Membrane Fractionation Processes for Removing 90% to 95% of the Lactose and Sodium from Skim Milk and for Preparing Lactose and Sodium-Reduced Skim Milk

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ABSTRACT: Pilot-scale microfiltration (MF), microfiltration-diafiltration (MDF), ultrafiltration (UF), ultrafiltration-diafiltration (UDF), and nanofilration (NF) membrane fractionation processes were designed and evaluated for removing 90% to 95% of the lactose and sodium from skim milk. The study was designed to evaluate several membrane fractionation schemes as a function of: (1) membrane types with and without diafiltration; (2) fractionation process temperatures ranging from 17 to 45 °C; (3) sources of commercial drinking water used as diafiltrant; and (4) final mass concentration ratios (MCR) ranging from about 2 to 5. MF and MDF membranes provided highest flux values, but were unsatisfactory because they failed to retain all of the whey proteins. UDF fractionation processes removed more than 90% to 95% of the lactose and sodium from skim milk. NF permeate prepared from UDF cumulative permeate contained sodium and other mineral concentrations that would make them unsuitable for use as a diafiltrant for UDF applications. A method was devised for preparing simulated milk permeate (SMP) formulated with calcium, magnesium, and potassium hydroxides, and phosphoric and citric acids for use as UDF diafiltrant or for preparing lactose and sodium reduced skim milk (L-RSM). MF retentates with MCR values of 4.7 to 5.0 exhibited extremely poor frozen storage stabilities of less than 1 wk at −20 °C, whereas MCR 1.77 to 2.95 MDF and UDF retentates and skim milk control exhibited frozen storage stabilities of more than 16 wk. L-RSM exhibited a whiter appearance and a lower viscosity than skim milk, lacked natural milk flavor, and exhibited a metallic off-flavor.

Keywords: lactose, nutrition, skim milk, sodium, ultrafiltration

Introduction

Recent evidence indicates that providing a "balanced" diet, restricting fat, cholesterol, and caloric intake, along with adequate exercise, minimizes the risk of becoming overweight or acquiring obesity, hypertension, diabetes, cancer, cardiovascular heart disease, stroke, osteoporosis, osteoarthritis, and other related diseases (Kim and Popkin 2005). Milk consumption reduces the incidence of osteoporosis, hypertension, kidney stones, gout, some cancers, obesity, and type 2 diabetes (Anonymous 2005; King 2005). Adequate restriction of daily sodium intake to 6 g would be expected to reduce the incidence of stroke and heart disease in humans by 13% and 10%, respectively (He and MacGregor 2004). The incidence rate of obesity and severe obesity, has been increasing steadily in the United States over the past 2 decades and is reaching epidemic proportions (Rand Health 2007). USDA's 2005 Dietary Guidelines for Americans recommends 3 cups of fat-free or low-fat milk per day to help meet dietary potassium requirements (King 2005). The American Dietetic Assn. recommends 3 servings of fortified low-fat or fat-free milk daily for meeting dietary requirements of 500 to 1200 mg of calcium and 200 to 600 International Units of vitamin D (Anonymous 2007).

Although milk is universally recognized as an excellent source of highly nutritious proteins and nutritionally important minerals, such as calcium, magnesium, phosphate, citrate, and potassium, it also provides 58 mg of sodium and 5 g of lactose per 100 g (Jenness 1988), both of which are significantly negative nutritional factors for human consumption. Lactose accounts for approximately 30% and 60% of the calories of whole milk and skim milk.

Ultrafiltration (UF) membrane fractionation technology is used extensively for fractionating whey for production of whey protein concentrates for use as nutritional and functional ingredients in a wide variety of formulated food product applications (Morr and Ha 1993). MF and UF fractionation of skim milk has been investigated extensively by a number of researchers (Domagk 1981; Edelsten and others 1983; Lonergan 1983; McGregor and White 1990). We are aware of only one commercially available ultrafiltered fluid milk product: Calorie Countdown® Fat Free Dairy Beverage, manufactured and distributed by HP Hood LLC, Chelsea, Mass., U.S.A. The label on this product claims that it "contains 75% less sugar and 75% fewer carbohydrates than milk."

This research study was designed to evaluate several pilot-scale MF and UF membrane fractionation processes for removing 90% to 95% of the lactose and sodium from skim milk without adversely altering its appearance, flavor, or consumer acceptance.

Materials and Methods

Materials

Commercial pasteurized skim (fat-free) milk purchased from Mayfield Dairy Farms, Inc., Athens, Tenn., U.S.A., and Greenville,

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S.C., U.S.A. was used for Trials 1 to 4. Laura Lynn® skim milk purchased from Ingles in Clemson and Seneca, S.C., U.S.A. was used for Trials 5 to 8. Commercial drinking water (Land O' Sky Steam Distilled Water®, Blue Ridge Pure Water®, and Dannon Spring Water®) was purchased from Ingles. Chemical reagents (J.T. Baker anhydrous calcium hydroxide, Mallinkrodt food-grade magnesium hydroxide powder, J.T. Baker 85% potassium hydroxide pellets, Mallinkrodt 85% phosphoric acid, and EMD OmniPur® anhydrous citric acid) were purchased from VWR Intl., Suwanee, Ga., U.S.A. Pure Vanilla Extract® (McCormick & Co., Inc., Hunt Valley, Md., U.S.A.) was purchased from Ingles. Avicel Plus Type CM 2159 Stabilzer® was provided by FMC BioPolymer, Newark, Del., U.S.A. and Sucralose® liquid concentrate and powder were obtained from McNeil Nutritional Div., McNeil, PPC, Inc., Fort Washington, Pa., U.S.A.

Membrane processing methods

Microfiltration, MDF, and UDF membrane processing schemes developed for this study were all similar, except that the MDF and UDF processes used diafiltration to remove higher percentages (90% or more) of the lactose and sodium from skim milk (Figure 1). One experiment was conducted using a nanofiltration (NF) membrane to remove additional minerals and lactose from UDF permeate for subsequent use as diafiltrant in subsequent UDF processing (Figure 1). The resulting NF retentate could possibly be further processed for the recovery of lactose, riboflavin, minerals, vitamins, or other minor milk constituents; or fermented for production of ethanol and other potentially valuable products (Morr 1992).

Membrane fractionation experiments were conducted with a pilot-scale CARRE® (Graver Technologies, Newark, Del., U.S.A.) membrane test unit equipped with stainless steel centrifugal and positive displacement pumps, piping, valves, pressure gages, flow meters, and a heat exchanger (Figure 2). Membranes used in the study were: (1) Tubular sintered stainless steel and titanium dioxide MF membrane modules with 0.1- and 0.02-μm nominal pore sizes and a membrane area of 0.525 m^2 (Scepter®; Graver Technologies); (2) A model M-U4040PES-D, PES (polyethersulfone) spiral-wound UF membrane element with a nominal molecular weight cut-off (MWCO) of 10000 daltons (10 kDa) and a membrane area of 7.43 m^2 (Applied Membranes Inc., Vista, Calif., U.S.A.); and (3) Model M-N4040A9 NF, 90% salt (NaCl) rejection NF membrane element with a membrane area of 7.43 m^2 (Applied Membranes Inc.). Retentate was circulated through the test unit at a rate of 61 kg/min, which was roughly 80% of the maximum flow rate recommended by the membrane manufacturer.

Instantaneous permeate samples were collected during short time intervals of several seconds in duration at various stages of each fractionation experiment. A single cumulative permeate sample was collected after combining and stirring the entire permeate fraction obtained for each fractionation experiment.

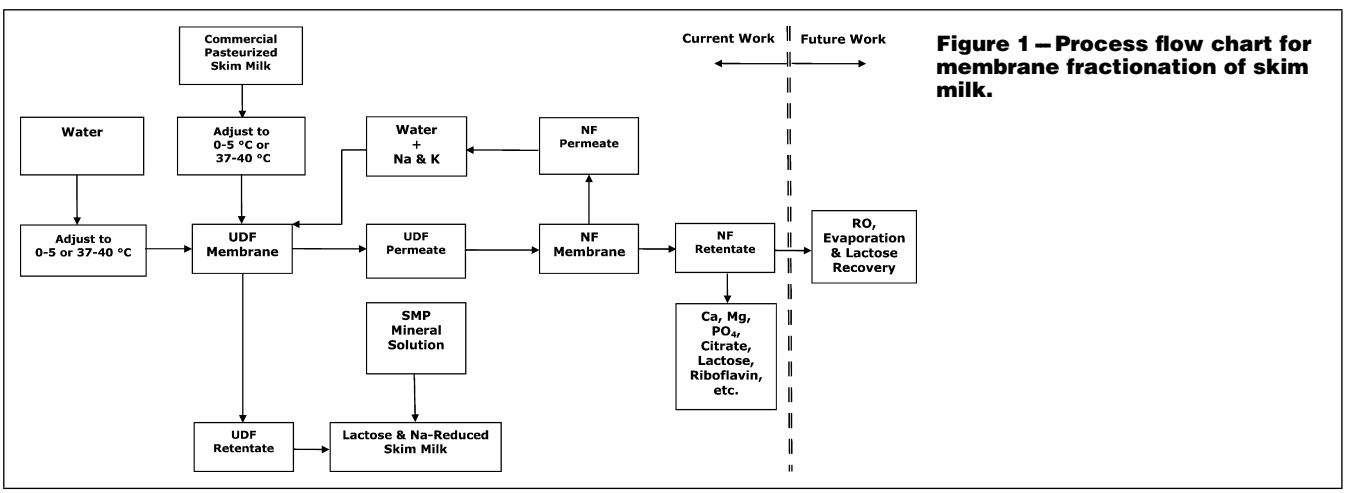

Figure 1—Process flow chart for membrane fractionation of skim milk.

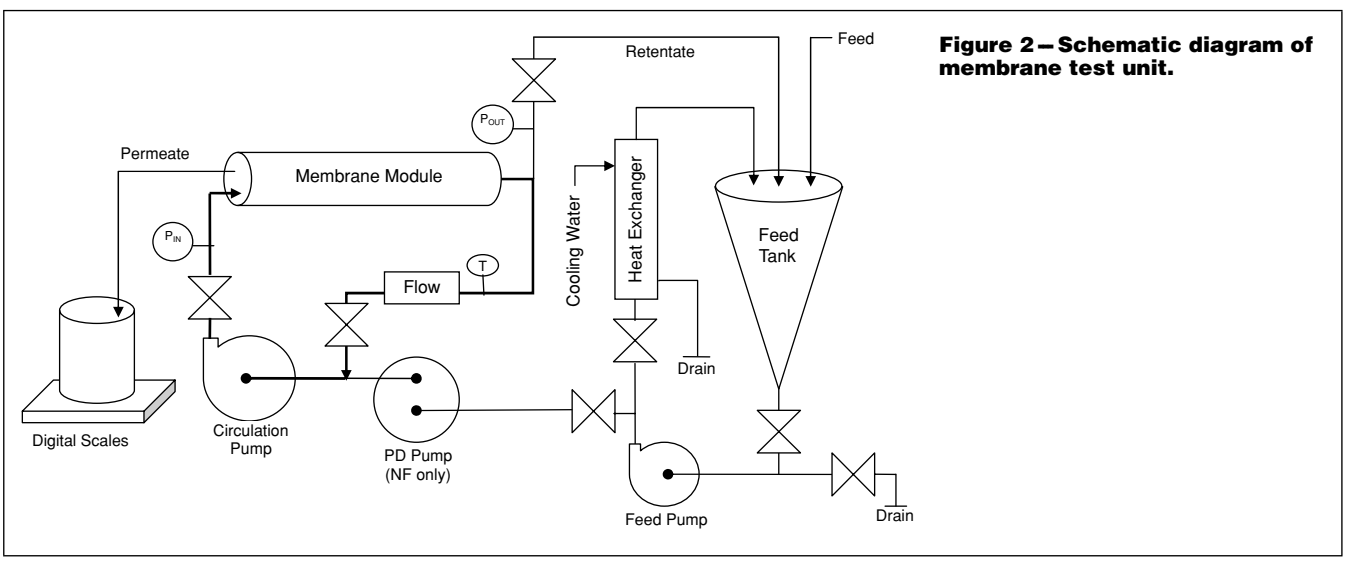

Figure 2—Schematic diagram of membrane test unit.

Membranes were cleaned in-place according to manufacturers' recommendations for cleaning and sanitizing agents, pH, temperature, and time. They were thoroughly cleaned and rinsed in-place with tap water between every fractionation experiment. The pumping system of the pilot-scale membrane test unit was controlled to provide a circulation rate of 61 kg/s.

Diafiltrants

University tap distilled water was used as diafiltrant for Trials 1 and 2; simulated milk permeate (SMP), formulated with university tap distilled water, was used as diafiltrant for Trials 3 and 4. The SMP was formulated to provide Ca, Mg, PO_4, and citrate ions at concentrations in milk (Jenness 1988) to stabilize the casein micelles and minimize possible solubilizaion of colloidal phosphate during membrane fractionation of skim milk. SMP was prepared by dissolving 85.32 g H_3PO_4, 96.78 g citric acid, 34.02 g $Ca(OH)_2$, and 11.34 g $Mg(OH)_2$ in about 1 kg of 0 to 5 °C distilled water. The solution was adjusted to pH 6 with chilled (0 to 5 °C) 1 N KOH solution, and then adjusted slowly and carefully to pH 6.6 with small increments of chilled 0.1 N KOH. Finally, the SMP was diluted to 60 kg with additional water. The resulting SMP contained no added sodium, chloride, sulfate, or carbonate ions as simulated milk ultrafiltrate (SMUF) prepared by Jenness and Koops (1962).

Membrane fractionation of skim milk

Skim milk, SMP, and commercial water diafiltrants were warmed to the processing temperature to be used for each fractionation experiment just before adding them to the membrane unit feed tank (Figure 2).

Since the diafiltration formula (Cheryan 1998) applies only for membrane processing at constant retentate volume or mass; we devised a numerical computer model using an Excel® (Microsoft Corp., Redmond, Wash., U.S.A.) spreadsheet to predict lactose and sodium removal for our UDF fractionation experiments. The total quantities of skim milk and diafiltrant to be used for each fractionation experiment were each divided into 0.5-kg increments. The predicted quantity of lactose removed in each 0.5-kg increment of permeate was estimated by multiplying the theoretical lactose concentration in the corresponding retentate fraction by the experimentally determined lactose permeation coefficient value of 0.776. Figure 3 and 4 illustrate the resulting prediction curves for lactose removal from skim milk for Trials 6 and 8, respectively.

Analytical

Lactose was determined with a Model DX-300 Dionex HPLC® (Dionex, Sunnyvale, Calif., U.S.A.), equipped with a pulsed

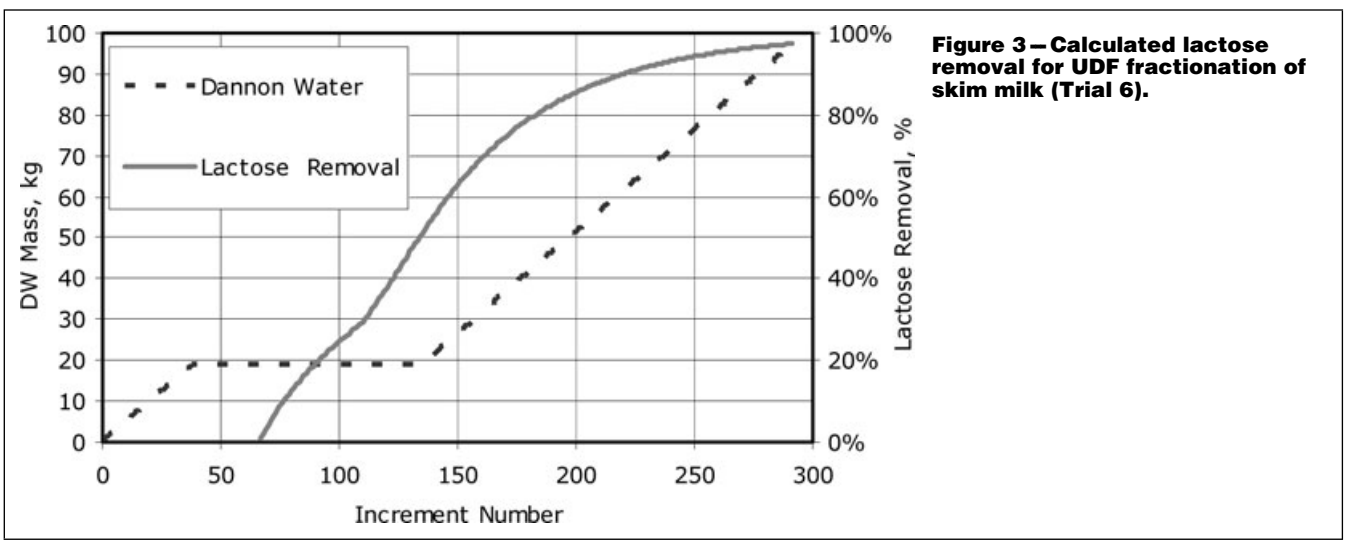

Figure 3—Calculated lactose removal for UDF fractionation of skim milk (Trial 6).

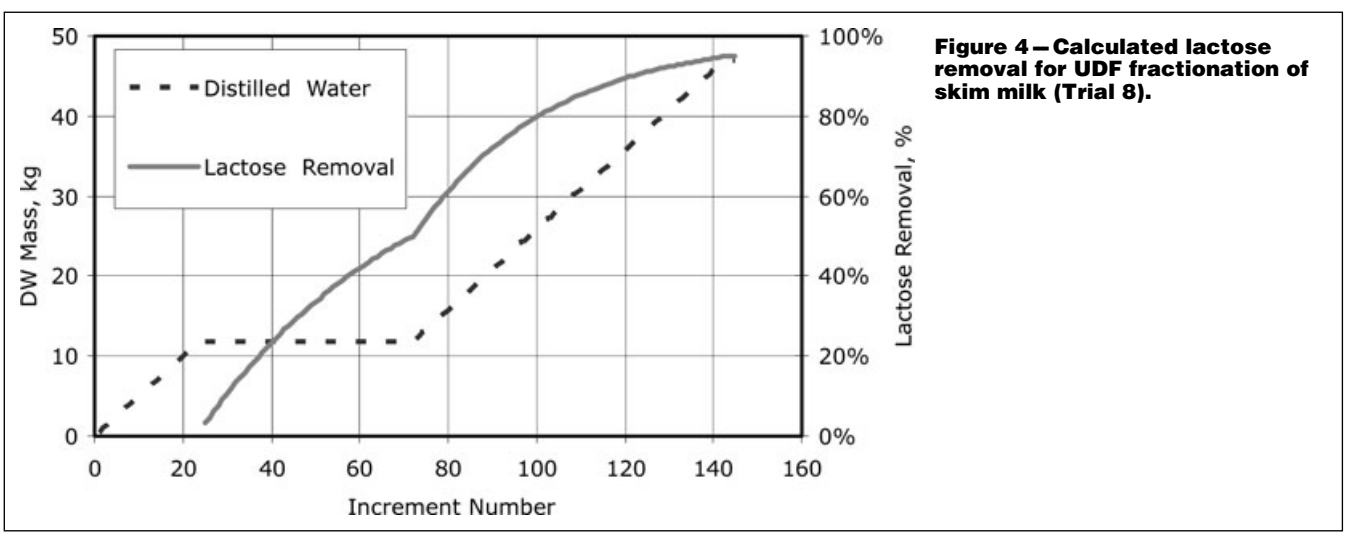

Figure 4—Calculated lactose removal for UDF fractionation of skim milk (Trial 8).

electrochemical detector using 0.2 N NaOH as the mobile phase or by the colorimetric method of Nickerson and others (1976), modified for using a micro-plate reader at 600 nm.

Proteins were determined by a modified micro-Kjeldahl nitrogen procedure (Official Methods of Analysis 1975), using an N conversion factor of 6.38. Noncasein N fractions were prepared by adjusting samples to pH 4.6 with 1 N HCl at about 20 °C and filtering with Whatman Nr 1 filters. Nonprotein N fractions were prepared by blending 30 g of 15% trichloroacetic acid with 120 g of sample at about 20 °C and filtering with Whatman Nr 1 filters (VWR Intl., Suwanee, Ga., U.S.A.) (Official Methods of Analysis 1975).

Minerals were determined by digesting samples in concentrated nitric acid and hydrogen peroxide at 125 °C and analyzing them with an ICAP 61 Vacuum Spectrometer® (Thermo Jarrell Ash Corp., Franklin, Mass., U.S.A.) in the Agricultural Service Laboratories, Clemson Univ. Citrate was determined as citric acid by the method of Toftegaard (1976). Citric acid dihydrate was used for preparing the standard curve; milk proteins were precipitated with perchloric acid; citrate was determined using a citric acid assay kit (Cat. Nr 139076; Boehringer Mannheim, Indianapolis, Ind., U.S.A.). CIE $L^*a^*b^*$ color space values were determined with a Hunter Lab UltraScan XE® Spectrophotometer using the appropriate software program (Hunter Assoc. Lab. Inc., Reston, Va., U.S.A.). Readings were made at 4 different loci within the 1-cm light path of 50-mL quartz cuvettes. The spectrophotometer was operated with Illuminant C, 10° observer, and specular (glossy) reflectance excluded. Surface reflectance was also determined with the UltraScan XE Spectrophotometer at 440 to 750 nm as a function of wavelength.

Frozen storage stability studies were conducted by freezing and storing multiple aliquots of skim milk and skim milk membrane retentate fractions in 10-mL capped plastic test tubes in a −10 °C lab freezer. Frozen samples were removed from the freezer at weekly or biweekly intervals, allowed to thaw by standing at room temperature, and examined visually for evidence of casein micelle destabilization.

Commercial drinking water, diafiltrants, skim milk, and UDF retentate fractions were evaluated for flavor, appearance, mouthfeel, aftertaste, viscosity, texture, and overall acceptability by an experienced, 3-member sensory evaluation panel. Coded samples were presented to the panelists in individual 120-mL plastic cups in a random order. The panelists were instructed to rinse their mouths before beginning and between samples with commercial drinking water that was provided in individual cups. A maximum of 6 samples was presented to the panel at each evaluation session. The panelists evaluated each of the samples independently for flavor, appearance, physical properties, and overall acceptability and recorded their observations on individual, preprinted evaluation forms. They subsequently discussed their evaluations as a group and arrived at a consensus evaluation for each of the samples.

Experimental Results

Selection of commercial water for use as diafiltrant

All 3 commercial drinking water products exhibited excellent flavor and overall acceptability. Dannon Spring Water®, which contained the highest Ca and Mg concentrations (Table 1), was used as diafiltrant in Trial 5 to assist in maintaining the integrity of the casein micelles and colloidal phosphate. Land O' Sky Distilled Water®, which contained the lowest Ca and Mg concentrations, was selected as diafiltrant for the Trial 8 UDF experiment to assist in maintaining the soluble casein complexes in their soluble state (Figure 5), which it was hoped would inhibit formation of the metallic flavor defect in the resulting skim milk UDF retentate fraction.

Membrane performance

Results for membrane fractionation experiments are summarized in Table 2. Total process time for the fractionation experiments ranged from 45 min (Trial 8) to 386 min (Trial 2), collected permeate mass ranged from 34.3 kg (Trial 4) to 127 kg (Trial 1), final retentate mass concentration ratios (MCR) ranged from 1.67 (Trial 5) to 5.19 (Trial 7), and mean membrane flux values varied from 61.5 (Trial 1) to 6.86 (Trial 7) kg/m^{2}h. As expected, mean membrane flux values were directly related to membrane pore size: 0.1-μm MF > 0.02-μm MF > 10-kDa UF > NF.

Mean membrane flux values for the 0.1-μm MF membrane were roughly double those for the 0.02-μm MF membrane (Trials 1 and 2). Mean membrane flux values for 0.02-μm MDF experiments ranged from 21.4 kg/m^{2}h (Trial 4) to 50.5 kg/m^{2}h (Trial 5) and, as expected, these values were also inversely related to the final MCR of the retentate. In contrast, the 10-kDa UF membrane exhibited a mean flux value of 17.0 kg/m^{2}h for the 34 to 35 °C fractionation experiment (Trial 6) compared to a mean flux value of only 10.8 kg/m^{2}h for the 17 to 21 °C fractionation experiment (Trial 8). Expected temperature dependent differences in viscosity, as well as those that may arise from the polymerization–depolymerization of casein micelles and association–dissociation of colloidal phosphate (Figure 5) may account for these differences in membrane flux values.

Membrane fractionation of skim milk

The 0.1-μm MF membrane rejected 99.6% of the casein, but only about 36% of the whey proteins (Trial 1) at 40 to 45 °C compared to 100% of the casein and only 69% of the whey proteins for Trial 2 with the 0.02-μm membrane. As expected, the 10-kDa MWCO UF membrane retained 100% of the skim milk proteins (Trials 6 and 8).

Results in Table 3 reveal that the 0.02-μm MF membrane (Trials 2 to 4) generally retained somewhat higher percentages of Ca, Mg, and P than the 0.1-μm MF membrane (Trial 1). Both membrane processes retained generally similar percentages of diffusible K, Na, and lactose. These results reveal that the 0.02-μm MF membrane was more effective than the 0.1-μm membrane for retaining colloidal Ca, Mg, and P (Figure 5).

SMP was used as diafiltrant for 0.02-μm MDF fractionation Trials 3 and 4. The Trial 4 MDF process, at 38 to 41 °C and a higher final MCR value of 2.95, retained substantially lower percentages of all minerals and lactose than were retained for the Trial 3 MDF process with its final MCR of 2.22 (Table 2). Trial 5 with the 0.02-μm MDF membrane, Dannon Spring Water® diafiltrant, and lower retentate temperatures of 27 to 38 °C, retained lower percentages of all minerals and lactose than was achieved in the fractionation experiments conducted at 34 to 35 °C.

Trial 6 UDF fractionation experiment, in which 34 to 35 °C skim milk and Dannon Spring Water® diafiltrant were used, achieved a final MCR of 1.77 (Table 2). This process retained slightly higher

Table 1 — Mineral composition of commercial drinking water products.

	(mg/kg)		
	Land O' Sky Distilled Water®	Blue Ridge Pure Water®	Dannon Spring Water®
Phosphorus	ND[a]	ND[a]	ND[a]
Potassium	ND[a]	0.73	1.07
Calcium	0.42	1.93	35.85
Magnesium	0.18	0.60	17.56
Sodium	0.16	2.12	8.46
Sulfur	ND[a]	0.67	2.83

[a]Not detected.

percentages of Ca, Mg, and P than the Trial 5 MDF process conducted at 27 to 38 °C, but exhibited a lower retention of lactose, 3.7%, than for any of the other membrane processes.

The UDF experiment (Trial 8) with 17 to 21 °C skim milk and Land O' Sky® distilled water diafiltrant retained less Ca (60.5%) and Mg (31.9%), and only slightly more K, Na, and lactose, than for the other UDF fractionation experiments (Table 3).

The NF membrane (Trial 7) retained 64.5% to 77.7% of the minerals and more than 99% of the lactose from the cumulative UDF permeate produced during Trial 6 (Table 3).

Mineral composition of skim milk and MDF retentate fractions

The mineral composition of Mayfield® skim milk was generally within the ranges of composition reported by Jenness (1988), except for its higher P and lower Na concentrations (Table 4). The SMP, used as diafiltrant in the Trial 4 experiment (Table 4), contained less Ca and K, but more Mg and P than in milk (Jenness 1988). It also contained 0.64% of the Na of the mean diffusible mineral composition of milk (Jenness 1988).

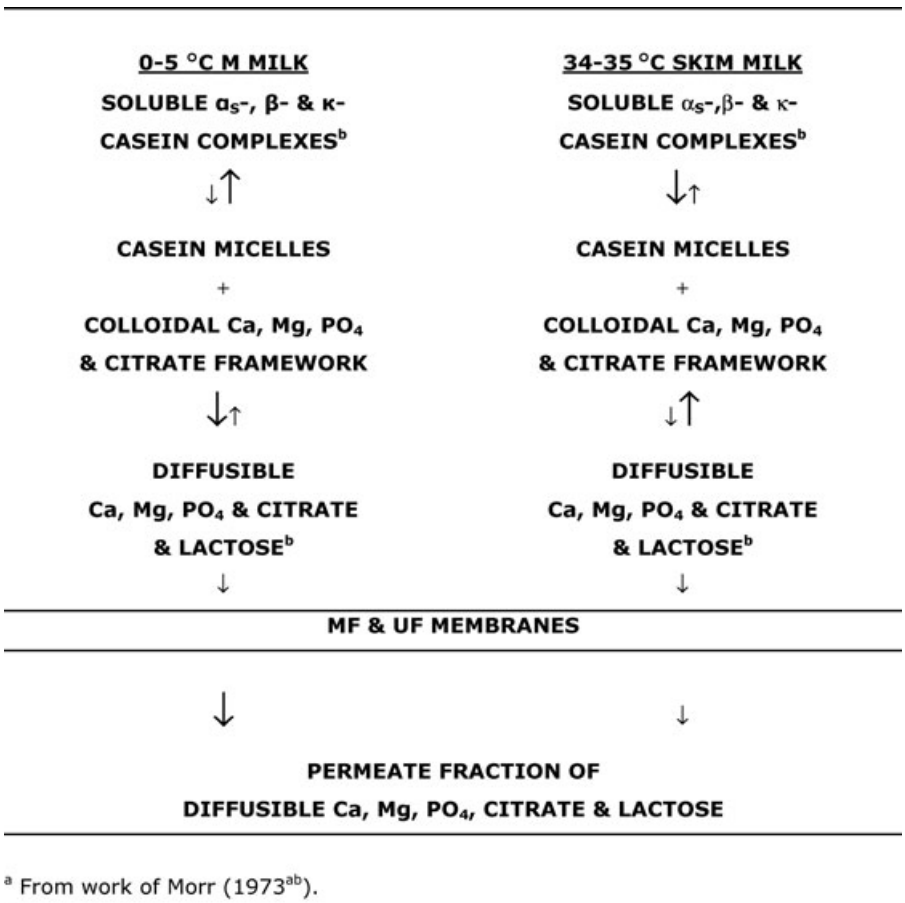

Figure 5—Temperature-dependent association and dissociation of casein micelles and minerals in skim milk.[ab]

[a] From work of Morr (1973[ab]).

[b] Not sedimented at less than 100,000 × g.

Table 2—Membrane fractionation of skim milk as a function of processing conditions.

Trail nr	Memb. process	Membrane type	Memb. area (m^2)	Process time (min)	Process temp. (°C)	Mass: Skim (kg)	Mass: Diafiltrant (kg)	Mass: Ret. (kg)	Mass: Perm. (kg)	Trans-memb. pressure[a] (kPa)	MCR	Mean flux (kg/m^2h)
1	MF	0.1-μm Scepter®	0.525	236	40 to 45	123	0	25	127.3	186 to 200	5.01	61.5
2	MF	0.02-μm Scepter®	0.525	386	34 to 35	136	0	29	107.5	267 to 282	4.72	31.8
3	MDF[b]	0.02-μm Scepter®	0.525	262	34 to 35	58.4	36.2	26.3	69.2	272 to 279	2.22	30.2
4	MDF[b]	0.02-μm Scepter®	0.525	183	38 to 41	52.0	34.5	17.7	34.3	83 to 152	2.95	21.4
5	MDF[c]	0.02-μm Scepter®	0.525	245	27 to 38	33.4	94.9	20.0	108.3	83 to 152	1.67	50.5
6	UDF[c]	10-kDa PES	7.43	53	34 to 35	35.4	96.2	20.0	111.6	134 to 243	1.77	17.0
7	NF[d]	90% Rejection	7.43	106	32 to 35	111.6[d]	0	21.5	90.1	220 to 1196	5.19	6.86
8	UDF	10-kDa PES	7.43	45	17 to 21	23.6	46.9	11.0	59.5	272 to 279	2.15	10.8

[a] 1/2 ($P_{inlet} + P_{outlet}$).
[b] SMP diafiltrant.
[c] Dannon Spring Water® diafiltrant.
[d] Trial 6 UDF permeate.
[e] Land O' Sky Distilled Water® diafiltrant.

Mineral composition of UDF retentate fractions

Adjustment of the Ca concentration in Trial 6 UDF retentate to a theoretical MCR of 1.0 yielded a value that was 66.4% of that of the skim milk (Table 5). The concentrations of the remaining minerals in the final retentate, adjusted to a theoretical MCR of 1.0, were all considerably lower than their respective values in skim milk (Table 5). For example, K and Na concentrations were only 3.45% and 4.73% of their respective skim milk concentration values.

A 2nd UDF experiment (Trial 8) was conducted using Laura Lynn® skim milk and Land O' Sky Distilled Water® diafiltrant, which contained lower concentrations of Ca and Mg (Table 6) than for previous experiments. The temperature of the retentate was maintained at 17 to 21 °C during UDF fractionation. This lower temperature range was chosen in an attempt to minimize the conversion of soluble casein and minerals to their colloidal forms (Figure 5). The concentrations of citrate in the skim milk and UDF retentate for this UDF experiment were consistent with the values listed by Jenness (1988). Our experimentally determined 8.1% retention value is consistent with our assumptions that 90% of the citrate in skim milk is diffusible and that our UDF membrane process should have removed 95% of the diffusible citrate.

The one NF membrane fractionation experiment (Trial 7) was conducted to fractionate skim milk UDF permeate produced in Trial 6 (Table 7). The cumulative NF permeate contained 5.7% to 49.2% of the minerals contained in Trial 6 skim milk UDF permeate. The NF membrane retained less of the K and Na than of the other minerals. The concentrations of minerals in the final, instantaneous NF permeate were consistently lower than those in the cumulative NF permeate, which is likely due to partial closing of the NF membrane pores during the fractionation experiment due to concentration polarization.

The concentrations of minerals in the final NF retentate, adjusted to an MCR of 1.0, represent only 64.5% to 77.7% of the minerals contained in the Trial 6 UDF permeate feed. The relatively high Na concentration in the NF permeate fraction would be expected to render it unsatisfactory for replacing commercial water as a UDF diafiltrant. However, it should be possible to reduce the Na concentration of the NF permeate fraction substantially by processing it through an RO membrane.

Mineral concentrations in the cumulative NF permeate, expressed as percentages of their respective concentrations in the NF feed (Trial 6 cumulative UDF permeate), ranged from about 5.7% for Mg to 46% to 49% for monovalent K and Na. These latter results confirm that NF permeate would almost certainly not be a satisfactory replacement for commercial drinking water as diafiltrant for UDF membrane processing (Figure 1). Subsequent fractionation of the NF permeate with an RO membrane might possibly produce an RO permeate more suitable for use as a UDF diafiltrant.

Table 3—Membrane retention of minerals and lactose.

Trail No.	Membrane process	*T* (°C)	MCR	Mineral retention (%)					Lactose retention (%)
				Ca	Mg	P	K	Na	
1	0.1-μm MF	40 to 45	5.01	67.7	41.3	57.8	22.8	22.5	18.3
2	0.02-μm MF	34 to 35	4.72	81.9	48.4	67.4	22.4	24.4	17.4
3	0.02-μm MDF[a]	34 to 35	2.22	76.9	56.8	67.7	30.7	14.0	13.7
4	0.02-μm MDF[a]	38 to 41	2.95	63.4	45.9	61.2	24.0	12.4	8.7
5	0.02-μm MDF[b]	27 to 38	1.67	61.3	41.0	45.5	3.7	4.6	5.3
6	10-kDa PES UDF[b]	34 to 35	1.77	66.4	46.4	48.6	3.44	4.73	3.7
7[c]	90%-Rejection NF	32 to 35	5.19	64.5	66.8	77.7	69.8	66.9	99.4
8	10-kDa PES UDF[d]	17 to 21	2.15	60.5	31.9	46.7	6.78	6.50	5.1

[a]SMP diafiltrant.
[b]Dannon Spring Water® diafiltrant.
[c]Trial 6 UDF permeate.
[d]Land O' Sky Distilled Water® diafiltrant.

Table 4—Mineral composition of skim milk MDF fractions.[a]

	(mg/kg)				
	Ca	Mg	P	K	Na
1. Mayfield® skim milk	1180	115	931	1580	413
2. Milk, range[b]	1110 to 1200	110 to 130	610 to 790	1130 to 1710	470 to 770
3. Milk, mean[b]	1180	120	740	1400	580
4. Milk, diffusible range[b]	344 to 372	72 to 85	323 to 420	1130 to 1710	470 to 770
5. Milk, diffusible mean[b]	366	78	392	1400	580
6. SMP mineral solution[c]	320.5 ± 16	83.2 ± 4.3	409.1 ± 20	864.4 ± 40	3.7 ± 0.10
7. Percent of skim milk diffusible, (#6 ÷ #5) × 100%	87.5	106.7	104.4	61.7	0.64
8. Final MDF retentate, MCR 2.95	2317 ± 23	166.6 ± 0.8	1658 ±17.7	1158 ± 23	180.8 ± 0.6

[a]0.02-μm Scepter® membrane, 38–41 °C, Trial 4.
[b]Jenness (1988).
[c]Diafiltrant formulated with Dannon Spring Water®.

Table 5—Mineral composition of skim milk UDF fractions.[a]

	(mg/kg)				
	Ca	Mg	P	K	Na
1. Skim milk, Laura Lynn®	1213	119	949	1570	408
2. Final, instantaneous UDF permeate	48.7	13.3	25.9	71.0	25.5
3. Final UDF retentate, MCR 1.77	1425	97.7	816	95.7	34.1
4. Final UDF retentate, MCR 1.0 (#3 ÷ 1.77)	805	55.2	461	54.1	19.3
5. Percent of skim milk, (#4 ÷ #1) × 100%	66.4	46.4	48.6	3.45	4.73

[a]10-kDa membrane using Dannon Spring Water® as diafiltrant, 34 to 35 °C, Trial 6.

Color and appearance of membrane fractions

Skim milk MF and UF permeate fractions exhibited a clear, transparent, greenish appearance that was presumed to be contributed by their riboflavin, which became visible upon removal of the casein micelles and colloidal phosphate. Skim milk MF retentate (MCR 4.7) appeared as a grayish colored, highly viscous fluid due to its tightly packed casein micelles and their colloidal phosphate complex constituents. MCR 2 Skim milk UDF retentate exhibited a whiter appearance than skim milk, presumably due to the light scattering properties of the colloidal casein micelles and also to the removal of most of the riboflavin by membrane fractionation. The NF retentate fraction (Trial 7) of the cumulative skim milk UDF permeate (UDF Trial 6) had a clear, yellow-colored appearance, whereas the NF permeate (Trial 7) was a clear, colorless solution.

UDF retentate reflected more light than skim milk at all wavelengths, especially in the 440 to 500 nm and in the 600 to 750 nm ranges (Figure 6). Skim milk has a combined lower $-a^*$ value and a higher $+b^*$ value compared to the UDF retentate indicating that it has a somewhat yellowish coloration (Figure 6 and Table 8). The coloration intensity is slight, however, because the L^* values are both very high at about 81.7 and 82.7. Overall, skim milk is very white with a hint of yellowness, whereas UDF retentate is primarily white with no chromatic appearance. Both of these samples would position near the L^* axis in the $-a^*$, $+b^*$ quadrant of the CIE color solid. These results confirm the perception that UDF retentate is noticeably whiter than skim milk.

Flavor and overall acceptability of water, SMP, and UDF retentate fractions

Land O' Sky Distilled Water® exhibited a low intensity, unidentified off-flavor, whereas the other 2 commercial water sources exhibited excellent flavors. SMP mineral solutions exhibited a slightly salty flavor.

UDF retentate fractions and L&S-RSM lacked sweetness and natural milk flavor, due presumably to the removal of lactose and natural milk flavor compounds during their preparation. Additionally, they exhibited a moderate intensity of a metallic off-flavor that was more pronounced when they were produced at higher processing temperatures than at the lower temperature as in Trial 8. This off-flavor is likely contributed by the casein micelles and their colloidal phosphate components, which is probably suppressed by lactose and natural milk flavors in skim milk. The larger-sized casein micelles that contain higher concentrations of colloidal phosphate in UDF retentate fractions recovered from 37 to 40 °C skim milk (Figure 5) may be responsible for their higher intensity of metallic off-flavor.

MDF and UDF membrane permeate fractions, which contained most of the lactose and natural milk flavor compounds (organic acids, aldehydes, ketones, alcohols, and so on), exhibited a pleasant, slightly sweet, milk-like flavor. In contrast, NF permeate of skim milk UDF permeate was clear, colorless, and exhibited a mildly bland flavor that lacked sweetness.

UDF retentate fractions and L&S-RSM lacked viscosity, due presumably to removal of lactose during membrane fractionation. This problem was overcome by addition of 0.1% of food stabilizer to L&S-RSM prior to pasteurization.

Frozen storage stability of UDF membrane retentate fractions and L&S-RSM

Frozen storage of MCR 4.7 to 5 skim milk MF retentate fractions (Trials 1 and 2) for more than 1 wk resulted in extensive casein micelle destabilization upon thawing. Similarly, frozen storage of skim milk MDF retentate (MCR 1.77) fractionated at 34 to 35 °C (Trial 6) with Dannon Spring Water® as diafiltrant, also resulted in extensive casein micelle destabilization. Skim milk control for the Trial 8 UDF experiment exhibited slight destabilization after 6 mo of frozen storage and thawing, whereas its MCR 2.15 UDF retentate remained completely stable for more than 6 mo. L&S-RSM prepared from Trial 6 UDF retentate fraction exhibited greater frozen storage stability than that of the UDF retentate, per se.

The processing temperatures used for membrane fractionation of skim milk may indirectly influence the frozen storage stability of MDF and UDF retentate by shifting the equilibrium between diffusible and colloidal minerals (Figure 5). Mineral composition results revealed that about 60%, 32%, and 47% of the Ca, Mg, and P, respectively, were retained during UDF at 17 to 21 °C (Trial 8)

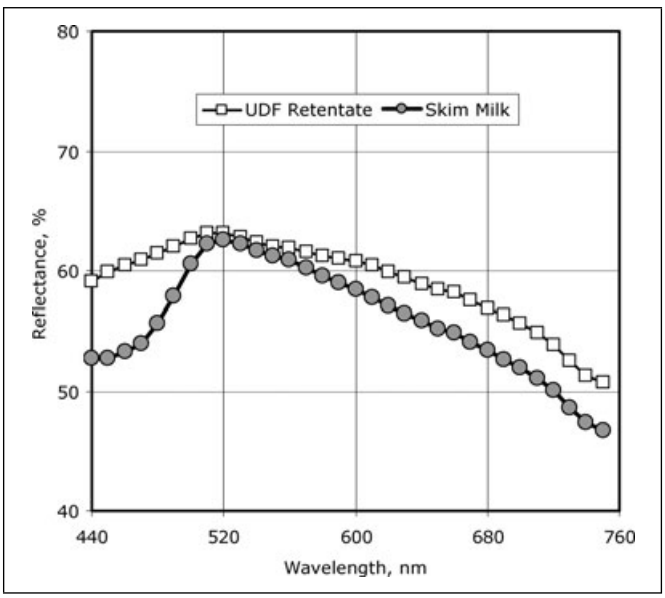

Figure 6 — Surface reflectance of skim milk & UDF retentate as a function of wavelength.

Table 6 — Mineral composition of skim milk UDF fractions.[a]

	(mg/kg)					
	Ca	Mg	P	K	Na	Citrate
1. Skim milk, Laura Lynn®	1220	119	965	1548	415	1695
2. Final UDF retentate, MCR 2.15	1586	81.7	968.5	226.5	58	300
3. Final UDF retentate, MCR 1.0 (#2 ÷ 2.15)	738	38	450.5	105	27	139.5
4. Percent of skim milk (#3 ÷ #1) × 100%	60.5	31.9	46.7	6.8	6.5	8.2
5. Land O' Sky Distilled Water®	0.42	0.18	ND[b]	ND[b]	0.16	—
6. SMP mineral solution[c]	823	159	317	669	14.2	—

[a]10-kDa PES membrane, Land O' Sky Distilled Water® diafiltrant, 17 to 21 °C, Trial 8.
[b]Not detected.
[c]Formulated with Land O' Sky Distilled Water.®

Table 7—Mineral composition of NF membrane fractions of Trial 6 UDF permeate.[a]

	(mg/kg)				
	Ca	Mg	P	K	Na
1. Cumulative UDF Trial 6 permeate, feed	92.4	24.1	84.7	283.6	80.5
2. Cumulative NF permeate	13.2	1.38	8.5	129.2	39.6
3. Percent permeated, (#2 ÷ #1) × 100%	14.3	5.7	10.0	45.6	49.2
4. Final instantaneous NF permeate	4.74	0.94	5.44	242.1	67.5
5. Final NF retentate, MCR 5.19	309.5	83.4	341.6	1027	280.0
6. Final NF retentate, MCR 1.0, (#4 ÷ 5.19)	59.6	16.1	65.8	197.9	53.9
7. Percent of cumulative UDF permeate feed, (#5 ÷ #1) × 100%	64.5	66.8	77.7	69.8	67.0

[a]NF Trial 7, 32 to 35 °C.

Table 8—C.I.E color space values for skim milk and final UDF retentate.[a]

	*L**	*a**	*b**
Skim milk[b]	81.69 ± 0.041	−4.81 ± 0.005	6.05 ± 0.024
UDF retentate[b]	82.71 ± 0.013	−2.728 ± 0.095	2.078 ± 0.005

[a]10-kDa membrane, Trial 8.
[b]Mean values ± standard deviation, $n = 4$.

compared to about 66%, 46%, and 49% of the Ca, Mg, and P, respectively, for UDF at 34 to 35 °C (Trial 6). The greater retention of Ca, Mg, and P during the skim milk fractionation experiment at 34 to 35 °C may be due to incorporation of diffusible Ca, Mg, and P into the casein micelles (Figure 5). This higher proportion of colloidal phosphate in the casein micelles of UDF retentate produced at 34 to 35 °C may be responsible for its shorter frozen storage stability and for imparting a more pronounced metallic flavor than was observed for UDF retentate produced at 17 to 21 °C (Trial 8).

Discussion

Buchheim and Prokopek (1976) and Karlsson and others (2005) were unable to find any differences in skim milk casein micelles before and after ultrafiltration by electron microscopic, dynamic light scattering, and rheological techniques. Similarly, Lonergan (1983) determined that ultrafiltration of skim milk to a VCR (volume concentration ratio) of six, dilution with distilled water to VCR 1, and ultrafiltration to VCR 6 did not alter the size distribution, mineral composition, or hydration of casein micelles, and did not alter the relative distribution of micellar and soluble casein. However, Srilaorkul and others (1991) reported that the largest proportion of casein micelles was changed from the 80 to 100 nm range in skim milk to 60 to 80 nm in milk concentrated 5-fold by UF. At higher degrees of concentration, the numbers of casein micelles with diameters less than 80 nm increased, but those with diameters more than 100 nm decreased. Consequently, both the volume distribution and average diameter of the casein micelles were altered. They determined that there was a decrease in casein micelle diameter from 118 nm in normal skim milk to 92 and 87 nm in milk concentrated 3- or 5-fold, respectively. They speculated that these changes may have been due to alterations in the Ca and P composition of the milk retentate as a result of ultrafiltration. Our results confirmed that the composition of the key minerals in skim milk UF and MF retentate are substantially different from those of skim milk.

The decrease in size of casein micelles reported by Srilaorkul and others (1991) may be due to very high shear forces during circulation of the more viscous MCR 5.0 membrane retentate through the membrane fractionation unit for prolonged time periods. However, we would not expect such changes in casein micelles with our MDF and UDF processing schemes as we were operating at lower MCR and retentate viscosity values. This factor would be an advantage of UDF processing over UF processing for removing lactose from skim milk.

Considerable studies have been conducted to investigate the factors that determine frozen storage stability of skim milk and milk concentrates prepared by vacuum evaporation. For example, Wells and Leeder (1963) and Lonergan and others (1981) reported that casein remains stable in frozen milk until after a significant portion of the lactose has been crystallized and that hydrolysis of the lactose or addition of sugars to milk prior to freezing effectively stabilized casein micelles in frozen milk concentrate. Morr (1964) demonstrated that soluble casein complexes in colloidal phosphate-free skim milk (Pyne and McGann 1960; Morr and others 1971) were more stable than casein micelles in frozen skim milk and concentrated skim milk. Thus, it appears that the colloidal stability of the casein micelles, which range in size from about 50 to 250 nm (Farrell 1988), is the primary factor for controlling the frozen storage stability of skim milk and that soluble lactose is a mediating factor. Casein micelles are subject to a temperature-dependent equilibrium with ionic Ca, Mg, P, and citrate as well as soluble α_s-, β-, and κ-casein complexes and colloidal phosphate (Figure 5).

The results of the present study indicate that casein micelles in MCR ≥ 4 skim milk MF and UF retentate were destabilized by frozen storage of 1 wk or less, agreed generally with those of Lonergan and others (1981). Casein micelle destabilization in prolonged frozen storage milk may be due to the formation of ionic and/or hydrophobic bonding that is favored as more and more of the lactose is crystallized and the availability of free, unfrozen water is decreased.

Inorganic colloidal phosphate components associated with casein micelles may be responsible for the metallic flavor observed in skim milk MDF and UDF retentate fractions that contain very low lactose concentrations. The higher intensity of metallic flavor observed for skim milk UDF retentate produced at 34 to 35 °C than for skim milk fractionated at 17 to 21 °C may be due to formation of colloidal calcium phosphate at the warmer temperature. The enhanced level of metallic flavor in UDF retentate compared to MDF retentate may also be due to a more complete removal of lactose in the former product. Lactose molecules may function in this regard by shielding the colloidal phosphate particles from the taste buds in the mouth, thereby preventing the former from imparting the metallic flavor to UDF retentate fractions. Additional studies will be needed to elucidate this and other possible mechanisms responsible for imparting metallic flavor to reduced lactose skim milk fractions.

The results from this study (Table 6) demonstrate that about 60% of the Ca, 32% of the Mg, and 47% of the PO_4 in skim milk were recovered in MF and UF membrane retentate fractions compared to only about 8% of the citrate. Since citrate-deficient UDF retentate resembles skim milk in general appearance, light reflectance as a function of wavelength (Figure 6), body, mouthfeel, and frozen

storage stability, it is likely that citrate ions or ion complexes may not be a key structural component of casein micelle-colloidal phosphate complexes that provide the framework for casein micelles in milk. Additional research would be needed to further elucidate the role of citrate in forming and stabilizing the colloidal casein micelles and their colloidal mineral components.

Conclusions

Several different membrane fractionation processes were evaluated for their ability to remove high percentages of lactose and sodium from commercial skim milk. A computer-based, mathematical model was developed to predict lactose removal from skim milk during UDF fractionation experiments. A simple procedure was developed for preparing a simulated milk permeate (SMP) that contains the major, nutritionally important minerals (Ca, Mg, K, PO_4, and citrate) at concentrations similar to those in skim milk for use as diafiltrant and for formulating L&S-RSM. L&S-RSM prepared by blending SMP and UDF retentate contained all of these nutritionally important minerals in concentrations comparable to those in skim milk, but contained less than 5% of the Na and lactose of skim milk. UDF retentate and L&S-RSM were both noticeably whiter and exhibited a lower viscosity than skim milk, lacked sweetness and natural milk flavor and imparted a metallic off-flavor. These flavor and viscosity deficiencies of L&S-RSM were overcome largely by adding low concentrations of stabilizer, sucralose, and vanilla extract. Additional research is needed to investigate further the mechanism responsible for imparting a metallic off-flavor to L&S-RSM and also to gain an understanding of the mechanism responsible for destabilizing casein micelles in frozen storage skim milk and L&S-RSM. L&S-RSM offers considerable potential as a source of high-quality proteins and nutritionally important minerals that contain very low concentrations of residual lactose and sodium for individuals at risk of or suffering from overweight or obesity.

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