Depletion of Whey Proteins and Calcium by Microfiltration of Acidified Skim Milk Prior to Cheese Making[1]

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ABSTRACT

Pasteurized skim milk was microfiltered (0.2-μm membrane) on a system equipped to provide uniform transmembrane pressure of 262 kPa (inlet:outlet pressure differential of 138 kPa, and crossflow velocity of 7.5 m/s). Retentates were gradually acidified to pH 6.6, 6.3, and 6.0 with glucono-delta-lactone during processing to promote solubilization of micelle-bound colloidal minerals into the serum phase of milk for subsequent transfer into permeate. Compositional characteristics of highly concentrated skim milk retentates (concentration factor 8 to 9) and composited permeates were determined to quantify retention and permeation of whey protein and Ca at specified pH conditions and to evaluate the suitability of retentate for cheese making. Final retentates contained an average 27.7% total solids, 20.2% total protein, 17.9% casein, 2.2% whey protein, 4.9% lactose, 2.3% ash, 0.62% Ca, and 0.4% fat. Dry basis retentate Ca and whey protein content significantly differed with pH level and contained 2.8, 2.4, and 1.9 weight percentage of Ca; and 7.2, 7.7, and 8.1 weight percentage of whey protein at pH levels of 6.6, 6.3, and 6.0, respectively. Microfiltration at pH 6.0, as compared with pH 6.6, reduced retentate Ca content by 20.1% but whey protein content was 12.6% higher. Retentate and butter oil were used to produce Mozzarella cheese with a normal Ca content and partial whey protein incorporation. Skim milk microfiltration, combined with in-process pH adjustment, is a useful method to produce highly concentrated retentate reduced in Ca and whey protein content with good potential for cheese manufacture.

(**Key words:** calcium, composition, microfiltration, whey protein)

Abbreviation key: CF = concentration factor, **GDL** = glucono-Δ-lactone, **ΔP** = pressure differential, **TMP** = transmembrane pressure, **UTMP** = uniform transmembrane pressure, **WP** = whey proteins.

INTRODUCTION

Developments in UF technology have led to the invention of cheese-making processes that fully incorporate whey proteins (**WP**) into cheese by elimination of whey drainage (20). Cheese manufacturing procedures have also been developed for low, intermediate, and high concentration UF retentates and have been successful in soft cheese manufacture (21). The main difficulty in the manufacture of semi-hard and hard cheese types is that as the economic benefits of incorporating more WP accrue, increased mineral and WP contents can lead to flavor, textural, and functionality defects (21). Frequent observations of retarded proteolysis and poor meltability of Mozzarella cheese (16) have been made when mineral and WP contents were increased. Elucidation of the effects of WP inclusion in cheese has been an active research area (15, 16, 17), and it is established that native-state WP in UF cheese are resistant to proteolysis and act as filler (10), while the proteolytic susceptibility of denatured WP in UF cheese is debatable (16, 17). The total effect of WP incorporation on cheese characteristics is dependent on the WP state (native or denatured), concentration, and cheese variety (15, 16, 17).

High concentrations of CN, Ca, and P in UF retentate increase buffering capacity and affects cheese-making aspects such as lactic acid production, coagulation kinetics, curd rheology, enzyme activity, and water holding capacity (18, 21). Micelle-bound Ca and P increasingly solubilize into the serum phase of milk at lower pH (18), thus UF at lower pH can transfer additional Ca and P into permeate (6) for subsequent production of cheese with proper mineral contents. In research on UF Mozzarella, UF at lower retentate pH led to some improvement in the resulting cheese texture (8, 19) and proved the importance of normalized mineral content, but effects of increased WP content were still evident. In addition, permeate flux declines more rapidly at lower pH, but production of retentate better suited to cheese making is preferable to production of defective cheese (21).

Hydrodynamic conditions such as inlet-outlet pressure differential (**ΔP**), crossflow velocity, and trans-

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[1]Use of trade names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by the authors, Cornell University, or the Northeast Dairy Foods Research Center.

membrane pressure (**TMP**) are important aspects in the performance and fouling characteristics of dairy filtration systems (7). When membrane fouling begins, the two major WP, α-LA and β-LG, are the initial and principal foulant proteins of both polysulfone (28) and alumina-based membranes (30). Calcium and P salts then assist in bonding caseins to the initial WP film and formation of a secondary filtration layer, which further entraps minerals and CN, resulting in cake layer buildup (2, 30). High crossflow velocity is essential to limit membrane fouling (9) but also leads to a high TMP that promotes fouling. To overcome this problem the uniform TMP (**UTMP**) concept was developed by Tetra Pak Filtration Systems (Aarhus, Denmark) effect a low and constant TMP along the entire permeating surface for reduction of membrane fouling. Reviews of the UTMP concept (22) and membrane fouling processes are available elsewhere (13).

Microfiltration is a class of filtration that uses larger membrane pore sizes and lower pressures than UF. Microfiltration with 1.4-μm pore size membranes has been used for microbial epuration of skim milk (22) with skim milk permeate used in cheese making or as market milk. Another process development relies on the fact that WP are small molecules (3 to 5 nm) as compared with CN micelles (15 to 600 nm) and can be separated by use of 0.1 to 0.2-μm pore size membranes (11, 23). This separation produces CN-enriched retentate and practically sterile permeate containing significant amounts of native-state α-LA and β-LG, having potential for manufacture of WP products with enhanced functionality (4). Reviews of this process can be found in the literature (14).

Effects of temperature and pH on skim milk microfiltration (0.1-μm membrane) combined with diafiltration and UF were studied by Turgeon et al. (29). It was indicated that filtration temperature has little effect on retentate composition but that microfiltration concentration plus diafiltration at pH 6.0 can decrease ash and increase protein content versus filtration at pH 6.6. Use of diafiltration has some benefits for CN purification but necessarily increases energy use and produces large volumes of dilute permeate with added processing or disposal costs.

We hypothesized that combining protein selective skim milk microfiltration with in-process acidification during concentration would produce retentate more suitable to semi-hard cheese production than would UF retentates. Advantages of such a process combination would include production of permeate containing native-state WP with consistent and enhanced functionality, decreased rennet usage (19), increased cheese yield (25), and subsequent generation of concentrated whey with modified protein composition.

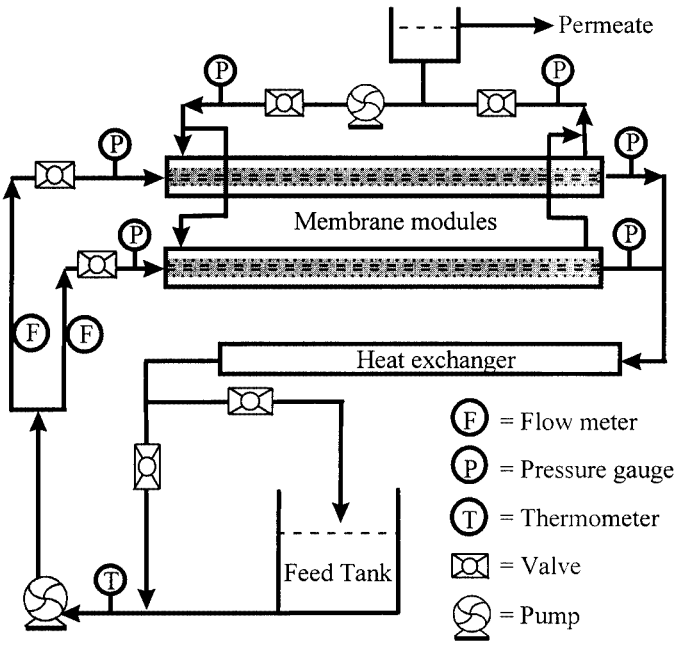

Figure 1. Schematic of microfiltration membrane system incorporating a uniform transmembrane pressure loop.

Development of a cheese-making process using highly concentrated microfiltration retentate is being investigated as part of a skim milk fractionation and utilization plan. Objectives of this research were to determine the compositional characteristics of highly concentrated microfiltration retentates [weight concentration factor (**CF**) 8 to 9] produced under three pH conditions, to quantify WP and Ca retention and permeation, and to initially evaluate suitability for Mozzarella cheese making.

MATERIALS AND METHODS

Microfiltration System

A batch concentration microfiltration system with UTMP capability (Figure 1) was constructed at the Cornell Food Science Department pilot plant facilities. The system was equipped with two alumina-based ceramic membranes (0.2-μm nominal pore diameter; 0.4 m^2 total surface area) (Membralox® P19-40; US Filter Corp., Warrendale, PA) and flow meters (Series 55-200; Wallace & Tiernan, Belleville, NJ) that were placed upstream from each membrane. The operating dead volume of the system was 16 L for retentate and 12 L for permeate. Skim milk retentate was circulated through the system by a 7.5-HP centrifugal feed pump (Reliance Electric Co., Minneapolis, MN) until reaching the desired CF with retentate temperature maintained by a shell and tube heat exchanger. A 2-HP centrifugal pump (Gould Century, St. Louis, MO) was used in the UTMP

loop to circulate permeate concurrently with retentate flow in the annulus between each membrane and module casing, which was packed with plastic beads (US Filter Corp., Warrendale, PA). Pressures on inlet and outlet permeate ports were controlled by butterfly valves placed before and after the permeate pump. Permeate was collected as overflow from an elevated balance tank.

Operational Conditions

Two hundred kilograms of HTST pasteurized skim milk was obtained from the Cornell University dairy plant, heated to 50°C, and put into the preheated microfiltration system maintained at 50°C (±2°C). Retentate and permeate inlet pressures were set to 448 and 186 kPa, respectively; outlet pressures were set to 310 and 48 kPa, respectively, for a constant inlet-outlet ΔP of 138 kPa and TMP of 262 kPa. Toward the end of concentration, the retentate outlet pressure slowly declined and widened ΔP, so permeate outlet pressure was concurrently decreased. Retentate flow velocity was maintained at 7.5 m/s.

Glucono-Δ-lactone (**GDL**) (Glucona America, Janesville, WI) was used for retentate acidification, as it hydrolyzes by a temperature-dependent process to form gluconic acid and offers a very predictable and controlled method of lowering retentate pH. Serpelloni et al. (26) presented data on GDL addition rates and response of milk pH. However, to provide a reference for GDL addition at high retentate CF for both microfiltration and subsequent cheese making, we prepared standard curves using CF 8 to 9 microfiltration retentate at four temperatures with three GDL concentrations (Figure 2). The resultant pH decline was asymptotic over time and was more rapid at higher temperatures. Gradual acidification of retentate during microfiltration was done by dissolving GDL in 4°C skim milk feed and adding it to the circulating retentate. One-half the desired pH reduction occurred between the start of the process and CF 2; the remaining pH reduction occurred up to CF 6. Approximately 1.0 or 1.6 g of GDL/L of skim milk was required for retentate to reach pH 6.3 and 6.0, respectively.

Retentate was concentrated to CF 8 to 9 with nine processing trials; 2 at pH 6.6, 2 at pH 6.3, and 5 at pH 6.0. Permeate flux, retentate pH, and retentate temperature were monitored during processing. Retentate was collected at the end of filtration and was frozen at –40°C along with samples of retentate and permeate for analysis. System flush liquid was sampled after 20 kg of deionized water was circulated at 60°C for 10 min with permeate ports closed. Membranes were cleaned using a cycle of 1.5 weight percent NaOH and 1.5 weight percent nitric acid with use of the UTMP system as a backwashing mechanism. Membranes were considered clean when water flux recovered to the original value.

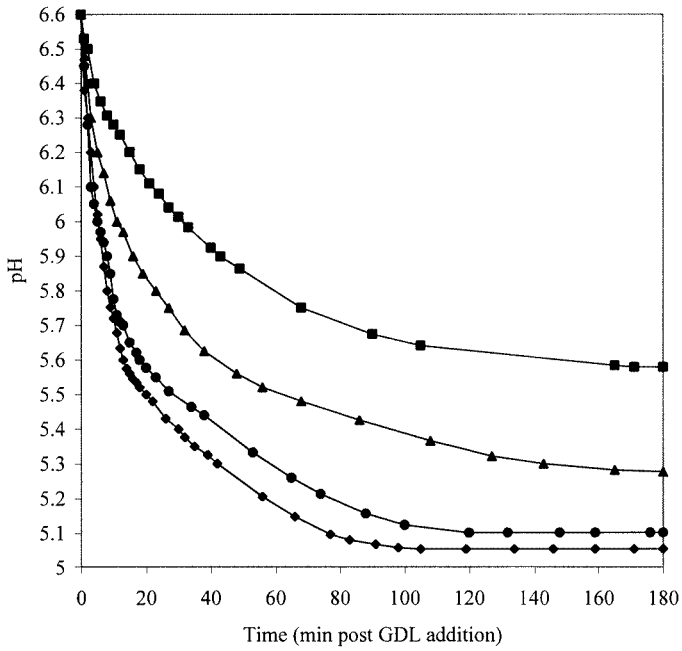

Figure 2. Standard pH response curves for glucono-Δ-lactone (GDL)-induced acidification of pH 6.6 concentration factor 8 to 9 skim milk microfiltration retentate. (■ = 30°C retentate with 1.5 weight percentage GDL, ▲ = 35°C retentate with 2.5 weight percentage of GDL, ● = 40°C retentate with 3.5 weight percentage of GDL, and ◆ = 45°C retentate, 3.5 weight percentage of GDL).

Compositional Analysis

Skim milks, retentates, permeates, and system flush liquids were analyzed in duplicate for composition. Total solids were determined gravimetrically by forced-air oven drying. Total nitrogen and NPN were determined (1) along with noncasein N by macro-Kjeldahl (12). Fat was measured by Mojonnier ether extraction (1). Ash was determined by drying samples in a forced-air oven at 100°C and then placing the dish in a muffle furnace for 20 h at 550°C. Lactose was calculated by difference. Total Ca was measured by an atomic absorption analysis procedure adapted from Brooks et al. (5) in which samples were mixed with 12% TCA and filtered through Whatman #541 (Whatman International, Maidstone, United Kingdom) filter paper. Lanthanum oxide (5% solution) and deionized water were added to the filtrate, after which the sample was aspirated into an atomic absorption spectrophotometer (Model 2380; Perkin-Elmer Corp., Norwalk, CT) fitted with a Ca lamp (Fisher Scientific, Springfield, NJ).

Statistical Analysis

Composition of skim milk feed, retentates, permeates, and system flush liquids were compared against pH level by analysis of variance with Fisher's least significant difference test of means at a significance level of $P = 0.05$. Statistical analysis was performed using Minitab 11 for Windows (Minitab, Inc., State College, PA).

RESULTS AND DISCUSSION

Composition

Skim milk was microfiltered until reaching CF 8 to 9 at pH of 6.6, 6.3, and 6.0. Use of GDL was effective in achieving the desired retentate pH levels in a controlled manner without inducing localized coagulation. Retentates had no off-flavors or odors during or after the filtration process. Viscosity of microfiltration retentates increased with TS and protein content: 2.4 cP at 10.7% TS, 2.9 cP at 12.0% TS, 4.1 cP at 14.5% TS, and 6.8 cP at 16.7% TS. All measurements were at 20°C using a rotational, controlled shear rate (0 to 2000^{s-1} to 0) rheometer (Viscotester VT500; Haake AG, Karlsruhe, Germany) fitted with a Coutte attachment and NV sensor system. This is an agreement with observations by Prokopek et al. (24) using UF retentates, viscosity of higher CF retentates increases dramatically: 10 cP at 20% TS, 33 cP at 25% TS, and 70 cP at 28% TS. High retentate viscosity has important consequences for membrane fouling and maintenance of permeate flux as is discussed below.

The mean composition of retentate and permeate at each pH level is shown in Table 1; as compared with normal skim milk, concentrations of all retentate components increased except for lactose and NPN. Similar compositional results were found in skim milk UF retentates at 80 to 85% volume reduction (3), but substantially increased CN:true protein ratios were realized in microfiltration retentates. Retentates were not fully depleted of WP by the end of concentration, which constituted 9 to 11% of true protein content. This WP content will have implications in cheese making, both for increased yields but with their reduction allowing for development of better cheese meltability than when similar UF concentrate (containing all WP) is used.

Retentate Ca:total protein and Ca:CN ratios are also shown in Table 1; both ratios decreased with lower pH. The depletion of retentate Ca into permeate replaced normal losses of Ca to cheese whey (typically 35 to 40% by weight), whereas Ca loss from microfiltration retentate cheese making was only 4 to 8% by weight (unpublished data). A lower retentate Ca:total protein ratio is essential to achieve a proper level of Ca in cheese having good melt and stretch properties (16).

Microfiltration permeate composition (Table 1) was similar to cheese whey in several aspects such as lactose, total protein, and ash content but did not contain measurable fat, glycomacropeptide, or CN (SDS-PAGE not shown). The lack of fat is positive, especially to enhance the functional properties of WP concentrate or isolate obtained from concentrated microfiltration permeate. A review of the properties and composition of WP isolate obtained from microfiltration permeate is found elsewhere (4).

The mean dry basis composition of retentate and permeate at each pH level is in Table 2 to allow treatment

TABLE 1. Weight percentage composition of concentration factor 8 to 9 skim milk retentate and composite permeate from microfiltration[1] conducted at pH 6.6, 6.3, and 6.0.

Component (weight %)	Skim milk[2]	Final retentate			Composite permeate		
	6.6	6.6	6.3	6.0	6.6	6.3	6.0
Total solids	8.91	27.72	28.86	27.29	5.90	5.82	6.04
Fat	0.05	0.41	0.41	0.39	0.0	0.0	0.0
Ash	0.69	2.39	2.39	2.17	0.40	0.41	0.45
Ca	0.11	0.78	0.69	0.53	0.028	0.036	0.047
Lactose	5.02	4.70	4.98	4.93	4.91	4.84	5.01
Total protein[3]	3.13	20.22	21.08	19.80	0.59	0.57	0.57
True protein[3]	3.01	20.13	20.97	19.73	0.41	0.39	0.39
Casein[3]	2.39	18.14	18.76	17.52	0.0	0.0	0.0
Whey protein[3]	0.61	1.99	2.21	2.21	0.41	0.39	0.39
Ca to total protein ratio	3.59	3.85	3.28	2.53	...	...	...
Ca to casein ratio	4.71	4.29	3.69	3.04	...	...	...

[1]Mean values of components from microfiltration done at three pH levels (n = 2 at pH 6.6, n = 2 at pH 6.3, and n = 5 at pH 6.0).

[2]Mean composition (n = 9).

[3]Computed as (N × 6.38).

TABLE 2. Dry weight percentage composition of skim milk, concentration factor 8 to 9 retentate and composite permeate from microfiltration[1] conducted at pH 6.6, 6.3, and 6.0.

Component (weight %)	Skim milk[2]	Final retentate						Composite permeate					
	pH 6.6	pH 6.6	SEM	pH 6.3	SEM	pH 6.0	SEM	pH 6.6	SEM	pH 6.3	SEM	pH 6.0	SEM
Fat	0.60	1.48^{A}	0.09	1.43^{A}	0.09	1.41^{A}	0.05	0.0	. . .	0.0	. . .	0.0	. . .
Ash	7.79	8.63^{A}	0.16	8.35^{B}	0.16	7.92^{C}	0.10	6.74^{A}	0.09	6.97^{B}	0.09	7.47^{C}	0.06
Ca	1.26	2.80^{A}	0.12	2.40^{B}	0.12	1.94^{C}	0.07	0.48^{A}	0.03	0.62^{B}	0.03	0.78^{C}	0.02
Lactose	56.43	17.06^{A}	0.91	17.36^{A}	0.91	18.07^{A}	0.58	83.35^{A}	0.37	83.21^{A}	0.37	82.90^{A}	0.23
Total protein[3]	35.26	72.81^{A}	0.92	73.02^{A}	0.92	72.54^{A}	0.58	10.01^{A}	0.07	9.76^{B}	0.07	9.52^{C}	0.04
True protein[3]	33.50	72.54^{A}	0.82	72.72^{A}	0.82	72.27^{A}	0.49	7.02^{A}	0.09	6.69^{B}	0.09	6.46^{C}	0.05
Casein[3]	26.60	65.40^{A}	0.84	64.88^{A}	0.84	64.16^{A}	0.48	0.0	. . .	0.0	. . .	0.0	. . .
Whey protein[3]	6.88	7.17^{A}	0.16	7.66^{B}	0.16	8.10^{C}	0.09	7.02^{A}	0.09	6.69^{B}	0.09	6.46^{C}	0.05
Casein:true protein ratio	79.48	90.12^{A}	0.27	89.43^{AB}	0.27	88.79^{B}	0.19	. . .	. . .	. . .	. . .	. . .	. . .

[1]Mean values of components from microfiltration done at three pH levels (n = 2 at pH 6.6, n = 2 at pH 6.3, and n = 5 at pH 6.0).

[2]Mean composition (n = 9).

[3]Computed as (N × 6.38).

[A,B,C]Means that differ across rows (within categories) are indicated by unlike superscripts ($P < 0.05$).

differentiation. Both retentate and permeate compositions differed significantly in ash, Ca, and WP as a consequence of pH adjustment ($P < 0.05$) and led to a significant change in the retentate CN:true protein ratio as pH decreased ($P < 0.05$). Dry basis composition of system flush liquids was similar to corresponding retentate (not shown).

Table 3 details the percentage weight transfer of individual skim milk components into permeate at each pH level with significant differences found in the transfer of ash, Ca, total protein, true protein, and WP as a result of pH adjustment ($P < 0.05$). Change in Ca and WP transfer into permeate is shown in Figure 3, in which from pH 6.6 to 6.0, retentate Ca content was decreased by 20.1% as WP retention was increased by 12.6%. Results indicated that gradual retentate pH change could be used to effectively change the colloidal mineral content of microfiltration retentate while maintaining 87.4% of nominal (pH 6.6) WP permeation. Changes in ash and Ca levels support the findings of St-Gelais et al. (27) in their study of compositional change in UF retentates at different temperatures and pH levels.

Membrane Performance

Typical permeate flux for microfiltration at three pH levels is shown in Figure 4. Permeate flux usually began at 60 to 70 kg/h/m^2 and declined to 5 to 18 kg/h/m^2 at the end of processing. Mean permeate flux was calculated according to the formula of Cheryan (7): FM = FF + 0.33(IF –FF), where FM, FF, and IF are mean permeate flux, final flux, and initial flux, respectively. For pH levels tested, mean permeate fluxes were 32.5, 28.8, and 23.1 for pH 6.6, 6.3, and 6.0, respectively, and as compared with microfiltration at pH 6.6, these values represented a significant decrease of 11.4% at pH 6.3 and 28.8% at pH 6.0 ($P < 0.05$).

Reduction in retentate pH will increase micellar Ca and P solubilization and, subsequently, increase their concentrations in resulting permeate (6). Attia et al. (2)

TABLE 3. Weight percentage transfer of skim milk components to permeate by microfiltration conducted at pH 6.6, 6.3, and 6.0.[1]

Component	pH 6.6	SEM	pH 6.3	SEM	pH 6.0	SEM
Total weight	87.70^{A}	0.83	87.30^{A}	0.83	86.84^{A}	0.52
Total solids	58.35^{A}	0.95	57.50^{A}	0.95	58.72^{A}	0.60
Ash	49.15^{A}	1.49	52.85^{AB}	1.49	56.40^{B}	0.94
Ca	21.90^{A}	0.60	28.05^{B}	0.60	37.62^{C}	0.38
Lactose	86.40^{A}	1.52	85.15^{A}	1.52	86.16^{A}	0.96
Total protein[2]	16.60^{A}	0.25	16.00^{AB}	0.25	15.84^{B}	0.16
True protein[2]	12.25^{A}	0.27	11.55^{B}	0.27	11.32^{B}	0.16
Whey protein[2]	59.55^{A}	1.00	56.75^{AB}	1.00	54.40^{B}	0.63

[1]n = 2 at pH 6.6, n = 2 at pH 6.3, and n = 5 at pH 6.0.

[2]Computed as (N × 6.38).

[A,B,C]Means that differ across rows are indicated by unlike superscripts ($P < 0.05$).

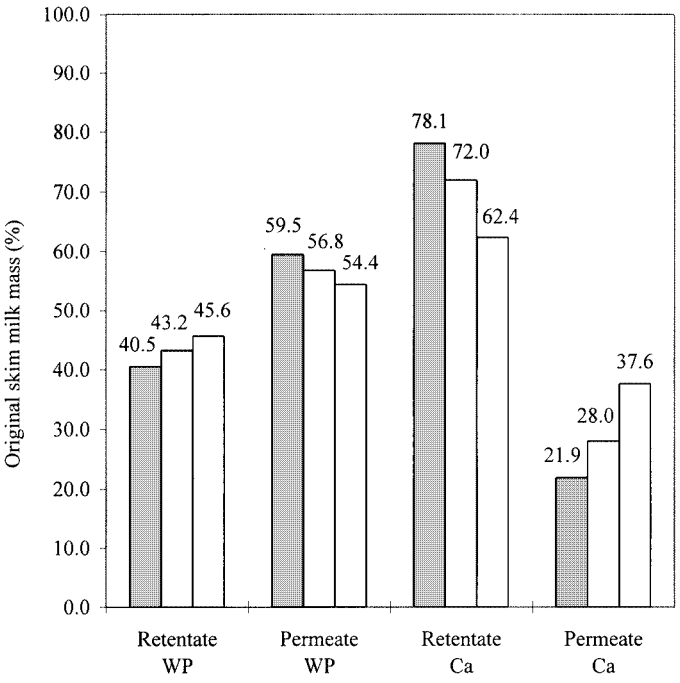

Figure 3. Mean percentage of original mass of skim milk Ca and whey proteins (WP) in retentate and permeate as a function of microfiltration pH. Order within each category is pH 6.6 (dark gray bar), pH 6.3 (light gray bar), and pH 6.0 (white bar).

determined that increased availability of these minerals in the serum phase allows for additional CN bonding to the membrane surface with subsequent reductions in permeate flux. Additionally, increasing retentate concentration leads to lower retentate velocity and shear stress at the membrane wall, further decreasing flux (25). In the present work, decreased WP permeation at lower pH levels could be attributed to increased mineral adsorption and protein complexation on the membrane surface (2) in addition to increased retentate CF.

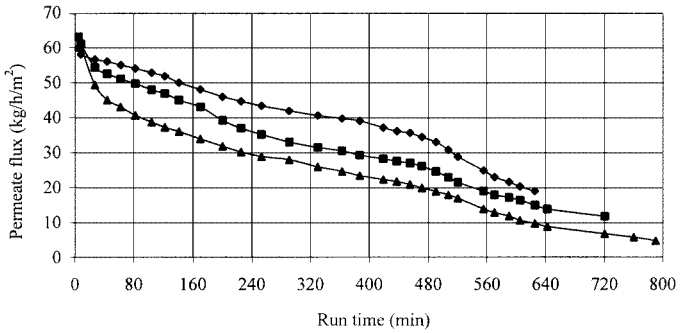

Figure 4. Effect of gradual reduction in retentate pH on typical permeate flux during skim milk microfiltration. (◆ = pH 6.6, ■ = pH 6.3, and ▲ = pH 6.0).

CONCLUSIONS

Compositional characteristics were established for CF 8 to 9 microfiltration retentates at pH 6.6, 6.3, and 6.0. Use of microfiltration produced an increase in the CN:true protein ratio and reflected membrane permeation of WP. Microfiltration at pH 6.0, as compared with pH 6.6, decreased retentate Ca content by 20.1% and increased WP content by 12.6%. These results demonstrated that low pH microfiltration can be used to effectively change mineral balances while maintaining 87.4% of nominal WP permeation. Permeate flux decreased significantly as a result of operation at lower pH. Reduction in retentate Ca was also needed to achieve the proper Ca:total protein ratio typically found in cheese. High retentate flow velocity and maintenance of UTMP were likely needed to slow fouling development and achieve retentate CF of 8 to 9.

Microfiltration of acidified skim milk with 0.2-μm membranes to reduce Ca and WP levels could be used to produce a very suitable, concentrated retentate for use in cheese manufacture. Because Ca salts have been widely implicated as being detrimental to production of UF cheese with desired textural and functional properties, subsequent cheese-making work has focused on the use of CF 8 to 9, pH 6.0, microfiltration retentate as the source of SNF. Preliminary cheeses made from this cheese milk have shown promising results, and a manufacturing method is being developed that has potential for production of both traditional or new cheese varieties with desirable properties. This overall approach to retentate cheese making will allow for production of cheese with normal Ca levels and significantly reduced amounts of WP as compared with full concentration UF cheese. In addition, production of permeate that has consistent composition, is sterile, is extremely low in fat, and contains the major WP in a highly functional state will also be possible with this method.

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