Use of Cold Microfiltration Retentates Produced with Polymeric Membranes for Standardization of Milks for Manufacture of Pizza Cheese

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ABSTRACT

Pizza cheese was manufactured with milk (12.1% total solids, 3.1% casein, 3.1% fat) standardized with microfiltered (MF) and diafiltered retentates. Polymeric, spiral-wound MF membranes were used to process cold (<7°C) skim milk, and diafiltration of MF retentates resulted in at least 36% removal of serum protein on a true protein basis. Cheese milks were obtained by blending the MF retentate (16.4% total solids, 11.0% casein, 0.4% fat) with whole milk (12.1% total solids, 2.4% casein, 3.4% fat). Control cheese was made with part-skim milk (10.9% total solids, 2.4% casein, 2.4% fat). Initial trials with MF standardized milk resulted in cheese with approximately 2 to 3% lower moisture (45%) than control cheese (~47 to 48%). Cheese-making procedures (cutting conditions) were then altered to obtain a similar moisture content in all cheeses by using a lower setting temperature, increasing the curd size, and lowering the wash water temperature during manufacture of the MF cheeses. Two types of MF standardized cheeses were produced, one with preacidification of milk to pH 6.4 (pH6.4MF) and another made from milk preacidified to pH 6.3 (pH6.3MF). Cheese functionality was assessed by dynamic low-amplitude oscillatory rheology, University of Wisconsin MeltProfiler, and performance on pizza. Nitrogen recoveries were significantly higher in MF standardized cheeses. Fat recoveries were higher in the pH6.3MF cheese than the control or pH6.4MF cheese. Moisture-adjusted cheese yield was significantly higher in the 2 MF-fortified cheeses compared with the control cheese. Maximum loss tangent (LT_{max}) values were not significantly different among the 3 cheeses, suggesting that these cheeses had similar meltability. The LT_{max} values increased during ripening. The temperature at which the LT_{max} was observed was highest in control cheese and was lower in the pH6.3MF cheese than in the pH6.4MF

cheese. The temperature of the LT_{max} decreased with age for all 3 cheeses. Values of 12% trichloroacetic acid soluble nitrogen levels were similar in all cheeses. Performance on pizza was similar for all cheeses. The use of MF retentates derived with polymeric membranes was successful in increasing cheese yield, and cheese quality was similar in the control and MF standardized cheeses.

Key words: microfiltration, cheese yield, texture, functionality

INTRODUCTION

Use of microfiltration (MF) in the dairy industry has focused on removing bacteria, spores, and somatic cells from milk; separating micellar CN from serum proteins; and removing residual milk fat from whey for whey protein isolate manufacture (Maubois, 2002). Within the past 20 yr, MF has emerged as a new technology for separating milk components as a result of the development of ceramic membranes and the use of uniform transmembrane pressure to reduce membrane fouling (Saboya and Maubois, 2000). Traditionally, ceramic membranes have been used for MF. Microfiltration with ceramic membranes has been used to concentrate CN, and these retentates have been used to standardize cheese milks for manufacture of Cheddar (St-Gelais et al., 1995; Neocleous et al., 2002a) and Mozzarella cheeses (Brandsma and Rizvi, 1999, 2001a,b; Garem et al., 2000). In the last several years, a new generation of polymeric spiral-wound membranes has been developed. Industry sources indicate that these membranes are considerably cheaper than ceramic membranes and have lower operating costs (DSS Silkeborg AS, 2005). When using ceramic membranes for MF, the process is typically carried out at 50 to 55°C (Maubois, 2002). Recently, many dairy applications that use membrane processing have been performed at a low temperature (<7°C) with polymeric spiral-wound membranes (Govindasamy-Lucey et al., 2004, 2005), which allows raw milk or whey to be filtered, reduces the likelihood of denaturation of serum proteins, and reduces microbial growth during processing.

Received February 20, 2007.

Accepted May 30, 2007.

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When whole or skim milk is processed with an MF membrane that has an average pore size of 0.1 to 0.2 μm , CN is retained in the retentate, whereas small molecules (such as lactose, soluble calcium) and smaller, nonmicellar proteins (such as serum proteins) pass through the membrane into the permeate stream (Saboya and Maubois, 2000; Maubois, 2002). Consequently, the permeate stream contains serum proteins without the added ingredients that are derived from cheese making, such as lactic acid, rennet, culture, annatto color, or glycomacropeptide (Britten and Pouliot, 1996). In addition, if the CN stream is concentrated, this material can be used to standardize cheese milk instead of skim milk powder, which is widely used in the US cheese industry.

Although in 1996 the US Food and Drug Administration approved the use of cold ($<7^{\circ}\text{C}$) UF of milk in Cheddar and Mozzarella cheese manufacture (Code of Federal Regulations, 2003), currently the use of MF retentates is not specifically permitted in the United States in the manufacture of cheeses with standards of identity. It can be used in pizza cheese, a non-standard-of-identity cheese. Pizza cheese is functionally and organoleptically similar to low-moisture, part-skim Mozzarella cheese, but it is a nonpasta filata, stirred-curd cheese manufactured with mesophilic cultures (*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis*; Chen and Johnson, 2001). Pizza cheese is commonly manufactured from milk with a CN:fat ratio of ~ 1.0 to 1.05, and milk can be standardized by cream removal or addition of condensed or UF milk or NDM.

The use of MF retentates in cheese milk may require modification of the cheese-manufacturing protocol, depending on the solids and protein contents of the cheese milk, to maintain the original functionality. We are not aware of any cheese-making trials that have been published in which polymeric MF membranes and cold-temperature processing were used to produce MF retentates. One of the objectives of this study was to use cold MF with these new crossflow, spiral-wound polymeric (polyvinylidene difluoride) membranes to produce retentates with an increased CN:true protein ratio (reduced serum protein content). In addition, we wanted to investigate the impact of using this type of retentate to standardize milk for pizza cheese manufacture and to determine the impact on cheese yield, composition, proteolysis, and functional characteristics. Previous studies showed that the addition of MF retentates to standardize cheese milks during Cheddar cheese manufacture lowered the moisture contents of the resultant cheeses (St-Gelais et al., 1995; Neocleous et al., 2002a). Differences in cheese moisture contents also alter the functional and ripening properties, so modifications to cheese-making procedures were used to attain MF forti-

fied cheeses with moisture contents similar to that of control cheese. Because of the higher CN content, the total calcium content also tends to be higher in MF cheeses (Brandsma and Rizvi, 1999; Neocleous et al., 2002a,b). Thus, the MF cheeses in this present study were manufactured by preacidifying milk to 2 different pH, 6.4 and 6.3, to remove some of the colloidal calcium content.

MATERIALS AND METHODS

MF and Standardization

Raw whole milk and cream were obtained from the University of Wisconsin Dairy Plant (Madison, WI) on the day before cheese making. Raw whole milk was skimmed (fat content $0.13 \pm 0.01\%$). Microfiltration was carried out by concentrating ~ 940 kg of skim milk to $\sim 16.4\%$ solids (11% CN). The MF concentration was performed at $<7^{\circ}\text{C}$ by recirculating the milk through Snyder MF 75925 (Snyder, model FR3B, size 3838) and Sepro MF (Sepro, size 3838-32) polyvinylidene difluoride membranes. These spiral-wound membranes were connected in parallel (each with a molecular weight cutoff of 800,000 Da and pore size of 0.2 μm , Membrane Systems Specialists Inc., Wisconsin Rapids, WI). During the MF process, milk was cooled in a spiral tubing system that was submerged in a chilled ($\sim 4^{\circ}\text{C}$) water tank to maintain the milk at a low temperature ($<7^{\circ}\text{C}$). During the MF process, the flux gradually decreases, so to increase the flux and remove more serum proteins during the MF process, 190 L of water was added twice; that is, 2 diafiltration (DF) steps were carried out during concentration. Table 1 shows the composition of the different fractions that were obtained during the MF and DF steps. The retentates were then stored overnight at 4°C and blended the following morning to give standardized cheese milks.

Two types of cheese milks were prepared: control and milk standardized with MF retentates. Control milk was standardized by blending part-skim milk with cream to an average of 10.92% (2.38 ± 0.05 CN) solids with a mean CN:fat ratio of ~ 1.0 . The MF cheese milk was prepared by blending the skim milk MF and DF retentate (9.5% on a weight basis) with whole milk ($12.06 \pm 0.06\%$ TS, $2.41 \pm 0.05\%$ CN, and $3.39 \pm 0.05\%$ fat) to obtain milk with 12.10% solids (3.14% CN) with a mean CN:fat ratio of 1.0.

Cheese Manufacture and Sampling Procedures

The same day as blending, nonpasta filata, low-moisture, part-skim pizza cheese was manufactured by licensed Wisconsin cheese makers at the University of Wisconsin-Madison dairy processing pilot plant. Two

Table 1. Composition of skim milk, microfiltered (MF) retentate before diafiltration (DF), MF permeate before DF, MF retentate after 2 DF steps, and MF permeate after 2 DF steps¹

Fraction	TS (%)	Total fat (%)	Total protein ² (%)	True protein ³ (%)	CN ⁴ (%)	CN to total protein (%)	CN to true protein (%)	Lactose (%)
Skim milk before MF	9.03 ± 0.17	0.13 ± 0.01	3.27 ± 0.07	3.08 ± 0.07	2.52 ± 0.08	76.97 ± 1.07	81.80 ± 1.02	4.79 ± 0.18
MF retentate before DF	12.47 ± 0.12	0.24 ± 0.04	6.56 ± 0.07	6.36 ± 0.06	5.34 ± 0.06	81.33 ± 0.09	83.95 ± 0.17	—
MF permeate before DF	6.08 ± 0.14	0.03 ± 0.03	0.41 ± 0.02	0.21 ± 0.03	0.05 ± 0.01	11.41 ± 2.67	22.13 ± 6.73	—
MF and DF retentate	16.39 ± 0.36	0.43 ± 0.13	12.86 ± 0.60	12.74 ± 0.61	10.99 ± 0.71	85.39 ± 1.61	86.23 ± 1.53	1.42 ± 0.04
MF and DF permeate	4.44 ± 0.26	0.01 ± 0.00	0.31 ± 0.04	0.17 ± 0.04	0.04 ± 0.00	11.48 ± 1.62	21.71 ± 4.87	3.36 ± 0.30

¹Means (n = 4) and standard deviations.²Total % N × 6.35.³(Total % N – % NPN) × 6.35.⁴(Total % N – % noncasein N) × 6.36.

types of MF standardized cheeses were manufactured. One vat used an almost identical manufacturing protocol as the control milk [i.e., milk was standardized by MF and preacidified to 6.4 (**pH6.4MF**)], and in another, MF standardized milk was made with milk preacidified to pH 6.3 (**pH6.3MF**). Prior to cheese making, blended milks were pasteurized at 74°C for 19 s and cooled to 5°C. Milk for each individual vat was sampled for chemical analysis and then weighed on a floor scale (model 31-1822-FD, Toledo Scale Co., Toledo, OH) before being gravity fed into each individual vat. The control, pH6.4MF, and pH6.3MF vats were filled, respectively, with 227 ± 0, 174 ± 3, and 174 ± 3 kg of pasteurized blended milk. In all experimental vats, milk volume was based on CN content in comparison with the control vat to keep actual cheese yield similar in all vats. Lactic acid (88%, wt/wt; Chr. Hansen, Milwaukee, WI) was diluted (wt/wt) at a rate of 4 parts water to 1 part lactic acid and the diluted acid was added to the cold milk (~5°C). The addition of diluted lactic acid to the standardized milks lowered the initial pH from 6.63 ± 0.04 to 6.43 ± 0.01, 6.44 ± 0.01, and 6.31 ± 0.01 for the control, pH6.4MF, and pH6.3MF cheese milks, respectively. The milk temperature was then raised to the ripening temperatures of 34.4 and 31.1°C for the control and MF cheese milks, respectively. The lower set temperature was used for the MF standardized cheeses to increase the moisture content. The starter was a direct-to-vat set culture comprising *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* (DVS 970, Chr. Hansen). The amount of starter added to all vats was based on the CN content of the standardized milks (6.24 ± 0.1 g of starter/kg of CN). Following a 60-min ripening period, double-strength chymosin (Chymostar, Danisco USA Inc., Madison, WI) was added to the control and experimental milks at a rate of 1.47 ± 0.02 mL/kg of CN.

The coagula were cut at similar firmness as subjectively evaluated by an experienced licensed Wisconsin

cheese maker. The control, pH6.4MF, and pH6.3MF coagula were cut with knives at the pH values of 6.33 ± 0.02, 6.37 ± 0.08, and 6.28 ± 0.02, respectively. The control coagulum was cut with 9.5-mm knives, whereas both the MF coagula were cut with larger knives (12.7 mm). The temperatures of both the control and experimental vats were raised to the cooking temperature of 36.7°C over a 20-min period. After reaching the cooking temperature, each vat was cooked at that temperature without stirring for ~10 min. The whey was drained for ~10 min, weighed on a floor scale (model 8140, Toledo Scale Co.), and sampled. After completion of draining, 36.3 kg of water at 18.3 and 15.5°C was added to the control and experimental curd, respectively. Lower temperature water was used to increase the moisture content of the standardized cheeses. The curd was left in the water slurry for a period of ~20 min, with slurry temperatures of 26.1 and 23.9°C for the control and experimental vats, respectively. The water was then drained slowly over a 15-min period, weighed on a floor scale (model 8140, Toledo Scale Co.), and sampled. The curd from all vats was then salted in 3 equal applications over a 15-min period at a rate of 119 ± 2 g of salt/kg of CN in milk. The curd was packed into 9-kg Wilson hoops and pressed for 3 h at ~23°C. Press whey was collected, weighed (model MSG-500 Series Electronic Scales, Mars Scale Group, Niagara Falls, NY), and sampled from the first salt application through 1 h of pressing. After pressing, the cheeses were placed in a cooler (7°C) overnight. The following morning, the cheeses were removed from the molds and weighed (model CW-80I-2A, Rice Lake Weighing Systems, Rice Lake, WI). Cheeses were vacuum-packaged in Cryovac standard clear bags (9Fv86, Cryovac North America, Duncan, SC) and aged at 7°C.

Compositional Analyses

All compositional analyses for each sample were carried out in triplicate. Milk, whey, wash water, and press whey samples were analyzed for fat (Mojonnier method;

Table 2. Compositions of pasteurized standardized milk, drain whey, press whey, wash water, and cheese (n = 4)

Component	Treatment ¹			SEM	P-value
	Control	pH6.4MF	pH6.3MF		
Standardized cheese milk					
TS (%)	10.92 ^b	12.02 ^a	12.17 ^a	0.062	<0.0001
Milk fat (%)	2.35 ^b	3.07 ^a	3.10 ^a	0.009	<0.0001
Total protein ² (%)	3.11 ^b	3.93 ^a	4.00 ^a	0.038	<0.0001
True protein ³ (%)	2.92 ^b	3.75 ^a	3.82 ^a	0.038	<0.0001
CN ⁴ (%)	2.38 ^b	3.11 ^a	3.17 ^a	0.036	<0.0001
NPN (%)	0.031 ^a	0.029 ^b	0.029 ^b	0.0003	<0.05
CN to total protein (%)	76.50 ^b	79.15 ^a	79.26 ^a	0.283	<0.001
CN to true protein (%)	81.58 ^b	83.02 ^a	83.15 ^a	0.254	<0.01
Whey protein ⁵ (%)	0.54 ^b	0.64 ^a	0.64 ^a	0.010	<0.001
Whey protein in serum phase ⁶ (%)	0.56 ^b	0.68 ^a	0.69 ^a	0.010	<0.001
Lactose (%)	4.55 ^a	4.32 ^b	4.30 ^b	0.031	<0.01
Lactose in serum phase ⁷ (%)	4.78 ^a	4.60 ^b	4.59 ^b	0.031	<0.01
CN:fat ratio	1.01	1.02	1.02	0.012	NS ⁸
Total calcium (mg/100 g of milk)	93.7 ^b	119.9 ^a	120.8 ^a	2.47	<0.001
Total calcium (mg/g of protein)	30.1	30.5	30.2	0.677	NS
Drain whey					
TS (%)	6.47	6.44	6.51	0.029	NS
Milk fat (%)	0.25 ^b	0.31 ^a	0.28 ^{ba}	0.010	<0.05
Total protein (%)	0.85 ^b	1.02 ^a	1.10 ^a	0.039	<0.05
Lactose (%)	4.79 ^a	4.59 ^b	4.62 ^b	0.028	<0.01
Press whey					
TS (%)	8.72	8.92	9.04	0.270	NS
Milk fat (%)	0.14	0.14	0.14	0.012	NS
Total protein (%)	0.65	0.69	0.72	0.017	0.07
Lactose (%)	1.90	1.83	1.88	0.052	NS
Wash water					
TS (%)	1.34	1.12	1.12	0.099	NS
Milk fat (%)	0.10	0.09	0.10	0.011	NS
Total protein (%)	0.14	0.14	0.15	0.012	NS
Lactose (%)	0.86	0.74	0.77	0.051	NS
Cheese					
Moisture (%)	47.37	47.85	47.80	0.422	NS
Fat (%)	22.82	22.67	23.09	0.205	NS
Total protein ⁹ (%)	24.55	24.46	24.73	0.243	NS
Salt (%)	1.63	1.71	1.53	0.078	NS
Moisture in nonfat substances (%)	61.38	61.87	62.15	0.479	NS
Fat in DM (% dry weight basis)	43.37	43.46	44.24	0.359	NS
Salt in moisture (%)	3.44	3.57	3.20	0.185	NS
Lactic acid at 1 mo (%)	1.22	1.22	1.22	0.051	NS
Lactose at 1 mo (%)	0.013	0.011	0.013	0.004	NS
pH at 1 d	5.38	5.38	5.31	0.032	NS
Total calcium (mg/100 g of cheese)	764	768	728	7.60	<0.05
Total calcium (mg/g of protein)	31.1 ^a	30.8 ^a	28.8 ^b	0.215	<0.001

^{a,b}Means within the same row not sharing a common superscript differ ($P < 0.05$).

¹pH6.4MF = cheese milk acidified to pH 6.4 and standardized with microfiltered (MF) retentates; pH6.3MF = cheese milk acidified to pH 6.3 and standardized with MF retentates.

²Total % N $\times$ 6.35.

³(Total % N – % NPN) $\times$ 6.35.

⁴(Total % N – % noncasein N) $\times$ 6.36.

⁵True protein – CN.

⁶Whey protein in serum phase = % whey protein/(100% – % fat – % CN).

⁷Lactose in serum phase = % lactose/(100% – % fat – % CN).

⁸Nonsignificant (F -test for full statistical model, $P > 0.05$).

⁹Total % N $\times$ 6.31.

AOAC, 2000), protein (total percentage N $\times$ 6.35, Kjeldahl method; AOAC, 2000), CN (AOAC, 2000), lactose (AOAC, 2000), TS (Green and Park, 1980), and NPN (AOAC, 2000).

The cheeses were sampled after 1 wk for compositional analysis. At the time of sampling, a 2.5-cm-thick slab was cut off the block; this slab was further sampled for each analysis. This cheese sample was completely

ground and used for analysis. Cheese samples were analyzed for moisture (Marshall, 1992), fat (AOAC, 2000), pH by the quinhydrone method (Marshall, 1992), protein by the Kjeldahl method (AOAC, 2000), and salt by the chloride electrode method (model 926; Corning Glass Works, Medfield, MA; Johnson and Olson, 1985). Proteolysis was monitored during ripening by measuring the amount of 12% TCA soluble nitrogen at 1, 2, 4, 8, and 12 wk (AOAC, 2000).

Total calcium in milk and cheese was determined by a modified method of Dolan and Capar (2002). Milk (1.0 mL) or ground cheese (0.4 to 0.5 g) samples were transferred to 55-mL Teflon-lined heavy duty vessels capable of operating at up to 260°C (HP-500 vessels, CEM Corporation, Matthews, NC). The milk or cheese samples were digested with 10 mL of 69% (wt/wt) nitric acid by using a pressurized microwave wet digestion system (MARS 5 Xpress, CEM Corporation). The vessels were sealed, placed in the microwave oven, and digested by heating at 10°C/min to 200°C and then holding at that temperature for 20 min. The microwave power applied was varied depending on the number of vessels in the system, with 600, 900, and 1,200 W used for 10 to 15, 16 to 25, and 26 to 40 vessels, respectively. After digestion was completed, the vessels were cooled and the digested samples were transferred to test tubes. Approximately 2 mL of the digest was then transferred to a 100-mL volumetric flask and diluted to volume with deionized water.

The calcium contents of the diluted and digested samples were measured by inductively coupled argon plasma emission spectroscopy (Vista-MPX Simultaneous ICP-OES, Varian Inc., Palo Alto, CA). The wavelength of plasma emission used to measure the calcium content was 315.9 nm.

SDS-PAGE

Denaturing, nonreducing SDS-PAGE was carried out by a modified method of Laemmli (1970). Sodium dodecyl sulfate-polyacrylamide separating gels (12.5%) were cast in a Mini-Protean III Cell unit (Bio-Rad Laboratories, Hercules, CA) and subjected to electrophoresis at a constant voltage of 200 V at ~25°C for 35 min. Prior to loading, the samples (skim milk, MF permeate before DF, and MF and DF permeate) were centrifuged at $3,026 \times g$ for 20 min. Aliquots of the supernatants were mixed with an appropriate amount of the sample buffer so as to obtain a final protein concentration of 0.4 and 2 µg/µL in the mixture for the permeate and skim milk samples, respectively. The mixture was then boiled for 5 min. Each sample (5 µL, 10 to 16 µL, and 24 to 30 µL of skim milk, MF permeate before DF, and MF and DF permeate, respectively) were loaded onto the gels.

Following electrophoresis, gels were stained with Coomassie Brilliant Blue G-250 (0.1 g/100 mL) dissolved in an aqueous solution of methanol (50 mL/100 mL) and acetic acid (10 mL/100 mL). Gels were destained with an aqueous solution of methanol (30 mL/100 mL) and acetic acid (10 mL/100 mL). Apparent molecular masses were estimated by comparison with standard markers ranging from 6.9 to 201 kDa (prestained SDS-PAGE standards, Bio-Rad Laboratories).

Fat and Nitrogen Recovery and Yield

A mass balance was carried out for each vat of cheese. Milk, drain whey, wash water, and press whey were weighed to ± 0.1 kg and cheese was weighed to ± 0.01 kg. The percentages of fat or nitrogen recovered in the cheese, drain whey, wash water, and press whey were calculated as the total amount of fat or nitrogen in the each one of these products divided by the total amount of fat or nitrogen in the original standardized milk and multiplied by 100.

Actual yield was calculated for each vat of cheese as the weight of the cheese divided by the weight of the original standardized milk multiplied by 100. Actual cheese yield was also adjusted to the target cheese moisture content; for pizza cheese, this was 46%. The approach recently described by Govindasamy-Lucey et al. (2006) was used to determine the predictive cheese yield and the recoveries of fat, CN, and other solids in cheese. Predictive cheese yields were calculated for each vat by using the Van Slyke cheese yield equation (Van Slyke and Price, 1936; equation [1]):

$$\text{Van Slyke cheese yield} = \frac{[(RF \times \% \text{ fat in milk}) + (RC \times \% \text{ CN in milk})] \times RS}{\left(\frac{100 - \% \text{ moisture of cheese}}{100} \right)} \quad [1]$$

where *RF* is the fraction of fat recovered in the cheese, *RC* is the fraction of CN recovered in the cheese, and *RS* reflects the proportion of other milk solids and salt recovered in the cheese in relation to the amount of CN and fat in the cheese. The *RF* values were determined experimentally as fat recovery for each cheese type during the cheese-making trials. Both *RC* and *RS* were calculated according to the method described by Govindasamy-Lucey et al. (2006).

Rheological Properties During Rennet Coagulation of the Cheese Milks

Rennet-induced milk gels are viscoelastic, and their small-deformation rheological properties can be determined by dynamic low-amplitude oscillatory rheometry

by measuring the storage modulus (G'), loss modulus (G''), and loss tangent (LT ; Zoon et al., 1988; Van Vliet et al., 1989; Lucey, 2002). The rheological characteristics of the standardized cheese milks [control and MF cheese milks (pH6.4MF and pH6.3MF)] during renneting were measured at 34.4 ± 0.1 and $31.1 \pm 0.1^\circ\text{C}$, respectively, in a UDS 200 Physica rheometer (Physica Messtechnik, Stuttgart, Germany) operating in oscillation mode at a frequency of 0.1 Hz and a strain of 1% as described by Govindasamy-Lucey et al. (2005). The measuring system consisted of 2 coaxial cylinders (diameters 25.0 and 27.5 mm). A profiled (serrated) bob was used in this couette-type fixture.

To determine the resistance of the coagulum to cutting, we studied the large-deformation properties of rennet gels by using the constant shear rate technique for determining an apparent yield stress and shear deformation at yielding, as described previously for acid milk gels (Lucey et al., 1997). Gels were made in situ as described above and sheared at a constant shear rate of 0.01 s^{-1} at 34.4°C (for control milks) and 31.1°C (for MF cheese milks) starting at the cutting time specified by our cheese maker. The gels were subjected to constant shearing, and yielding of the gel was defined as the point at which the shear stress started to decrease (Lucey et al., 1997).

Small-Amplitude Oscillatory Rheology Tests on Cheese

Rheological properties of the cheeses were studied with a UDS 200 Physica rheometer (Physica Messtechnik) as described by Govindasamy-Lucey et al. (2005). Cheese samples were subjected to a long heating profile (**LHP**) and short heating profile (**SHP**) in the rheometer. In both tests, a strain of 0.2% was applied to the cheese samples at a frequency of 0.1 Hz. In the SHP, developed by Hassan (2001), the heating rate used in the test was adjusted to be similar to the nonlinear heating rate used in the UW-MeltProfiler (Muthukumarappan et al., 1999). In the LHP, the cheese samples were heated at a constant rate of $1^\circ\text{C}/\text{min}$ from 5 to 80°C . In both tests, G' (or stiffness), G'' , and LT parameters were measured as a function of temperature. We also calculated the temperature at which LT was equal to 1 (i.e., where $G' = G''$) because this indicates the transition from a solid to a liquid-like system (i.e., a crossover point). The maximum LT (LT_{max}) and the temperature at which the LT_{max} occurred during heating with the LHP were also recorded.

Melt Profile Analysis

Melt and flow properties of the cheese samples were evaluated in a UW-MeltProfiler as described by Muthu-

kumarappan et al. (1999). Cheese samples were sliced into discs of 7 mm thick and 30 mm in diameter. Samples were stored in a plastic bag in the refrigerator at -6°C for at least 3 h before testing. The change in cheese height as a function of sample temperature was measured until the sample temperature reached 63°C . Degree of flow (**DOF**) was calculated as the change in height of the cheese sample at 60°C as compared with the cheese height at the beginning of the test.

Sensory Analysis

Each cheese was evaluated for bitterness, saltiness, acidity, oxidized flavor, other off-flavors, firmness, and smoothness on a 0- to 7-point scale. Cheeses were shredded on a pilot plant-scale shredder (Urschel model CC, Alard Equipment Corp., Williamson, NY). The cheeses were baked on pizza in a forced-air commercial oven (Impinger Ovens, Lincoln Foodservice Products Inc., Ford Wayne, IN) at 260°C for 5 min and the performance (e.g., oiling off, strand elasticity, flow off crust, mouth feel, chewiness, and flavor, e.g., acidity and saltiness) of each cheese was subjectively evaluated by a panel of 6 to 8 panelists, which included at least 3 experienced cheese graders.

Experimental Design and Statistical Analysis

Four replicate cheese-making trials were carried out; in each trial, 3 standardized milks (i.e., part-skim milk or control, pH6.4MF, and pH6.3MF) were used to make pizza cheese. A 3×4 completely randomized block design, which incorporated all 3 treatments and 4 blocks (replicate trials), was used for analysis of the response variables relating to milk, cheese, and whey composition. Analysis of variance was carried out with PROC GLM of SAS (version 9.1; SAS Institute, 2002–2003). In the ANOVA model, the 3 different standardized milks (different treatments) were analyzed as a discontinuous variable, whereas cheese-making day (i.e., different batch of milk) was blocked. Scheffé's multiple-comparison test was carried out to evaluate differences in the treatment means at a significance level of $P < 0.05$.

A split-plot design was used to monitor the effects of treatment and time of aging and their interactions on pH, proteolysis (12% TCA soluble nitrogen, expressed as a percentage of total nitrogen), LT_{max} values, temperature of LT_{max} , temperature of the crossover point, DOF, and sensory attributes during ripening. For the whole-plot factor, treatment was analyzed as a discontinuous variable, whereas cheese-making day was blocked. For the subplot factor analysis, age was treated as a continuous variable. The interactive term treatment $\times$ cheese-making day was treated as the error

term for the treatment effect. Analysis of variance for the split-plot design was carried out with PROC GLM of SAS. Fisher's least significant difference test was carried out to evaluate differences in the treatment means at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Composition of MF and DF Retentate and Permeate

The composition of all the different streams collected during the MF and DF steps is shown in Table 1. The TS, total protein, and CN contents of the final MF and DF retentates were 16.39 ± 0.36 , 12.86 ± 0.60 , and $10.99 \pm 0.71\%$, respectively (Table 1). The retentate was concentrated to ~4-fold based on the CN content at the end of the MF and DF runs. At the end of the concentration process, 48% of serum protein was removed, based on total protein (36% on a true protein basis). The lactose content in the retentate was greatly reduced (Table 1) because of the 2 DF steps that were carried out during MF processing. A trace amount of fat was detected in the permeate before DF (0.03%) and in the final MF and DF permeate (0.01%); the MF membrane was very efficient at removing residual fat from whey. In both the permeate samples (before and after DF), a small amount of CN was present (0.04 to 0.05%), as measured by Kjeldahl analysis.

One of the concerns in carrying out the MF process cold was that β -CN may dissociate from the micelle and pass through the membrane into the permeate stream. Consequently, the permeate stream could contain some CN. Thus, the protein in the final MF and DF permeate stream was analyzed by SDS-PAGE (Figure 1). A trace or a very faint band corresponding to β -CN protein was seen in the permeate sample, suggesting that during the MF and DF steps, a very small amount of β -CN was passing through the membrane. The presence of β -CN probably accounts for the trace amount of CN that was measured in the permeate by Kjeldahl analysis. In the composite MF and DF permeate stream, ~22% of the true protein was CN (Table 1). However, the amount of true protein present in the MF and DF permeate was only 0.17% (Table 1). Thus, the ratio of CN:true protein in the permeate stream was only approximately one-fifth of a very small amount (0.17%).

Composition of Standardized Cheese Milks and Whey

As expected, the TS, fat, total protein, true protein, and CN contents were significantly higher in the milks standardized with MF retentates (both pH6.4MF and pH6.3MF) than the control milks (Table 2). The standard plate counts for the control, pH6.4MF, and

pH6.3MF cheese milks were 3.08×10^1 , 1.98×10^1 , and 1.88×10^1 cfu/mL, respectively. The CN:true protein ratio was significantly higher in the MF standardized cheese milks because some of the serum protein was removed from the MF permeate. Even though ~48% of serum protein was removed based on total protein (36% on a true protein basis) during the MF and DF processes (because only ~10% of retentates were used in the preparation of MF standardized cheese milks), the serum protein contents in the MF standardized cheese milks were significantly higher ($P < 0.001$) than in control milks because of the higher overall concentration of protein. The concentration of serum protein in the serum phase was also lower in the control milks than in the MF retentate-standardized milks (Table 2). Neocleous et al. (2002a) also reported that serum protein concentrations increased in the MF retentate-standardized cheese milks used for Cheddar cheese manufacture, and this increase could indicate that some of the high molecular weight milk serum proteins may not have passed through the MF membrane (Jost et al., 1999). The lactose content of the control milk was significantly higher than that of the MF standardized milks. The reduced lactose contents of the MF retentates were a result of the DF step that was used and not of the increased fat and CN contents in the MF standardized cheese milks, because the lactose levels in the serum phase (lactose/100 – % fat – % CN) were also higher in the control milks. Higher fat and protein contents and lower lactose levels were found in the drain whey obtained from the MF milks than from the control milks, which was expected because these milks began with higher fat and protein levels. Guinee et al. (1994) predicted that fat and protein losses in whey would increase linearly with increasing milk protein level as a result of the reduced whey volume in higher protein milks that were produced by using UF. There were no significant differences in the composition of press whey or wash water derived from the control and MF standardized milks.

Cheese Composition

In preliminary studies, when the same manufacturing protocol was used to manufacture both the control and MF standardized cheeses, the moisture contents of the MF standardized cheeses (45%) were much lower than those of the control cheeses (~47 to 48%, data not shown). This result is in agreement with previous work (St-Gelais et al., 1995; Neocleous et al., 2002a). The manufacturing protocol was then modified to increase the moisture of the MF standardized cheeses to be comparable with the control cheeses by using a lower setting temperature, increasing the curd size, and low-

Figure 1. Sodium dodecyl sulfate-PAGE electrophoretograms of proteins from skim milk, microfiltered (MF) permeate before diafiltration (DF), and MF and DF composite permeate. Lane 1 = standard markers ranging from 6.9 to 201 kDa; lane 2 = 15 μ L of skim milk; lanes 3 to 6 = MF permeate before DF; lanes 7 to 10 = MF and DF composite permeate. For the MF permeate before DF, the 4 lanes correspond to (left to right) 10, 12, 14, and 16 μ L of sample that was loaded; for the MF and DF composite permeate, the 4 lanes correspond to (left to right) 24, 26, 28, and 30 μ L of sample that was loaded.

ering the wash water temperature during manufacture of the MF cheeses. Because MF cheeses tend to have an increased colloidal calcium content due to a high concentration of CN, the MF cheeses were manufactured by preacidifying the milk to 2 different pH values. Preacidification to remove some of the colloidal calcium content was carried out on milk at 5°C before adjusting the milk to the ripening temperature and inoculating it with starter. Neocleous et al. (2002b) found that Cheddar cheese made with MF standardized milks was harder because the cheeses had lower moisture and higher calcium contents. Differences in hardness in their study were eliminated when the manufacturing protocol was adjusted so that the moisture contents of the control and MF cheeses were similar.

After changes were made to the manufacturing procedure, the moisture contents of the 2 MF cheeses were similar to those of the control cheeses (Table 2). There was no significant difference in the composition of all the cheeses once the moisture content was corrected (Table 2). There was a slightly higher fat in DM value for the pH6.3MF cheeses than for the control or pH6.4MF cheese, but it was not significantly different. The fat in DM values of the cheeses were within the typical range of values expected for this stirred-curd pizza cheese (0.43 to 0.44). There was very little residual lactose ($\sim$ 0.01%) in any of the cheeses after 1 mo because of the wash step and the rapid fermentation

of residual lactose by the mesophilic cultures. The lactic acid levels (at 1 mo) were similar in all cheeses (1.22%).

When MF was used to increase the CN content of milk, the cheeses had a lower moisture content than the control cheeses. Cheese making can be considered a concentration process in which the CN and fat in milk are concentrated, mainly by the syneresis of curd particles (which is influenced by cutting conditions, heating, stirring, and acid development). However, because of the increased concentration of CN in MF standardized cheese milks, less concentration of CN is required during the syneresis process of cheese making to obtain an equivalent concentration factor for CN. Thus, the extent of syneresis in MF standardized cheese milks must be reduced compared with control cheese milks by altering cheese-making conditions that are known to influence syneresis (e.g., cutting conditions). In the MF standardized cheese milks, a CN concentration factor of $\sim$ 7.8 times was required during cheese making, compared with 10.3 times in the control cheese milks, to obtain cheeses with a similar protein content ($\sim$ 24.5%).

Fat and Nitrogen Recoveries in Cheese

The fat and nitrogen recoveries in cheese are given in Table 3. There was significantly higher fat recovery in the pH6.3MF cheeses than in the control or pH6.4MF cheese, which were similar (Table 3). This was also

Table 3. Fat and nitrogen recoveries for stirred-curd pizza cheeses (n = 4)

Component recovery	Treatment ¹			SEM	P-value
	Control	pH6.4MF	pH6.3MF		
Fat recovery (%)					
Cheese	90.04 ^b	89.82 ^b	91.83 ^a	0.373	<0.05
Drain whey	9.16	8.40	7.66	0.334	NS ²
Press whey	0.149	0.163	0.138	0.020	NS
Wash water	0.710	0.641	0.629	0.087	NS
Total	100.06	99.02	100.26		
Nitrogen recovery (%)					
Cheese	73.75 ^b	76.01 ^a	76.56 ^a	0.461	<0.05
Drain whey	23.48	22.05	22.24	0.553	NS
Press whey	0.406	0.489	0.502	0.023	NS
Wash water	0.842	0.847	0.876	0.024	NS
Total	98.48	99.40	100.18		
Drain whey ³ (% mass)	74.52 ^a	69.80 ^b	70.49 ^b	0.427	<0.001
Press whey ³ (% mass)	1.67 ^b	2.20 ^a	2.31 ^a	0.046	<0.001
Wash water ³ (% mass)	14.24 ^b	17.17 ^a	17.02 ^a	0.178	<0.0001

^{a,b}Means within the same row not sharing a common superscript differ ($P < 0.05$).

¹pH6.4MF = cheese milk acidified to pH 6.4 and standardized with microfiltered (MF) retentates; pH6.3MF = cheese milk acidified to pH 6.3 and standardized with MF retentates.

²Nonsignificant (F -test for full statistical model, $P > 0.05$).

³Amount of drain whey, press whey, and wash water obtained from 100 kg of cheese milk.

reflected in the reduced amounts of fat loss, although it was not statistically significant, in the drain whey derived from pH6.3MF cheeses. Higher fat losses can occur if gels are cut too firmly (which is more likely to occur with very high CN milks) because of tearing or cutting when gels are too soft followed by rapid stirring (Johnston et al., 1991; Johnson et al., 2001). Preacidifying to a lower pH (i.e., 6.3 vs. 6.4) presumably decreases the colloidal calcium phosphate content, which encourages more rearrangements and this increases the rate of increase in G' . This might make the system better able to trap fat by covering the exposed fat globules on cut surfaces. This could account for the slightly higher fat recovered in the pH6.3MF cheeses than in the pH6.4MF cheeses.

The amounts of nitrogen recovered in the pH6.4MF and pH6.3MF cheeses were higher (~3%) than in the control cheeses (Table 3), with both the MF standardized cheeses having similar nitrogen recoveries. In addition, the CN:true protein ratio was higher in the MF standardized milks (Table 2). It is the CN that makes the matrix and that is retained in curd particles, whereas serum proteins are lost in proportion to the amount of whey removed. This higher CN:true protein ratio in MF milk is probably responsible for the observed increase in nitrogen recovery in these cheeses. St-Gelais et al. (1995) and Neocleous et al. (2002a) reported that the CP recovered in cheese increased with an increasing concentration factor for MF retentates used to standardize the milk.

Cheese Yield

Actual and moisture-adjusted yields for both the MF fortified cheeses were significantly ($P < 0.05$) higher than for the control cheeses (Table 4) because of the higher CN and fat contents in the MF standardized milks. Van Slyke cheese yield equations were developed for all 3 cheese types according to the method of Govindasamy-Lucey et al. (2006; Table 4). RC values were calculated for the control, pH6.4MF, and pH6.3MF cheeses and were found to be 0.957, 0.956, and 0.961, respectively. Thus, all calculations (RS values and Van Slyke cheese yield) were carried out by using an average RC value of 0.958. In this study, RF was determined experimentally from the cheese trials and by using the constant value of $RC = 0.958$; RS was calculated as described by Govindasamy-Lucey et al. (2006). The Van Slyke cheese yield formula very closely predicted the experimentally obtained actual cheese yield.

Whey Composition

There was a slight, but not significant, reduction in the amount of total whey (including drain whey, press whey, and wash water) produced when MF standardized milks were used for cheese making compared with control milks (Table 5). Lactose levels were significantly ($P < 0.01$) higher in the whey derived from control milks than from MF standardized milks. This was expected because the lactose contents in the milks standardized with MF retentates were lower than those in the control

Table 4. Actual and calculated cheese yield values for pizza cheese (n = 4)

Item ¹	Treatment ²			SEM	P-value
	Control	pH6.4MF	pH6.3MF		
RF value	0.900	0.898	0.918	—	—
RC value	0.957	0.956	0.961	—	—
RS value	1.110	1.105	1.093	—	—
Actual yield (%)	9.28 ^b	12.16 ^a	12.33 ^a	0.070	<0.0001
Van Slyke cheese yield (%)	9.27 ^b	12.15 ^a	12.32 ^a	0.070	<0.0001
Averaged moisture adjusted yield to 46% moisture (%)	9.21	11.96	12.14	—	—

^{a,b}Means within the same row not sharing a common superscript differ ($P < 0.05$).

¹RF is the fat recovered in cheese, determined experimentally from cheese trials. RC was calculated as described in Govindasamy-Lucey et al. (2006). RS is the recovery of noncasein, nonfat solids in cheese. It was calculated as described in Govindasamy-Lucey et al. (2006). Van Slyke cheese yield was calculated by equation [1], with milk and cheese composition data given in Table 2.

²pH6.4MF = cheese milk acidified to pH 6.4 and standardized with microfiltered (MF) retentates; pH6.3MF = cheese milk acidified to pH 6.3 and standardized with MF retentates.

milks (Table 2). The true protein contents in all the wheys were not statistically different. The whey produced from the MF standardized milks had a significantly lower proportion of lactose in the TS of whey (~69%) compared with the control milks (~72%). True protein levels, as a percentage of TS, were significantly higher in the whey produced from the MF standardized milks (pH6.4MF and pH6.3MF were ~11.8 and 12.9%, respectively) than from the control milks (~9.6%). This is in agreement with previous work in which true protein levels, as a percentage of TS, were higher in the whey produced from milks standardized with UF retentates compared with control milks (Govindasamy-Lucey et al., 2005).

Coagulation Properties

The rheological properties of rennet-induced cheese milk gels are shown in Table 6. Our cheese makers cut the coagula based on firmness, which they evaluated subjectively, rather than based on time. Microfiltered standardized milks gelled faster than control milks,

and the cheese makers cut both the pH6.4MF (21.4 min) and pH6.3MF (16.5 min) gels sooner than the control gels (24.3 min; Table 6). The gelation time, as determined by the rheometer, was significantly ($P < 0.01$) longer in the control milk than in the pH6.4MF or pH6.3MF milks (Table 6). The gelation time was significantly shorter in the MF standardized milks, presumably because of the higher CN content; because rennet was added on a CN basis, gelation occurred faster, in agreement with the results of Creamer et al. (1987). The use of a lower preacidification pH (6.3) resulted in significantly shorter gelation and cutting times, probably because of the increased rennet activity and reduced electrostatic repulsion between renneted micelles at the lower pH value. Even though there was no significant difference in G' values at cutting between the control and MF standardized milk, our cheese makers cut the cheeses made from MF standardized milk when they were slightly softer (as reflected by the slightly lower G') because of their concerns about the rapid rate of gel firming in milk with a higher solids content (based on their past experience). They believed

Table 5. Mean (n = 4) lactose and true protein composition in whey normalized to concentrations that would be produced from 100 kg of standardized milk

Treatment ¹	Total amounts in cheese whey						
	Total whey ² (kg)	Lactose (kg)	True protein (kg)	TS (kg)	Total fat (kg)	Lactose/TS (%)	True protein/TS (%)
Control	90.43	3.72 ^a	0.495	5.16 ^a	0.202	72.15 ^a	9.60 ^b
pH6.4MF	89.16	3.37 ^b	0.577	4.88 ^b	0.232	69.11 ^b	11.83 ^{ba}
pH6.3MF	89.16	3.43 ^b	0.642	4.99 ^{ba}	0.215	68.76 ^b	12.87 ^a
SEM	0.327	0.023	0.027	0.040	0.009	0.407	0.562
P-value	NS	$P < 0.01$	NS	$P < 0.01$	NS	$P < 0.01$	$P < 0.05$

^{a,b}Means within the same column not sharing a common superscript differ ($P < 0.05$).

¹pH6.4MF = cheese milk acidified to pH 6.4 and standardized with microfiltered (MF) retentates; pH6.3MF = cheese milk acidified to pH 6.3 and standardized with MF retentates.

²Total whey = drain whey + press whey + wash water.

Table 6. Effects of higher solids on the rheological properties of rennet-induced milk gels ($n = 4$)

Parameter	Treatment ¹			SEM	<i>P</i> -value
	Control	pH6.4MF	pH6.3MF		
Gelation time ² (min)	18.90 ^a	17.33 ^b	13.42 ^c	0.351	<0.01
Storage modulus (<i>G'</i>) at cutting ³ (Pa)	5.24	2.87	3.67	1.122	NS
Cutting time ⁴ (min)	24.3 ^a	21.4 ^b	16.5 ^c	0.732	<0.01
Yield stress ⁵ (Pa)	19.79 ^b	39.14 ^a	42.01 ^a	1.811	<0.01
Yield strain ⁵	2.25 ^b	2.63 ^a	2.56 ^a	0.063	<0.05

^{a-c}Means within the same row not sharing a common superscript differ ($P < 0.05$).

¹pH6.4MF = cheese milk acidified to pH 6.4 and standardized with microfiltered (MF) retentates; pH6.3MF = cheese milk acidified to pH 6.3 and standardized with MF retentates.

²Point when the gels had a storage modulus of ≥ 0.1 Pa.

³*G'* value of the gels at the cutting time selected subjectively by our cheese makers.

⁴Cutting time selected subjectively by our cheese makers.

⁵Yield was defined as the point at which the shear stress started to decrease when gels were subjected to a constant shear rate of $\sim 0.01 \text{ s}^{-1}$.

that the rapid rate of gel firming in higher protein milks would make it difficult for them to cut the coagulum if the cutting time were delayed, and this might result in higher fat losses.

The yield stress value (i.e., the force required to break or rupture the gel network) was significantly higher for both of the MF standardized milks than for the control milk (Table 6); the yield stress values were approximately double (~ 40 Pa) those of the control milk (20 Pa). The higher concentration of structure-forming protein in the gel matrix of the MF standardized milks was probably responsible for the higher yield stress. Because of the very slow shearing rate used in this test, some bond formation and rearrangements probably occurred in MF standardized milks because of the rapid rate of gel firming in these samples, which helped to increase the apparent yield stress of these milks. Yield strain values were significantly ($P < 0.01$) lower in gels made from the control milk, reflecting a shorter gel texture than in the milks with a higher solids content. This is in agreement with our previous work (Govindasamy-Lucey et al., 2004, 2005) in which yield strain values were higher in gels made from milks standardized with cold UF retentates. Preacidifying the milk to pH 6.3 had no significant effect on the yield strain or yield stress compared with preacidification to pH 6.4.

pH and Proteolysis

There were no significant ($P > 0.05$) differences in pH values between cheeses at any time point during ripening (Table 7 and Figure 2). The pH values of all 3 types of cheeses slightly decreased with age in the first 4 wk and then slightly increased. The initial pH decrease could be due to the fermentation of any residual lactose. The slight pH increase was due to the solubilization of residual colloidal calcium phosphate and

the formation of phosphate ions, which can combine with H^+ ions, resulting in buffering and an increase in cheese pH (Lucey and Fox, 1993; Lucey et al., 2003). Changes in pH during ripening in many cheeses are due to the interplay between these 2 reactions (Hassan et al., 2004).

The amount of 12% TCA soluble nitrogen formed during ripening was similar for all 3 types of cheeses (Table 7 and Figure 3). The use of milks with higher CN contents or lower preacidification pH values did not result in any significant difference in 12% TCA soluble nitrogen, probably because both the starter:CN and rennet:CN ratios were kept the same for both the control and concentrated milks (Govindasamy-Lucey et al., 2005). In previous studies, when rennet was added on a milk volume basis, increasing the milk protein concentration was shown to result in slower proteolysis in cheeses during ripening (Green et al., 1981; Green, 1985; Spangler et al., 1990; Guinee et al., 1994). In cheeses made from UF concentrated milks, a lower extent of proteolysis was observed, even when the rennet concentration was standardized on a CN basis (Creamer et al., 1987). During milk concentration by UF, the concentration of whey proteins and proteinase-peptidase inhibitors may increase in cheese (Hickey et al., 1983). In MF, whey proteins and inhibitors can permeate the membrane, so their concentration in cheese is presumably lower than in milk concentrated by UF. Therefore, flavor development issues are less likely to occur in MF standardized cheese compared with UF cheese. Lower acidification pH values might have been expected to increase chymosin retention (Holmes et al., 1977), but no differences were observed in 12% TCA soluble nitrogen between samples. As expected, the amount of 12% TCA soluble nitrogen increased with age (Table 7 and Figure 3).

Table 7. Mean squares, probabilities (in parentheses), and R^2 for pH, 12% TCA soluble N (proteolysis), and rheological properties during ripening of pizza cheese at 7°C for 12 wk

Item	pH	Proteolysis (%)	LHP temperature at which loss tangent = 1 ¹	LHP max loss tangent ²	LHP temperature at which loss tangent = max ³	SHP temperature at which loss tangent = 1 ⁴	SHP max loss tangent ⁵	Degree of flow ⁶
Whole plot								
Treatment (T)	0.007 (0.620)	0.074 (0.36)	7.92 (<0.01)	0.223 (0.208)	2.25 (<0.05)	3.22 (0.20)	0.115 (0.49)	3.93 (0.57)
Day of cheese making (D)	0.014 (0.43)	0.547 (<0.05)	11.10 (<0.01)	0.584 (<0.05)	9.41 (<0.01)	7.68 (0.06)	0.715 (<0.05)	29.21 (0.06)
Error (T × D)	0.013	0.060	0.324	0.108	0.262	1.49	0.141	6.40
Subplot								
Age (A)	0.012 (<0.05)	33.33 (<0.0001)	104.74 (<0.0001)	9.54 (<0.0001)	29.34 (<0.0001)	44.43 (<0.0001)	10.99 (<0.0001)	1081.17 (<0.0001)
A × A	—	4.65 (<0.0001)	—	4.82 (<0.0001)	6.89 (<0.01)	13.24 (<0.01)	5.40 (<0.0001)	627.64 (<0.0001)
A × T	0.003 (0.52)	0.027 (0.88)	1.03 (0.31)	0.133 (0.26)	1.28 (0.22)	0.794 (0.49)	0.053 (0.61)	4.79 (0.70)
A × A × T	—	0.022 (0.90)	—	0.090 (0.40)	0.901 (0.34)	0.68 (0.54)	0.041 (0.69)	4.60 (0.71)
Error	0.003	0.223	0.846	0.095	0.819	1.11	0.107	13.21
R^2	0.64	0.97	0.94	0.87	0.87	0.85	0.88	0.80

¹Temperature at which loss tangent value = 1, as measured in the long heating profile (LHP) method.²Maximum loss tangent values, as measured in the LHP method.³Temperature at which loss tangent value is a maximum, as measured in the LHP method.⁴Temperature at which loss tangent value = 1, as measured in the short heating profile (SHP) method.⁵Loss tangent value at temperature = 60°C, as measured in the SHP method.⁶Degree of flow was calculated with the UW-MeltProfiler. **Figure 2.** The pH for control cheese (○), cheese made from micro-filtered (MF) standardized milk adjusted to pH 6.4 (▼), and cheese made from MF standardized milk adjusted to pH 6.3 (□) during 12 wk of ripening of pizza cheeses at 7°C. Vertical bars represent standard deviations.

Rheological Properties

The storage modulus (G') represents the solid-like or elastic character of a viscoelastic material such as cheese. For the LHP, the G' for all cheeses decreased as the temperature was increased (Figure 4A to 4C), in agreement with other studies (Rosenberg et al., 1995; Lucey et al., 2003). As the cheeses aged, the G' values at ≤30°C for the 3 cheese types did not appear to be very different. However, as the temperature was increased from 30 to 80°C, the G' values of the cheeses of various ages became distinctly different (Figures 4A to 4C), with lower G' values observed for older cheeses. Similar trends were also seen for the G' profiles in the control and MF standardized cheeses (Figures 4A to 4C). The

Figure 3. The 12% TCA soluble N as a percentage of total N for control cheese (○), cheese made from microfiltered (MF) standardized milk adjusted to pH 6.4 (▼), and cheese made from MF standardized milk adjusted to pH 6.3 (□) during 12 wk of ripening of pizza cheeses at 7°C. Vertical bars represent standard deviations.

Figure 4. Changes in the G' (A–C) and loss tangent (D–F) as a function of temperature, determined from the long heating profile for the control cheese (A and D), cheese made from microfiltered (MF) standardized milk adjusted to pH 6.4 (B and E), and cheese made from MF standardized milk adjusted to pH 6.3 (C and F) at 1 (●), 2 (▽), 4 (■), 8 (◇), and 12 (▲) wk of ripening of pizza cheeses at 7°C ($n = 4$).

G' values of control cheese in the SHP also decreased as a function of temperature, and older cheeses had lower curves, especially at $\geq 35^{\circ}\text{C}$ (results not shown). The LT values at temperatures $\leq 30^{\circ}\text{C}$ remained constant during ripening (Figure 4D to 4F), with a value of ~ 0.3 , and at $\geq 30^{\circ}\text{C}$ the LT increased to a maximum at ~ 60 to 65°C and then decreased. The LT values in-

creased at $\geq 40^{\circ}\text{C}$ for the SHP and increased with cheese age (results not shown). An increase in LT indicates a change in the character of the cheese from a solid-like to a viscous or liquid-like character. The LT values (at temperatures $> 30^{\circ}\text{C}$) increased with age of the cheese, but the values for cheeses aged for 4, 8, and 12 wk appeared similar (Figure 4D to 4F). Similar profiles for

Figure 5. Changes in the (A) maximum loss tangent, (B) temperature at the maximum loss tangent, and (C) crossover temperature determined from the long heating profile as a function of ripening time for control cheese (○), cheese made from microfiltered (MF) standardized milk adjusted to pH 6.4 (▼), and cheese made from MF standardized milk adjusted to pH 6.3 (□) during 12 wk of ripening of pizza cheeses at 7°C. Vertical bars represent standard deviations.

LT values as a function of temperature were observed for MF cheeses (Figure 4E and 4F). There was no significant difference in the LT_{max} values at each ripening point for any of the cheeses (Table 7 and Figure 5). The LT_{max} value for all cheeses increased as the cheeses aged up to ~4 wk. With longer aging times, the LT peak continued to increase slightly in all cheeses (Figure 5). The age-related increase in LT and decrease in G' at

Figure 6. Changes in the (A) maximum loss tangent and (B) temperature of the crossover point determined from the short heating profile as a function of ripening time for control cheese (○), cheese made from microfiltered (MF) standardized milk adjusted to pH 6.4 (▼), and cheese made from MF standardized milk adjusted to pH 6.3 (□) during 12 wk of ripening of pizza cheeses at 7°C. Vertical bars represent standard deviations.

high temperatures is due to the loss of insoluble calcium from CN particles as well as ongoing proteolysis (Lucey et al., 2003). The value of the LT at higher temperatures has been used as index of meltability (Ustunol et al., 1994; Mounsey and O'Riordan, 1999). A high LT indicates a more liquid-like system, which could then flow and melt.

The temperature at which the LT_{max} occurred was affected by treatment (Table 7). Similar trends were observed for the LT_{max} from the LHP and SHP (Figures 5A and 6A). At each ripening point, the temperature at which the LT_{max} occurred for the pH6.3MF cheeses was slightly lower than for the control or pH6.4MF cheese (Figure 5B). This trend was also observed for the temperature at the crossover point in the LHP (Figure 5C). The crossover point (i.e., when $LT = 1$), which has been used as another indicator of cheese meltability (Sutheerawattanonnda and Bastian, 1998), was similar for the control and MFpH6.4 cheeses but was slightly

lower for the MFpH6.3 cheeses (Table 7 and Figure 5C). The temperature at which the LT_{max} occurred decreased as a function of age (Figure 5C), suggesting that less thermal energy needed to be given to aged cheese to reach the state at which it was most fluid-like (or most meltable). This indicated that the older cheese melted at a lower temperature, probably because of the loss of intact CN (caused by ongoing proteolysis) and the loss of cross-linking material (caused by the shift from insoluble to soluble calcium), both of which occur during cheese ripening (Lucey et al., 2003, 2005). Presumably, the lower temperature of LT_{max} or the crossover point in the pH6.3MF cheese compared with the control or pH6.4MF cheese was due to the lower total calcium per gram of protein (or more likely a lower insoluble calcium) content of the pH6.3MF cheese.

In the SHP, the LT_{max} (i.e., LT value at $\sim 60^{\circ}\text{C}$) for all cheeses increased with cheese age during the first 4 wk and then showed little change or a slight increase for the rest of the ripening period (Figure 6A). In the SHP, the temperature at which the crossover occurred for all cheeses decreased from $\sim 54^{\circ}\text{C}$ in cheeses aged for 1 wk to $\sim 47^{\circ}\text{C}$ in cheeses aged for 12 wk (Figure 6B). There were no significant differences in the crossover temperatures among all the cheese types (Table 7). The use of the SHP allowed a direct comparison with the melting properties from the UW-MeltProfiler (i.e., same heating profile; Hassan, 2001).

UW-MeltProfiler

The meltability of cheese was compared by using the parameter DOF, which was the percentage change in the height of cheese when it was heated to 60°C compared with the original cheese height. The DOF of cheeses manufactured from MF standardized milks were similar to those of the control cheese (Table 7 and Figure 7). The results from the SHP profile (e.g., crossover temperature, LT_{max}) agreed with the melt profile trends, and no significant differences were observed among samples. The DOF of all cheeses increased over the first 4 wk, after which there was little or no further change.

Sensory Attributes

There were no differences in the attributes of bitterness, saltiness, acidity, firmness, off-flavor, and smoothness in the unmelted control and MF standardized cheeses (results not shown). When the control and MF standardized cheeses were shredded, the shred attributes were found to be similar. When the cheeses were baked on pizzas, they did not brown but rather formed yellowish blisters. This is typical for this pizza cheese

Figure 7. Age-related changes in the degree of flow calculated from the UW-MeltProfiler analysis for control pizza cheese ($\circ$), cheese made from microfiltered (MF) standardized milk adjusted to pH 6.4 ($\blacktriangledown$), and cheese made from MF standardized milk adjusted to pH 6.3 ($\square$) during 12 wk of ripening at 7°C . Vertical bars represent standard deviations.

because of the lack of residual sugar as a result of the starter cultures used, the length of fermentation time in the nonpasta filata manufacturing method, and the washing step. Immediately after melting, cheeses were tested for stretchability by lifting a piece of cheese with a fork and pulling the cheese until the strands broke. The strand length for all the cheeses was similar at all time points (results not shown). There were no differences in bitterness, saltiness, acidity, chewiness, off-flavor, and moistness when the control and MF cheeses were baked on pizzas in an Impinger oven (results not shown). Overall, no differences were detected in the functional performance of the control or MF fortified cheeses.

CONCLUSIONS

Polymeric MF membranes and cold-temperature processing can be used to produce retentates with an increased CN: true protein ratio (reduced serum protein content). During the MF and DF steps, a very small amount of β -CN passed through the membrane. In our present study, we used membranes with a pore size of $0.2\ \mu\text{m}$. This is a little larger than the $0.1\text{-}\mu\text{m}$ pore size that is sometimes used for the whey protein-CN split. If membranes of $0.1\text{-}\mu\text{m}$ pore size had been used, even less CN would have passed through the membranes into the permeate stream. To remove the residual CN, the permeate could be warmed to room temperature and then passed through the MF membranes. When this MF and DF stream was used to standardize cheese milks for pizza cheese manufacture, the cheeses were of lower moisture ($\sim 3\%$) than the control cheese, probably

because of the different synergetic properties in these gels. This lower moisture content has previously been observed with the use of MF concentrates for cheese making. It was necessary to adjust the cheese-making procedure for the cheeses manufactured from MF standardized milks to obtain moisture contents similar to those of the cheese made from unconcentrated milk. There were no differences in the chemical composition of cheeses once moisture was increased, although modifications were made to the manufacturing procedure. Fat recoveries were higher in the pH6.3MF cheese than in the control or pH6.4MF cheese. The amounts of nitrogen recovered in the MF fortified cheeses were higher (~3%) than those in the control cheese because there was a higher proportion of CN:true protein in the MF standardized milks. Rennet coagulation occurred sooner in the MF standardized milks because of the higher CN content and preacidification, which increased rennet activity and reduced electrostatic repulsion between renneted micelles. The rate of gel firming was faster for the MF standardized milks. Thus, the cutting process must be closely monitored so that cutting can be initiated when the gel is soft, or it is likely to become excessively firm. Higher fat losses would occur as a result of cutting when the gel is too firm. Gels formed from MF standardized milks had a higher yield stress, indicating greater resistance to the cutting process. Proteolysis in the MF fortified cheeses was similar to that of the control cheese, probably because both starters and rennet were added on a CN basis for both the control milk and milks with higher solids contents. In addition, there was likely to be less retention of whey protein and other proteinase-peptidase inhibitors in MF concentrated milks compared with UF concentrated milks. No significant differences in LT_{max} and DOF were found among the cheeses. Slightly lower temperatures of the LT_{max} and crossover point were observed in the pH6.3MF cheese compared with the control or pH6.4MF cheese. The pH6.3MF cheese melted at a lower temperature, probably owing to the slightly lower calcium content or loss of cross-linking material, because the total calcium content (per g of protein) of this cheese was lower than that of the control or pH6.4MF cheese. No differences were detected in the sensory characteristics between the control and MF fortified cheeses. The use of cold MF retentates for the standardization of milks for pizza cheese manufacture is an option for cheese makers to consider, because there is a significant increase in cheese yield. In addition, some modifications (starter, rennet, and salt, which were added on a CN basis rather than based on the volume of milk in the vat; the coagula from the MF standardized milks being cut sooner; and modifications such as lowered setting temperatures, increasing curd size, and

wash temperatures) were necessary in the manufacturing protocol to make cheeses of the desired composition and quality. The large increase in yield is important in cheeses such as pizza cheeses, which have traditionally been made from part-skim milk (i.e., milks with a low solids level, and therefore low cheese yields). We conclude that standardization of cheese milks with cold milk MF retentates produced using polymeric membranes increased pizza cheese yields without compromising quality or functional attributes.

ACKNOWLEDGMENTS

The authors would like to thank the following former and current Wisconsin Center for Dairy Research and University of Wisconsin Dairy Plant (Madison, WI) personnel for their assistance and support in cheese making and in analytical work: Bill Hoesly, Brian Leitzke, Lorraine Heins, Bill Tricomi, Cindy Martinelli, Amy Bostley, Kristen Houck, Cathy Landers, Juan Romero, Gene Barmore, Kate Lim, Karen Smith, Ray Michaels, Ken Norton, Gina Mode, and Bill Klein. We also thank Robert Fassbender from T. C. Jacoby (St. Louis, MO), and personnel from Membrane Systems Specialists (Wisconsin Rapids, WI) for all their assistance in setting up the MF units. We also wish to thank Jongwoo Choi for his help with the statistical analysis of the data and Dan Zhu for carrying out SDS-PAGE. We also thank Chr. Hansen Inc. (Milwaukee, WI) and Danisco USA Inc. (Madison, WI) for their donation of the starter cultures and coagulants used in this study. The financial support of the Wisconsin Center for Dairy Research, Center Industry Team; Wisconsin Milk Marketing Board (Madison, WI); and Dairy Management Inc. (Rosemont, IL) is greatly appreciated.

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