred in milk during fermentation, but not in retentate. Reasons for this difference were not offered. The authors also found no difference in optimal growth temperature between a five-fold whole milk retentate and milk. Optimal growth temperature for bacteria in both was 29 to 31 °C.
PART I: PREPARATION OF RETENTATE
INTRODUCTION

Part I of this study deals with development of procedures necessary for producing retentate with proper composition, acidity, and consistency. The new cheese making system requires removal of sufficient lactose during UF so that upon incubation with lactic starter culture, the pH of the retentate decreases to approximately 5.0 to 5.2 and no further. This is the pH of the finished uncured cheese curd. The final pH will be determined by the amount of residual lactose left in the retentate. Therefore it was necessary to determine the proper degree of diafiltration required to achieve the desired lactose level.

Proper functioning of the cheese making equipment requires that the retentate remain liquid after fermentation to pH 5.0 to 5.2. Experiments were performed to determine the highest incubation temperature that would allow fermentation to proceed without concurrent acid coagulation.
MATERIALS AND METHODS

Milk

Raw, whole milk was obtained from the Utah State University Dairy farm. The milk was pasteurized at 63 °C for 30 min and stored at 4 °C until ultrafiltered.

Acidification of Milk

All milk in this study was acidified prior to UF. Addition of acid was carried out at 4 °C to prevent localized coagulation of protein. Addition of 42 mL of concentrated HCl per 39 kg milk resulted in a pH of approximately 5.80 to 5.85 at a UF temperature of 54 °C.

Ultrafiltration and Diafiltration

Ultrafiltration was by batch method using an Abcor HFK-130, single stage, spiral wound, polysulfone membrane with a molecular weight cut-off of 10,000 daltons and 5 m² of filtering surface (Figure 1). A balance tank and centrifugal pump were used for recirculation. An inlet pressure of 420 kPa (60 psi) and outlet pressure of 280 kPa (40 psi) were used throughout the process.

Ultrafiltration proceeded at 54 °C until 60% w/w of the milk was removed as permeate. Diafiltration was begun at this point by introducing deionized water at 54 °C and proceeded at constant volume as described by Peri et al. (55). Diafiltration water was calculated as percent of original milk weight. Following diafiltration, an additional 20% of the original milk weight was removed as permeate, resulting in retentate of 5X concentration (19).
Figure 1. Ultrafiltration equipment used for fractionation of milk
For example, if one begins with 100 kg milk, and 65% diafiltration was desired, 60 kg permeate would first be removed. Then diafiltration water would be introduced into the holding tank at the same rate permeate is bled out. Volume of retentate during diafiltration would be kept constant by maintaining a constant level in the holding tank. When 65 kg of permeate had been removed, diafiltration water would be turned off. Finally, 20 kg of permeate would be removed, leaving 20 kg of 5X retentate.

Samples of retentate were collected immediately following UF and stored at 4 °C until analyzed.

Membranes were cleaned as follows: water rinse; alkaline wash (NaOH, pH 11.5) and chlorine (300 mL/39 L water)-30 min.; water rinse; acid wash (HNO₃, pH 1.5)-30 min; water rinse. Equipment was sanitized immediately before use with water containing 200 ppm chlorine. All water used for washing and sanitizing the UF membrane was deionized and at 54 °C.

Chemical Analysis

**Moisture**

Moisture was determined as weight loss from 2.5 to 3.0 g of milk, or 2.0 to 2.5 g of retentate weighed in an aluminum pan, evaporated on a steam bath, and dried 3 h at 98 to 101 °C in a forced draft oven (56). Moisture determinations were made at least in duplicate. Samples revealing discrepancies were repeated until close agreement was achieved.

**Fat**

Fat was estimated by Mojonnier method (51) using samples of approximately 10 g for milk and 5 g for retentate.
Protein

Protein was estimated by semi-micro Kjeldahl procedure for nitrogen (32) using automatic Kjeltec equipment. Determinations were made in duplicate and protein content was calculated by multiplying the nitrogen content of the sample by the factor 6.38.

Lactose

Lactose was estimated by Boehringer-Mannheim enzymatic-ultraviolet method and expressed as percent anhydrous lactose (33).

Buffer Capacity

Buffer capacity was estimated by accurately weighing approximately 1 g of retentate, diluting with 100 mL distilled water and titrating to pH 5.10 with .05 N HCl (19). Buffer capacity was expressed as milliequivalents of HCl absorbed per 100 g retentate.

Fermentation of Retentate Samples

Retentate samples were inoculated with a single strain Streptococcus cremoris, frozen concentrated starter culture (Biolac, 300 S. Main, Millville, Utah 84326), and incubated in thermostatically controlled water baths. Temperatures and times of incubation are reported with the results.

pH

pH values of whole milk at the time of UF, and retentate during fermentation were determined with a glass electrode and potentiometer (Model 811, Orion Research, Cambridge, MA 02139).
RESULTS AND DISCUSSION

Preliminary Determinations of Diafiltration Level and Retentate Incubation Temperature

A preliminary experiment was performed to determine the general effect of diafiltration level on post-fermentation pH of SX retentate. Data from the preliminary experiment were used to establish the diafiltration range necessary to produce retentate with a post-fermentation pH of about 5.0 to 5.2. A later experiment was carried out to describe effects of diafiltration within this range on retentate composition, buffer capacity, and post-fermentation pH.

Table 1 shows percent diafiltration and total solids content of the final 5X retentates. Figure 2 shows effect of diafiltration level and fermentation time on rate of acid development and final pH of the retentate. It is

Table 1. Total solids in retentates after five diafiltration levels

<table>
<thead>
<tr>
<th>Diafiltration Level (%)</th>
<th>Total Solids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>36.11</td>
</tr>
<tr>
<td>50</td>
<td>37.22</td>
</tr>
<tr>
<td>60</td>
<td>37.53</td>
</tr>
<tr>
<td>70</td>
<td>38.61</td>
</tr>
<tr>
<td>80</td>
<td>35.56</td>
</tr>
</tbody>
</table>

* Percent of original milk weight.
Figure 2. Acid development in fermenting retentates for five levels of diafiltration.
apparent from Figure 2 that final pH of fermented retentates was dependent upon degree of diafiltration. pH was lowest for 40% diafiltration, as this low level left the most lactose in the retentate for conversion to lactic acid during fermentation. pH was highest for 70 and 80% diafiltration, with little difference between them.

Ten hours after inoculation, all samples gelled into hard acid curds. Since the new cheese making process is dependent upon retentate remaining liquid after fermentation, a study was performed to determine the effect of incubation temperature on curd consistency near pH 5.1. All retentate samples (5X, 60% diafiltration) were inoculated with culture in covered plastic cups and incubated in water baths.

Table 2 records pH values and curd consistency after fermentation of retentate at three temperatures. Acid coagulation occurred only in retentate

<table>
<thead>
<tr>
<th>Incubation Temperature (°C)</th>
<th>pH</th>
<th>Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>5.11</td>
<td>Liquid</td>
</tr>
<tr>
<td>21</td>
<td>5.09</td>
<td>Liquid</td>
</tr>
<tr>
<td>25</td>
<td>5.05</td>
<td>Liquid</td>
</tr>
<tr>
<td>25</td>
<td>5.02</td>
<td>Liquid</td>
</tr>
<tr>
<td>30</td>
<td>4.98</td>
<td>Coagulated</td>
</tr>
<tr>
<td>30</td>
<td>4.98</td>
<td>Coagulated</td>
</tr>
</tbody>
</table>
samples incubated at the highest temperature of 30 °C. Lowest pH was determined in the sample incubated at 30 °C, since pH in milk and retentate is temperature dependent (8,76). However, in all subsequent experiments using a 25 °C incubation temperature, acid coagulation of retentate never occurred.

The endothermic nature of protein hydrophobic bonding has been documented (50). As temperature increases, water molecules "frozen" around aliphatic protein side chains tend to return to liquid water with an increase in their entropy. Protein side chains then form hydrophobic bonds with one another. The corresponding unfavorable enthalpy of formation from the side chains is more than counterbalanced by the increase in entropy from the water. In retentate, a substantial amount of water has been removed from the milk, proteins are in close proximity and are prone to such hydrophobic bonding. Many preliminary experiments conducted during the present study showed the tendency of fermented retentates to form a curd when subjected to temperatures much above 25 °C. However, some fermented retentates were warmed to 32 °C without coagulation. Apparently, other factors besides hydrophobic bonding of proteins play a role in coagulation of warm fermented retentates. As shown in Table 2, fermentation at 25 °C prevented coagulation. Therefore, retentates were incubated at 25 °C in all subsequent experiments.

Composition of Retentates for Four Diafiltration Levels

Diafiltration levels of 35, 50, 65, and 80% were chosen for investigation. It was determined that a pH of approximately 5.0 to 5.2, (that of uncured Cheddar cheese) could be achieved in fermented retentate within
this diafiltration range (see Figure 2). The experiment was repeated three times with three different lots of milk. Thus, twelve ultrafiltration trials were performed.

A regression analysis of the data was performed. Retentate total solids, protein, fat, lactose, buffer capacity, and post-fermentation pH were dependent variables, each treated as a function of percent diafiltration. Regression models with $R^2$ values were determined for each dependent variable. Histograms depicting dependent variables as functions of diafiltration level contain averages of three trials.

A regression analysis also was performed in which the final pH of fermented retentate was a dependent variable; a function of retentate total solids, protein, fat, lactose, and buffer capacity. Regression models with accompanying $R^2$ values were computed to predict the final pH of fermented retentate as a function of these variables.

Statistical computations are reported only for significant relationships.

**Total Solids Content of Retentates**

Percent total solids of retentates for four diafiltration levels are recorded in Figure 3. Since more lactose and salts are removed at higher diafiltration levels, total solids content decreased slightly ($p=0.0518$) with increasing diafiltration.

**Protein Content of Retentates**

Percent protein in retentates for four diafiltration levels is depicted in Figure 4. Little variation was apparent since protein is almost completely rejected during UF (19,28). Nitrogenous compounds passing through the membrane include only small amounts of urea, amino acids, and ammonia (29). Thus, nitrogen content varied only slightly with degree of diafiltration.
Figure 3. Percent solids in retentate resulting from four levels of diafiltration. Black bars represent standard error of mean. (n = 3)
Figure 4. Percent protein in retentate resulting from four levels of diafiltration. Black bars represent standard error of mean. (n = 3)
Fat Content of Retentates

Figure 5 depicts percent fat in retentates for four diafiltration levels. Little variation was observed between diafiltration levels, which confirms the findings of Ernstrom et al. that rejection of fat during UF is complete (19).

Lactose Content of Retentates

Percent lactose in retentates was significantly related (p=0.0001) to diafiltration level (Figure 6). Diafiltration at 35% produced retentates with an average lactose content of 1.60% for three trials. Conversely, retentates diafiltered at 80% had an average lactose concentration of 0.35%. A regression model for retentate lactose concentration as a function of percent diafiltration was computed:

\[ \text{Retentate [lactose]} = 2.355 - 0.027 \times \text{percent diafiltration} \]

\[ (R^2 = .82) \]

It is apparent that diafiltration may be used to remove lactose to precisely the level desired in retentate. Since its content is the most consistent of all major milk constituents (76), diafiltration control of lactose level in retentate is greatly simplified.

Buffer Capacity of Retentates

Figure 7 depicts buffer capacity of retentates resulting from four diafiltration levels. As the graph reveals, insignificant differences were observed. At a 5X concentration, buffer capacity is primarily dependent on protein content. As reported by Ernstrom et al. (19), milk protein retention
Figure 5. Percent fat in retentate resulting from four levels of diafiltration. Black bars represent standard error of mean. (n = 3)
Figure 6. Percent lactose in retentate resulting from four levels of diafiltration. Black bars represent standard error of mean. (n = 3)
Figure 7. Buffer capacity of retentate resulting from four levels of diafiltration. Black bars represent standard error of mean. (n = 3)
by UF is over 98%. Buffering salts exert less influence, as they are present in much lower concentrations and some are subject to removal during the UF process (19,23,77). For these reasons, buffer capacity remains fairly constant for milks with similar protein content and concentrated to the same extent by UF.

**Final pH of Fermented Retentates**

Final pH values of fermented retentates for four diafiltration levels are shown in Figure 8. The graph follows a pattern similar to the graph depicting lactose concentration (Figure 6). Final pH values for retentates were primarily determined by lactose levels, as buffer capacity for all twelve retentate samples was similar. Diafiltration level was highly correlated ($p = .0001$) with post-fermentation pH of retentates.

Statistically, final pH of fermented retentate was treated as a dependent variable determined by both percent diafiltration and retentate composition. Therefore, two regression models were computed.

When final pH was treated as a function of percent diafiltration only, the regression model was computed as follows:

$$\text{Final pH} = 4.158 + 0.017 \times \text{(percent diafiltration)}$$

($R^2 = .96$)
Figure 8. Final pH of fermented retentate resulting from four levels of diafiltration. Black bars represent standard error of mean. (n = 3)
When final pH was treated as a function of total solids, protein, fat, buffer capacity, and lactose, the regression model was computed as:

\[
\text{Final pH} = 2.281 - 0.266 \times \text{[total solids]} + 0.447 \times \text{[protein]} + 0.251 \times \text{[fat]} + 0.086 \times \text{[buffer capacity]} - 0.524 \times \text{[lactose]}
\]

\[(R^2 = .90)\]

These relationships show that extent of diafiltration determines retentate composition, which in turn determines the post-fermentation pH of the retentate.

An important goal of this investigation was to find the diafiltration level necessary to produce retentate with residual lactose content which, when fermented, would produce a final pH of about 5.0 to 5.2. The regression equation suggests an appropriate diafiltration rate is between 49.5 and 62.5% for the preacidified (pH 5.8) Holstein milk used in this study.

Residual Lactose in Fermented Retentates

Residual lactose in fermented retentates was determined for trials 1 and 3. Residual lactose was present only in retentates produced with 35% diafiltration, indicating that lactic acid was the limiting factor for bacterial growth in these samples rather than exhaustion of lactose.

Where availability of lactose is not the limiting factor for bacterial acid production, final pH cannot be predicted from the residual lactose. In such cases, final pH is determined primarily by retentate buffer capacity and acid tolerance of the bacteria. Lactose concentration can be used to predict final pH only in cases where all lactose in the retentate is converted to lactic acid.
In the context of this investigation, lactose concentration and diafiltration level were effective predictors, since the final pH sought was well above the limiting pH for bacterial activity.
CONCLUSIONS

Manipulation of diafiltration level during UF of milk can be used to adjust lactose levels in retentate. When lactose concentration is controlled, post-fermentation pH of retentate can be predicted.

The primary goal of this study was to create a cheese making procedure with high potential for becoming a continuous process. By fermenting properly diafiltered retentate to the pH of Cheddar cheese before curd formation, the cheese making process was greatly simplified. The primary goal after curd formation was simply one of removing moisture. Moisture removal was easier, since much of the water had been removed by UF. Elimination of cheddaring and milling of curd also contributed to process simplification. These factors will allow for design of smaller and less complicated cheese making equipment than that currently in use.

Data presented in Part I confirm that final pH of fermented whole milk retentate can be controlled by manipulation of lactose levels by diafiltration. Maintenance of a liquid retentate following fermentation is possible by adjusting incubation temperature to 25 °C.

The following conclusions may be drawn:

1. Post-fermentation pH in five-fold whole milk retentates decreased with decreasing diafiltration, when the buffer capacity remained relatively unchanged.

2. A diafiltration level of 49.5 to 62.5% resulted in a post-fermentation pH of 5.0 to 5.2 in 5X retentates made from acidified (pH 5.8) Holstein milk.

3. Degree of diafiltration during UF did not markedly affect buffer capacity of 5X retentates.
4. Protein concentration in retentates was not markedly affected by degree of diafiltration.

5. Fat concentration in retentate was not markedly affected by degree of diafiltration.

6. Lactose concentration in retentates decreased with increasing diafiltration.

7. Whole milk concentrated five-fold by UF remained liquid during fermentation down to pH 5.0, if incubated at a temperature of not more than 25 °C.
PART II: CHEESE MAKING
INTRODUCTION

Part II of this study describes the new cheese making process and presents data from several curd cooking treatments. Whole milk retentate, prepared as described in Part I, was made into curd utilizing equipment designed specifically for this study. The curd was then treated to remove moisture and converted into cheese.

Equipment such as cheddaring towers, draining and matting conveyors, and mechanized buckets are designed for batch operation and frequently are very large to accommodate a lengthy fermentation time. Devices such as these are designed and built to ferment curd as it is simultaneously made into cheese. The simpler process and accompanying equipment developed in this investigation have a potential to be truly continuous. Rennet curd made from highly concentrated whole milk retentate is much firmer at cutting than its counterpart formed from normal milk and may be used in automated systems as in this study without extensive damage to its integrity. Additionally, equipment for this process may be simplified, since fermentation does not have to be accommodated in its design. After rennet curd formation, the primary goal is one of simply removing curd moisture with a minimum removal of solids. Therefore, a shortened manufacturing time can be expected.

The purpose of this investigation was to make natural cheese from continuously produced curd, which was in turn made from prefermented whole milk retentate (5X).
MATERIALS AND METHODS

Preparation of Fermented Retentate

Retentate

Preacidified milk (pH 5.8 at 54 °C) was ultrafiltered and diafiltered so the resulting five-fold retentate contained residual lactose at the proper concentration. Retentate was inoculated with starter culture and incubated at 25 °C until the pH stabilized. The final pH of the retentate was approximately 5.0 to 5.2, the pH of uncured Cheddar cheese curd. At this point the liquid fermented retentate was ready for conversion into cheese curd.

Fermentation of Retentate

Retentates were inoculated with a single strain, Streptococcus cremoris, frozen concentrated starter culture (Biolac, 300 S. Main, Millville, Utah 84326) immediately following UF and fermented in covered 37.8 L stainless steel milk cans at 25 °C. Incubation was in a thermostatically controlled incubator equipped with refrigeration and heating units to prevent temperature fluctuation.

When a single batch (117 kg) of milk was ultrafiltered, an inoculum of 0.18% was sufficient. Larger amounts (234 kg) required that the milk be divided in half and UF done twice. Retentate from the first batch of milk was cooled while the second batch was ultrafiltered. After the second UF, all retentate was mixed together, samples were taken, and frozen starter culture was added. Early experiments with two UF steps showed that retentate inoculated at 0.18% developed a fruity odor. It is possible that during the cooling period for retentate obtained first, spoilage organisms
grew in numbers sufficient to out-grow the lactic bacteria during fermentation at 25 °C. When milk was ultrafiltered in two stages, 0.7% starter culture was used and spoilage problems were eliminated.

Retentate was used for cheese making when pH stabilized at approximately 5.0 to 5.2. Early experiments showed that at this point, all lactose had been fermented to lactic acid. Time required for complete fermentation varied from 18 to 36 h, depending on percent inoculum. Incubation time was reduced by starting the fermentation at 28 °C and reducing the temperature to 25 °C when the pH dropped to approximately 5.7.

Cheese Making Equipment

System Overview

Figure 9 is a flow diagram of the new cheese making process. Figure 10 shows assembled curd forming equipment.

Retentate Pump

A positive displacement rotary pump (Ladish Co., Tri-Clover Division, Kenosha, WI 53141) equipped with a specially designed AC-DC converter (Damrow Co., 196 Western Ave., Fond du Lac, WI 54935) was used. The converter was equipped with a rheostat so precise control of retentate flow was achieved. Retentate volume flow was 900 to 1200 mL/min. Rubber tubing was used to join the retentate pump to the retentate tank and in-line rennet mixer.
Figure 9. Flow diagram for cheese making process.
WHOLE MILK
Pasteurized at 63 °C for 45 min and cooled to 4 °C

WHOLE MILK
Acidified with conc. HCl at 4 °C to pH 5.8 (at 54 °C)

WHOLE MILK
Ultrafiltered and Diafiltered at 49.5 to 62.5%

5X RETENTATE
Inoculated with starter culture

5X RETENTATE
Incubated at 25 °C

FERMENTED SX RETENTATE
Passed through curd former, cut into cubes

FERMENTED SX RETENTATE
Liquid retentate pH 5.0 to 5.2

CUT CURD
Moisture removed by cooking in liquid or evaporation. Curd is drained, salted, pressed

FINISHED CHEESE
**Rennet**

Dilute, single strength calf chymosin (New Zealand Milk Products, Inc., 1269 North McDowell Blvd., Petaluma, CA 94952) was used as a coagulant for retentate during cheese making.

**Rennet Pump**

Rennet was introduced by a peristaltic pump with a MasterFlex controller (Model WZIRO31, Cole-Parmer Instrument Co., 7425 N. Oak Park Ave., Chicago, Ill. 60648). The dilute solution (9% by volume) was pumped at 10 mL/min through a small rubber tube inserted into the larger retentate tubing.

**In-line Mixer**

Efficient mixing of rennet and retentate was accomplished with an in-line mixer (Figure 11) designed and built especially for the cheese making system. At the beginning of this study, a passive in-line mixer was used, but complete mixing was not achieved. A portion of the passive mixer was then used as a rotating spindle with staggered fins. The spindle was positioned within a stainless steel cylinder and cup arrangement that fit onto the bottom of the primary curd forming cylinder. A small electric motor (Wire Guard Systems Inc., Los Angeles, CA 90055) was used to turn the spindle.

**Primary Curd Forming Cylinder**

Two cylinders were fitted together end to end to form the primary cylinder for curd formation. Each cylinder had an internal diameter of 7.5 cm and was 74 cm in length. Total length of the primary cylinder, from the bottom of the in-line mixer cup to the exit port was 166 cm. The lower cylinder was a transparent Plexiglass sight glass, which allowed viewing of
Figure 11. In-line mixer for blending retentate-rennet solution prior to its introduction into primary curd forming cylinder.
the retentate-rennet mixture as it traveled upward and formed a curd (Figure 12). The upper tube was stainless steel with internal Teflon coating. Both tubes were sprayed lightly with vegetable oil to prevent sticking. Rubber gaskets and clamps were used to join the cylinders, in-line mixer, and exit hood.

**Exit Hood and Cutting Apparatus**

As the newly formed curd reached the top of the primary cylinder, it was cut with a stationary grid of knives situated within the exit hood (Figures 13-16). The knives were spaced so that curd emerging from the exit port was cut into 8 mm strips. The strips were cut horizontally by hand with a spatula, so absolute uniformity was not achieved (Figure 17).

Initially, an elbow was attached to the top section of the primary cylinder so that the exit hood pointed downward. This was so curd cut automatically would fall into a cooking apparatus. Experiments conducted with the elbow however, revealed that curd distortion occurred as it traveled around the elbow, resulting in damage to curd integrity. A rotating double-edged blade was originally used to cut the curd as it passed through the knife grid. In experiments reported here, curd was cut by hand with a spatula, as the rotating blade would not operate correctly without the accompanying elbow. Hand-cutting allowed closer inspection of the emerging curd.

**Curd Moisture Removal**

Following cutting, the curd was placed into either acidified whole milk permeate or acidified deionized water for cooking (Figure 18). Cooking liquids were acidified to the pH of the fermented retentate to maintain the same pH as in the water phase of the curd. Preliminary cheese making trials
Figure 12. Transparent sight glass allowed viewing of curd forming process as retentate-rennet mixture traveled up primary column.
Figures 13 and 14. Curd emerged from the exit hood ready for cutting and moisture removal.
Figures 15 and 16. Curd emerging from the cutting device. Curd was firm and very easily cut. Note squeezing of curd at knife grid and expansion following exit. Also, sides of curd plug were slightly ragged. (See text for explanation)
Figure 17. Cut curds were of an excellent size for moisture removal. Hand cutting prevented achieving absolute uniformity of size. Note syneresis of curd at middle right.
Figure 18. Curds maintained their shape well during cooking. Few curd fines were evident. The formerly greenish-yellow transparent permeate gained an opaque white cast, due to fat and protein losses from curd.
using unacidified permeate or water resulted in high pH values in final cheese. Cooking was carried out in large aluminum pots (7.5 - 37.8 L capacities). Curd was gently stirred by hand as the temperature was slowly raised. Separate samples of curd and liquid cooking medium were drawn at predetermined time intervals, placed in sealed plastic bags, labeled, and stored at 4 °C until analyzed. Where indicated, some curd was taken from the cooking liquid and placed in a Groen steam-jacketed vacuum kettle (Model KC-8, Dover Corp., 1900 Pratt Blvd., Elk Grove Village, IL 60007) for moisture removal. A 64 cm vacuum was pulled on the sample. (Lengths of time and temperatures for vacuum treatments are reported with the results.) When sufficient moisture removal was accomplished, the curd was drained and salted (Figure 19). Salted cheese curd was pressed overnight in small cylindrical cheese hoops perforated to promote drainage. Pressed cheese was vacuum-sealed in plastic bags and stored at 10 °C until analyzed.

Chemical Analysis

Moisture

Moisture was determined as the weight loss from approximately 2.5 to 3.0 g of milk or permeate, or 2.0 to 2.5 g of retentate weighed in an aluminum pan, evaporated on a steam bath, and dried 3 h at 98 to 101 °C in a forced draft oven (56). Curd and cheese samples (2.5 - 3.0 g) were weighed into 50 mL pyrex beakers and dried for 16-18 h at 100 °C in a forced draft oven (56). All samples were cooled in a glass desiccator prior to final weighing.
Figure 19. Following moisture removal, curd was drained, salted, and pressed. Cheddaring and milling was not required.
Fat

The Mojonnier method (51) was used to determine the fat content in milk, permeate, freshly cut curd, and retentate. Samples of approximately 10 g for milk, 7 g for permeate, 2 g for curd, and 5 g for retentate were used. Method of homogenizing samples before weighing depended upon sample consistency. Permeate samples were warmed and homogenized briefly in a microblender. Retentate samples were warmed and mixed with a spatula. Curd samples were chopped and mixed with a spatula.

Fat in cheese was determined by a modified Babcock method using a sample size of 9 g (56).

Protein

Protein was estimated by semi-micro Kjeldahl procedure for nitrogen (32) using automatic Kjeltec equipment. Determinations were made at least in duplicate and protein content was calculated by multiplying the nitrogen content of the sample by 6.38.

Lactose

Lactose was estimated by Boeringer Mannheim enzymatic-ultraviolet method (33) or Shaffer-Somogyi method (68) and expressed as percent anhydrous lactose.

pH

pH values for whole milk at time of UF, retentate during fermentation, and final cheese were determined with a glass electrode and potentiometer (Model 811, Orion Research, Cambridge, MA 02139).
RESULTS

Operation of Curd Forming Equipment

Figure 10 shows the assembled curd forming apparatus. It was necessary first to establish, through trial and error, appropriate rates of flow for retentate and rennet solution as well as rennet concentration. The amount of rennet mixed with the retentate was such that adequate residence time was allowed in the primary cylinder for a firm curd to develop. Two factors aided in reducing retentate residence time. Firstly, milk retentate forms a firm curd more rapidly, and with less rennet than is required to coagulate normal milk (11, 26, 41, 43). The present investigation confirmed this observation. Secondly, the retentate was fermented to a pH of approximately 5.0 to 5.2 before addition of the enzyme. Therefore, the rennet was acting at a pH closer to its optimum as compared with conventional cheese making (20, 76). These two factors reduced residence time in the primary cylinder, and the amount of rennet required for operation of the curd former. On the other hand, the temperature of the retentate when poured into the supply tank was at the incubation temperature (25 °C), which was lower than the optimal setting temperature used for normal cheese making (35). This was necessary to prevent acid coagulation of the retentate. It may be assumed that if the primary cylinder could be equipped with a heat exchanger, the retentate-rennet mixture could be warmed, in which case rennet activity would increase, and retentate residence time could be shortened.

Many trials were carried out initially to determine optimal conditions for operation of equipment. Too much enzyme caused extensive proteolysis and resulted in a pasty curd. When too little enzyme was used, uncoagulated
retentate poured from the curd former exit. These problems were solved by adjusting retentate flow, rennet solution flow, and rennet concentration. In this study, satisfactory curd forming was achieved by pumping retentate at 900 to 1200 mL/min, and rennet solution at 10 mL/min. Residence time for curd forming in the primary cylinder ranged from approximately 6-8 min depending on retentate volume flow rate. At a retentate flow rate of 900 mL/min, rennet usage based on original milk weight was the same as used in conventional cheese making. When retentate flow rate was increased to 1200 mL/min, rennet was 25% less than used in conventional methods.

With the prototype described here, these parameters worked well. However, with an improved design, parameters may be adjusted and better operation may result.

The retentate pump with AC-DC converter gave excellent performance, allowing for accurate adjustment of retentate flow rate. The pump was easily disassembled for cleaning and reassembled for cheese making. Rubber tubing used to join the pump to the holding tank leaked very little, only where the rennet solution tube was inserted.

The rennet solution pump gave good performance, with a steady delivery of enzyme into the retentate maintained throughout operation.

The in-line mixer delivered thoroughly mixed enzyme solution and retentate. A small amount of retentate leaked from the bottom of the spindle onto the electric motor housing. Paper towels were wrapped around the bottom of the spindle to soak up liquid before it caused electrical damage. Upon disassembly of equipment following cheese making, the rotating spindle of the in-line mixer appeared to have collected curd on the primary shaft during operation. This problem may be solved by using a polished stainless steel spindle that would resist curd adherence.
Figure 12 shows curd being formed in the primary cylinder. Problems were encountered occasionally as curd tended to stick to interior surfaces of the upper cylinder. This stationary curd layer became thicker with continued operation. Correction of this problem was accomplished only by stopping the retentate and rennet pumps for approximately one minute, and allowing the retentate within the top portion of the cylinder to become firm while remaining still. Pumps were then turned on and proper functioning of the equipment was regained. It was suspected that adherence of curd to the interior of the cylinder was initiated at the junction of the upper and lower portions of the primary cylinder. It was difficult to join the tubes together and have a totally smooth interior at the junction. An improved design would provide a single tube for the primary cylinder, resulting in a smooth interior the entire length of the column.

Figures 13-16 show curd being cut as it emerged from the curd former. As seen in Figure 16, the curd plug was slightly squeezed at the knife grid, and expanded as it emerged. This distortion was a result of a decrease in the effective diameter of the cylinder at the point of the knife grid. To prevent this, the cylinder diameter may be slightly increased at the knife grid to account for space occupied by the knives themselves. Additionally, close inspection of Figure 16, reveals the side surface of the curd plug was slightly ragged; probably another consequence of reduced cylinder diameter. In spite of these shortcomings, the curd former produced curd that was nicely cut into cubes of an excellent size for cooking (Figure 17).

At this point, the process assumed a batch mode. Cut curd from the curd former was immediately placed into a liquid cooking medium for moisture removal. Therefore, all curd was not in the cooking medium for a
uniform length of time. For example, curd cut at the beginning of a trial was in the liquid longer than curd cut at the end of a trial. Usually about 30 min lapsed between beginning and end of curd cutting. Samples drawn during cooking were assumed to be uniform however, since curd was stirred continuously by hand during cooking. Cooking temperature was raised only after all curd had been placed into the liquid.

During cooking, temperature of curds and liquid was increased from 27 °C to approximately 38 °C over time periods that varied between experiments. Curd cubes maintained their shape well as revealed in Figure 18. Close inspection of a curd cube revealed a tough outer "skin", with an interior of seemingly higher moisture content. Cooking temperatures much over 38 °C gave the curd a stretchy, mozzarella consistency.

As cooking proceeded, fat and protein were lost into the liquid cooking medium. Figure 18 shows that the once transparent yellow permeate gained an opaque white cast during cooking. Curd fines were not observed in great amounts during cooking.

In selected experiments, a portion of curd was removed from the liquid and placed in a vacuum kettle evaporator for more rapid moisture removal. Although faster removal of moisture was effected, the kettle's agitator damaged the curd structure to a degree. This result underscores the need for specially designed equipment to remove moisture from curd produced by this process.

When cooking and/or evaporation was complete, curd was drained, salted, and pressed overnight. Figure 19 shows drained curd just before addition of salt. Cheddaring and milling were not required during manufacture.
Preliminary Experiment to Determine the General Change in Curd Moisture During Cooking

A preliminary experiment was conducted to establish the general rate of change in curd moisture. Whole milk was ultrafiltered and diafiltered at 58%. The retentate was inoculated with 0.18% starter culture and fermented until the pH stabilized at 5.27. The fermented retentate was run through the curd former and the cubed curd was placed in warm acidified permeate (pH 5.23, 27°C). When all curd was in permeate, the temperature was raised to 37°C over the first 20 min and held at that temperature for an additional 160 min. Curds and permeate were stirred continuously throughout cooking. Figure 20 shows the change in curd moisture during cooking. Most water was removed during the first 30 min of cooking. Very little moisture was removed during the final 2 h while the temperature remained at 37°C.

Chemical Analysis of Curd Cooked in Acidified Permeate

The goal of the next experiment was to determine loss of moisture, fat, and protein from curd during cooking and evaporation. Milk was ultrafiltered and diafiltered at 55%. The resulting 5X retentate was inoculated with 0.7% lactic starter culture and fermented until the pH stabilized at 5.17. Figure 21 depicts decreasing lactose concentration in the retentate during the first 20 h of fermentation. Lactose concentration decreased most rapidly during the first 8 h. Bacterial conversion of lactose was higher at the beginning of fermentation since the high buffer capacity of the retentate maintains pH near the optimum for growth (5.5 to 6.0) (31).
Figure 20. Curd moisture during cooking of curd in acidified permeate. Data presented in this graph are from a preliminary experiment to determine the general change in curd moisture. Zero on X axis represents point where all curd had been placed from curd formers into permeate.
Figure 21. Lactose concentration in retentate during initial 20 h of fermentation. Most lactose is fermented to lactic acid during the first 8 h of incubation. Incubation temperature was 25°C.
Curd was cooked only 105 min, since it was determined from the preliminary study that cooking any longer resulted in very little moisture removal. Cut curd was placed into acidified permeate (pH 5.17) at 27 °C. The temperature was raised to 32 °C over the next 15 min, and then to approximately 38 °C over the following 15 min. The temperature was held at 38 °C for the last 75 min. During cooking, curds were continuously stirred gently by hand. A portion of curd was drawn for evaporation 60 min after all curd had been placed in the permeate. The sample was placed on perforated shelves in a vacuum kettle evaporator (with rotating agitator removed). A 64 cm vacuum was pulled, but no heat was applied. The kettle was opened every 10 min and the cool curds were gently tumbled by hand. Evaporation of the curd proceeded for 30 min.

Figure 22 shows curd moisture during cooking in permeate acidified to pH 5.17, and during evaporation of a sample drawn from the permeate. Practically all of curd moisture removal occurred during the first hour of cooking, with most removed during the first 30 min. Stabilization of cooking temperature after the first 30 min probably helped prevent further syneresis. Evaporation resulted in a much more rapid decrease in curd moisture, since evaporation is more efficient in removing water, and solids are not lost during the process. Figure 23 shows increasing solids content of permeate during cooking. The curve is an approximate inverse of Figure 22, that of curd moisture loss during cooking. The permeate solids content began to stabilize after about an hour, the time that curd moisture removal slowed. From Figures 22 and 23, it is apparent that water removed from curd during cooking in permeate contained solids.

Figure 24 depicts the increasing curd fat content during cooking in acidified permeate. As moisture was removed, the relative amount of fat in
Figure 22. Curd moisture during cooking in acidified permeate and for a sample drawn from permeate and evaporated. Origin of X axis represents beginning of cutting at curd former exit. Zero on X axis represents point where all curd had been placed from curd former into permeate.
Figure 23. Percent solids in permeate during cooking of curd. Origin of X axis represents beginning of cutting at curd former exit. Zero on X axis represents point where all curd had been placed from curd former into permeate.
Figure 24. Percent fat in curd during cooking in acidified permeate and for a sample drawn from permeate and evaporated. Origin of X axis represents beginning of cutting at curd former exit. Zero on X axis represents point where all curd had been placed from curd former into permeate.
the curd increased. During evaporation, moisture removal was more rapid, and fat percentage increased accordingly. Fat losses into permeate were high as recorded in Figure 25. Soon after the cut curd was placed into permeate, the color of the liquid went from transparent yellow to opaque white, partially the result of fat losses from the curd. After 75 min of cooking, a yellowish fat scum formed on the surface of the permeate. From Figure 25, curd fat losses continued even after curd moisture had stabilized. Percent fat in dry matter values (FDM) of final cheese were slightly below legal standards (48.6 for permeate cook, 49.2 for permeate and evaporation). Higher fat content in the final cheese could result with no higher moisture content if cooking were simply terminated after an hour.

Figure 26 shows that protein content of curd increased during cooking in acidified permeate. As with fat, relative content of protein in curd increased as curd moisture level dropped. Evaporation accelerated this effect through a more rapid moisture removal. Protein content in permeate increased during cooking, but stabilized after 75 min (Figure 27). Significantly, protein and solids content of permeate followed a similar pattern during cooking, with Figures 26 and 23 showing a stabilizing of their concentrations after 75 min. Additionally, curd moisture loss during cooking practically stopped after 75 min (Figure 22). It seems that most proteins lost from curd during cooking are probably soluble whey proteins that leave the curd along with water.

Following cooking, curd from both permeate and permeate plus evaporation treatments was drained or removed from the kettle. Curd was salted at 2% based on weight of drained curd and pressed in small perforated cheese hoops overnight.
Figure 25. Percent fat in permeate during cooking of curd. Origin of X axis represents beginning of cutting at curd former exit. Zero on X axis represents point where all curd had been placed from curd former into permeate.
Figure 26. Percent protein in curd during cooking in acidified permeate and for a sample drawn from permeate and evaporated. Origin of X axis represents beginning of cutting at curd former exit. Zero on X axis represents point where all curd had been placed from curd former into permeate.
COOKING TREATMENT

- ○ PERMEATE
- ● PERMEATE AND EVAPORATION

PROTEIN IN CURD (%)

COOKING TIME (min)

Range: -30 to 105

Figure 27. Percent protein in permeate during cooking of curd. Origin of X axis represents beginning of cutting at curd former exit. Zero on X axis represents point where all curd had been placed from curd former into permeate.
Table 3 records data for final cheese from the experiment described above. Although moisture content of both cheese were higher than legal standards allow for Cheddar, the cheese FDM values were fairly close to the 50% required by law for Cheddar cheese.

Protein content of experimental cheese was low compared to conventionally produced Cheddar cheese. However, if excess moisture in experimental cheese were removed without simultaneous removal of protein, the protein content would approach the levels found in traditional Cheddar cheese. Moreover, if moisture could be removed from the curd without removing soluble whey proteins as discussed previously, yield increases may be realized.

Table 3. Data for final cheese. Curd cooked in acidified permeate only, or with evaporation. (Moisture, fat, and protein are percentages)

<table>
<thead>
<tr>
<th></th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>pH</th>
<th>pH after 7 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeate</td>
<td>45.7</td>
<td>26.4</td>
<td>24.5</td>
<td>4.8</td>
<td>4.7</td>
</tr>
<tr>
<td>Permeate and evaporation</td>
<td>44.3</td>
<td>27.4</td>
<td>24.0</td>
<td>4.8</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Experimental cheese had acid defects immediately after pressing. The high concentration of lactose in permeate used for cooking probably contributed to higher lactose concentration in the water phase of the cheese curd through an equilibration process. Permeate frequently contains lactose concentrations in excess of 4% (76). It is likely that fermentation of the excess lactose led to the drop in pH to 4.6 after seven weeks curing, as
indicated in Table 3. Additionally, inhibition of bacteria may have been reduced, as whey expressed during pressing contained visible amounts of salt.

Immediately after pressing cheese was acid in flavor and exhibited a slightly crumbly texture. Cheese made without an evaporation treatment broke along lines where curd cubes were not sufficiently fused. A weak salt flavor was evident in both cheese due to salt lost in whey during pressing.

**Chemical Analysis of Curd Cooked in Acidified, Deionized Water**

An experiment was conducted in which deionized, acidified water was used as a cooking liquid instead of acidified permeate. Curd was cooked in water only, and in water with an evaporation treatment. Yield was calculated for water cooking only, as the evaporated curd sample was necessarily too small to get accurate weights.

UF was with 55% diafiltration as described in the previous experiment. Retentate was inoculated at a rate of 0.7% and incubated until the pH stabilized at 5.17, which required approximately 18 h. A lactose determination on the retentate revealed no residual lactose at cheese making.

Cheese curd was produced with the curd former as previously described, with cut curd placed in two separate pots, each with a weighed amount of deionized water acidified to pH 5.17 and warmed to 32 °C. Curds in both pots were stirred gently by hand throughout cooking. When an adequate amount of curd had been weighed into each pot, the temperature of both was raised to 38 °C over 20 min. Curds cooked in water only were held at 38 °C for 90 min, for a total cooking period of 110 min. Curds
receiving an evaporation treatment were cooked at 38 °C for 10 min (total of 30 min in liquid), then drained and evaporated under a 64 cm vacuum at 34 °C for 45 min. When water removal for both treatments was terminated, curds were drained or removed from the evaporator, salted at 3% based on weight of drained curd, and pressed overnight. Curd was heavily salted to counteract the loss of salt during pressing, as observed in the previous experiment. All cooking water and final cheese were weighed in order to determine yield.

Table 4 records total solids, fat, and protein content of cooking liquid. Water from both treatments contains similar concentrations of the three components. This is interesting, as water for the evaporation treatment was drained after 30 min cooking while the non-evaporated sample was cooked continuously in water for 110 min. Most migration of curd solids (fat and protein) into the water occurred during initial stages of cooking. As a percentage present in initial curd, a similar amount of fat was lost into the water for the non-evaporated sample (27%) compared with the evaporated sample (29%). Protein lost during cooking of curd in water was lower than protein lost during cooking in permeate (0.4% for water cook, 0.5% for water cook plus evaporation; 0.9% for permeate). When the amount of curd per

Table 4. Composition of drained water used for cooking.

<table>
<thead>
<tr>
<th></th>
<th>Total Solids (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (non-evaporated)</td>
<td>1.2</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Water (evaporated)</td>
<td>1.3</td>
<td>0.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>
unit weight of cooking liquid was considered, the difference was magnified even more. As with fat, protein loss from curd into water was greatest during the first 30 min of cooking, since the evaporated sample was drained after this time period and both samples exhibited similar protein concentrations.

Table 5 shows the distribution of total solids, fat, and protein during cheese making for the non-evaporated treatment only, since the evaporated sample was too small to provide meaningful data. Curd and water were

<table>
<thead>
<tr>
<th></th>
<th>Total Solids (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drained Water</td>
<td>23.7</td>
<td>26.8</td>
<td>19.1</td>
</tr>
<tr>
<td>Pressed Cheese</td>
<td>79.6</td>
<td>58.9</td>
<td>83.3</td>
</tr>
<tr>
<td>Total Recovery</td>
<td>103.3</td>
<td>85.7</td>
<td>102.4</td>
</tr>
</tbody>
</table>

Table 5. Recovery of total solids, fat, and protein after cooking curd in water. (Values represent components as percentages in curd at beginning of cooking)

Weighed at the beginning of cooking. Water was weighed after the curd was drained. Cheese was weighed immediately following pressing. Total solids, fat, and protein content were then estimated for all water and cheese.

Recovery of total solids exceeded 100%. This was due in part to the high salting rate used. About a fourth of the fat in the original curd was lost into the water. A small amount of fat was lost during pressing and was unaccounted for. Protein lost into the water was approximately equivalent to soluble whey proteins expressed as a percentage of total proteins in whole
milk (76). It is apparent that a large amount of protein was lost when ultrafiltered cheese curd was cooked in permeate or water.

Data for final cheese made from both water cooking treatments are presented in Table 6. Moisture content for the evaporated curd was lower than the sample cooked only in water, reflecting the more rapid moisture removal effected by evaporation. A large difference was found in fat contents of the cheese representing the two treatments. Part of the difference was simply due to higher moisture in the curd cooked only in water.

Final pH values for cheese made from both treatments were within the acceptable range for uncured Cheddar cheese. Apparently, the absence of lactose in the cooking liquid was instrumental in maintaining a more acceptable pH than samples cooked in permeate.

FDM values for both cheese were lower than legal standards, reflecting excessive fat losses during cooking.

Although the experiment was not designed especially for yield determination, the following estimates are of interest. Yield of cheese from

<table>
<thead>
<tr>
<th></th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>pH (%)</th>
<th>FDM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>46.6</td>
<td>23.8</td>
<td>25.5</td>
<td>5.2</td>
<td>44.6</td>
</tr>
<tr>
<td>Water and Evaporation</td>
<td>43.0</td>
<td>26.1</td>
<td>25.4</td>
<td>5.2</td>
<td>45.8</td>
</tr>
</tbody>
</table>

*Fat as percent of dry matter
the experiment was 10.5% based on original milk weight (19). Theoretical cheese yield from milk of similar composition is 13.1% when calculated from the Van Slyke and Price formula (19). Low yield resulted from excessive fat and protein losses during cooking of curd.

Both cheese possessed a slightly crumbly texture, and a tendency to break along lines where curd had not fused sufficiently. Cheese exhibited a strong salt flavor as very little salty whey was expressed during pressing in contrast to the previous experiment. However, cheese was not acid, as with the cheese made from permeate-cooked curd. Flavor of both was close to that of conventionally produced Cheddar cheese.
DISCUSSION

The proposed method of making cheese from UF retentate prefermented to pH 5.0 to 5.2 prior to curd formation has definite commercial advantages. By reducing the volume of milk by UF, cheese manufacturing equipment can be reduced in size without decreasing production capacity. Prefermentation of retentate will simplify cheese making and thereby reduce complexity of the machinery.

Although the process was conducted on a laboratory scale, expansion to a larger industrial scale could prove easy due to its inherent simplicity. Ultrafiltration and diafiltration of milk will be more efficient and precise with the aid of flow meters, heat exchangers and other accessories. Reduction in the amount of time and water required for diafiltration may be accomplished by diafiltering at constant solids, rather than constant volume (P. Tortosa, Pasilac Inc., Minneapolis, MN, personal communication). Immobilized enzyme devices may be used for retentate coagulation, and a heat exchanger built into the curd former would hasten coagulation and reduce manufacturing time. Changes in design of the curd former could increase efficiency by reducing curd distortion.

The basic challenge in improving the system lies in preventing fat losses and removing curd moisture. Excessive fat lost from curd during cooking in liquid had a detrimental effect on cheese composition and minimized yield improvements. Evaporation of curd may have commercial potential since fat losses were greatly reduced using this treatment.

Methods of moisture removal presented in this paper included cooking in either permeate or water, and evaporation. Again, evaporation seems to be the most promising method. Cooking curd in liquid caused significant
total solids losses which precluded yield improvements. Also, permeate contained lactose in amounts that promoted fluctuations in curd acidity, thereby preventing precise control of cheese pH. Salts present in permeate used for cooking may have an effect on cheese quality. When water was used as a cooking medium, the acidity problem was eliminated, but large amounts of extra effluent were produced. With evaporation of moisture from curd, solids are not lost during processing, and pH control of the final product is simplified since the number of variables is reduced. The only effect is one of moisture removal.

Several major considerations must be made in designing evaporation equipment to remove curd moisture. A primary consideration is maintenance of curd integrity during processing. In the present study, evaporation was in a kettle evaporator with an agitator that was not designed for dealing with curd produced by the process. The result was a slight chopping of curds and subsequent matting of curd into larger lumps. Curd surface area was reduced and rapid moisture removal was prevented. An improved design for evaporation equipment would promote moisture removal by gently tumbling curds, repeatedly exposing all sides of each curd cube. The process could be at low temperature so that lactic bacteria survive and contribute to flavor during ageing. A problem that may occur with evaporation of curd involves curd salt concentration. When curd is cooked in a liquid medium, salts are washed out. With evaporation, solids, including salts, are concentrated. The high salt level may have a deleterious effect on cheese quality.

A limitation of this investigation was in the composition of milk used in the experiments. Milk used was from the same source and did not vary substantially in composition. For cheese plants using milks of different
composition, it is essential that pH of the fermented retentate be predicted from analysis of original milk. For example, milk with higher protein content than milk used in this study would have a higher buffer capacity that may require consideration when choosing a diafiltration rate. Determination of milk protein content and buffer capacity are procedures that can easily be done in a cheese plant.
CONCLUSIONS

1. Natural cheese can be made from prefermented whole milk UF retentate (5X).

2. Cut, natural cheese curd can be continuously produced from prefermented whole milk UF retentate (5X).

3. Acidified UF permeate can be used as a cooking liquid to reduce moisture in curd made from prefermented whole milk retentate (5X).

4. When permeate was used as described in conclusion 3, a significant amount of solids (fat and protein) was lost from the curd.

5. When permeate was used as described in conclusion 3, the pH of finished cheese was unacceptably low for Cheddar.

6. When permeate was used as described in conclusion 3, moisture in finished cheese produced from the curd remained unacceptably high for Cheddar cheese.

7. Acidified deionized water can be used as a cooking liquid to reduce moisture in curd made from prefermented whole milk retentate (5X).

8. When water was used as described in conclusion 7, a significant amount of solids (fat and protein) was lost from the curd.

9. When water was used as described in conclusion 7, the pH of finished cheese produced from the curd was similar to that of conventionally produced Cheddar cheese.

10. When water was used as described in conclusion 7, moisture in finished cheese produced from the curd remained unacceptably high for Cheddar cheese.

11. Evaporation accelerated moisture removal in cheese curd produced from prefermented whole milk retentate (5X).
REFERENCES


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Doctor of Philosophy

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Doctorate Grade Point Average: 3.52.

