Vatless Manufacturing of Low-Moisture Part-Skim Mozzarella Cheese from Highly Concentrated Skim Milk Microfiltration Retentates*

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ABSTRACT

Low-moisture, part-skim (LMPS) Mozzarella cheeses were made from concentration factor (CF) 6, 7, 8, and 9, pH 6.0 skim milk microfiltration (MF) retentates using a vatless cheese-making process. The compositional and proteolytic effects of cheese made from 4 CF retentates were evaluated as well as their functional properties (meltability and stretchability). Pasteurized skim milk was microfiltered using a 0.1- μ m ceramic membrane at 50°C to a retentate CF of 6, 7, 8, and 9. An appropriate amount of cream was added to achieve a constant casein:fat ratio in the 4 cheesemilks. The ratio of rennet to casein was also kept constant in the 4 cheesemilks. The compositional characteristics of the cheeses made from MF retentates did not vary with retentate CF and were within the legal range for LMPS Mozzarella cheese. The observed reduction in whey drained was greater than 90% in the cheese making from the 4 CF retentates studied. The development of proteolytic and functional characteristics was slower in the MF cheeses than in the commercial samples that were used for comparison due to the absence of starter culture, the lower level of rennet used, and the inhibition of cheese proteolysis due to the inhibitory effect of residual whey proteins retained in the MF retentates, particularly high molecular weight fractions.

(**Key words:** Mozzarella cheese, microfiltration, concentration factor, proteolysis)

Abbreviation key: CF = concentration factor, CM = commercial Mozzarella, GDL = glucono- δ -lactone, LMPS = low-moisture, part-skim, MF = microfiltration, MFM = microfiltration cheesemilk, MFR = mi-

crofiltration retentate, SN = soluble nitrogen, WP = whey proteins.

INTRODUCTION

The introduction of UF led to new developments in cheese manufacturing processes from UF retentates. The aim was to eliminate whey disposal problems by retaining whey proteins (WP), which resulted in increased cheese yield with increasing concentration factor (CF) (Maubois and Mocquot, 1975; Green et al., 1981; Iyer and Lelievre, 1987). However, the inclusion of WP was found to have negative effects on the development of proteolysis in Cheddar cheese, either by dilution of the casein substrate or direct inhibition of proteolytic enzymes (Creamer et al., 1987). Furthermore, WP in their native state in UF cheeses have shown resistance to proteases and peptidases in Cheddar cheese (de Koning et al., 1981) and in native and denatured states in cheese slurries (Harper et al., 1989). Other common defects observed in these cheeses included increased hardness in Cheddar cheese (Green et al., 1981) and reduced melting and stretching properties in Mozzarella (Lawrence, 1989), which made attempts to commercialize such cheeses between 1980 to 1989 unsuccessful (Horton, 1997). However, the use of ultrafiltration of milk prior to cheese manufacture found applications in cheese that are consumed fresh such as Quarg, Ricotta, cream, and goat milk cheeses (Lelievre and Lawrence, 1988), as well as some soft ripened cheese varieties such as Feta (Lawrence, 1989) and Camembert (Maubois and Mocquot, 1975; Lelievre and Lawrence, 1988).

Microfiltration (MF) can be used to selectively remove WP along with salts and lactose. The basis for separation in small pore size MF is that WP are small molecules compared with casein micelles (Walstra and Jenness, 1984) and can therefore be separated using a 0.1- to 0.2- μ m pore size membranes. The separation produces a casein-enriched retentate and a permeate containing WP that is free of caseins. Microfiltration produces a permeate stream that is invariant with the

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type of cheese subsequently made using the retentate and is free of cheese fines, fat, salt, rennet, starter culture, color, and glycomacropeptide. Most of the downstream costs of whey processing could be saved, since the purification unit operations are eliminated. The MF permeate thus produces a virgin whey protein stream that can be converted into virgin whey protein concentrates and isolates, with improved functionality over currently available cheese whey products.

The development of continuous and semicontinuous cheese making processes has been proposed and practiced by several authors. The Cascade system (Olson, 1975) consists of a vertical vat divided into 3 compartments for coagulation, cutting, and cooking. This system allows the process to flow continuously after the separation of whey and curd. The development of continuous mixing and molding systems has been the main focus in pasta-filata cheeses, whereby the curd is softened in hot water or steam and stretched in rotating screw devices. Another method was developed based on the cold milk renneting concept and continuous curd formation proposed by Berridge (1942). In this process, rennet and starter culture are added to cold milk. After a few hours, the milk is continuously heated to 46 to 48°C and the resulting curd follows a continuous draining and cooking process (Berridge, 1972). Maubois and Mocquot (1975) suggested a continuous process that used automated UF, renneting, molding, and salting steps for various types of cheeses if the appropriate equipment is designed. Another proposed concept based on this approach is recirculating UF retentates through enzyme immobilization in fixed supports (Kosikowski, 1975) for coagulation and flavor development.

Limited research has been conducted on the manufacture of cheeses from microfiltration retentate (MFR). Cheddar cheese made from low CF (1 to 2 times) microfiltered milk (St-Gelais et al., 1995; Neocleous et al., 2002a,b) reported slower proteolysis in the cheeses when compared with a control due to low moisture in the nonfat substance, low residual chymosin in the cheese, and the retention of high molecular weight whey proteins such as α_2 -macroglobulin that may inhibit chymosin activity. Most of the differences in composition, proteolysis, and hardness between control and MF cheeses were eliminated by increasing the quantity of rennet added and by standardizing the curd cooking time (Neocleous et al., 2002a). A study (Brandsma and Rizvi, 2001a) conducted on low-moisture, part-skim (LMPS) Mozzarella cheese from CF 8 to 9 MFR depleted of whey proteins and calcium demonstrated that MF cheeses can be produced with composition similar to that of commercial Mozzarella (CM) samples. Microfiltration Mozzarella exhibited substantial textural and functional development between 30 to 60 d of age as

opposed to CM cheese that experienced these changes between 7 to 30 d of age. The use of starter culture in the MF cheese resulted in improved rheological and functional properties.

The pH at which the MF is conducted affects the partition of Ca between the retentate and the permeate streams and thus the final cheese quality. Brandsma and Rizvi (1999) demonstrated that by lowering the pH of the skim milk to 6.0 with glucono- δ -lactone (GDL) during MF, Ca levels were depleted by 35 to 40% in the retentates, achieving a Ca:casein ratio within the range of commercial Mozzarella cheese samples. Glucono- δ -lactone was also used to acidify the cheesemilk to attain the desired pH for setting the curd. Mistry and Kosikowski (1986) demonstrated that acidification of UF retentate via use of lactic starter cultures was hindered by strong buffering capacity. Therefore, to eliminate this variable, a chemical acidulant can be used to provide more consistent and reliable acid development.

The objective of this study was to develop a continuous manufacturing process for LMPS Mozzarella cheese from CF 6 to 9 skim milk MFR and evaluate the yield, chemical composition, component recoveries, proteolysis, and functional properties of the final product.

MATERIALS AND METHODS

Vatless Cheese Making from Skim Milk MFR

The protocol and schematic of a vatless cheese making process are depicted in Figures 1 and 2. The major steps are explained in detail below.

Feed. A quantity of 1611 kg of high-temperature, short-time pasteurized skim milk was obtained from the Cornell Dairy Plant and held overnight at 4°C. Glucono- δ -lactone (Glucona America, Janesville, WI) was added to skim milk at 4°C 1 h prior to MF. The rate of addition was selected at 1.6 g of GDL/kg skim milk based on prior work by Brandsma and Rizvi (2001b). Glucono- δ -lactone gradually acidifies the retentate, rendering a final pH of 6.0, which reduces the calcium:casein ratio from 4.4 in skim milk to 3.0 in the MFR.

Microfiltration system and operational conditions. A Megaloop continuous MF system with uniform transmembrane pressure capability was used. The system was equipped with 38 ceramic membrane elements (0.1- μ m nominal pore diameter, 1020 mm in length) with a total surface area of 9.2 m². The dead volume of the system was 116 and 30 L for the retentate and the permeate sides, respectively. Prior to the addition of skim milk to the system, 130 L of water, purified by reverse osmosis, was circulated until a temperature of 50°C was achieved and the retentate and permeate pressures were stabilized. Warm skim milk was then added to the system, the temperature was maintained

Figure 1. Vatless manufacturing process for low-moisture, part-skim Mozzarella cheese from milk microfiltration retentates concentration factors 6 to 9 times. GDL = Glucono- δ -lactone.

Figure 2. Diagram of vatless manufacturing process for low-moisture, part-skim Mozzarella cheese from milk microfiltration retentates concentration factors 6 to 9 times. *Images from Dairy Processing, Alfa Laval/Tetra Pak, 1995.

at 50°C, and the retentate and permeate inlet pressures regulated to 372.3 and 96.5 kPa, respectively, while outlet pressures were set to 282.7 and 6.9 kPa, respectively, for a constant axial pressure differential (ΔP) between the retentate inlet and outlet of 275.8 kPa. These conditions allowed a uniform transmembrane pressure of 89.63 kPa. Retentate crossflow velocity was kept at 4.9 m/s. During the concentration process, permeate flux, retentate pH, retentate temperature, and retentate and permeate inlet and outlet pressures were monitored every 10 min. Flux was determined by measuring the weight of the permeate every 10 min.

Microfiltered cheesemilk (MFM). The skim milk was concentrated to a retentate of CF 6, and 20 kg was collected and mixed with pasteurized cream (approximately 40% fat) at 4°C to standardize the casein:fat ratio to 1.1. The resulting MFM was cooled to 36°C. This casein:fat ratio was kept constant in the 4 treatments. The same procedure was repeated for CF 7 and 8. When the skim milk retentate reached CF 9, the system was shut down, and 20 kg of retentate was collected, cream was added, and the MFM was treated the same as the previous 3 CF.

Vatless coagulation. A stirred curd, no brine cheese making method (Barbano et al., 1994) was used to make LMPS Mozzarella cheese. Following the MF process and the standardization with cream, each MFM was tempered to 36°C prior to the coagulation process. The order of cheese making was randomized, with the 4 CF MFM made on the same day. To attain a final cheese pH of 5.3, GDL (1.7% wt/wt) was added to the MFM prior to renneting, and this addition level was kept constant for the 4 CF. Rennet usage level was set 80 μ L/kg of cheesemilk according to previous work by Brandsma and Rizvi (2001b) resulting in a rennet:casein ratio of 0.596 μ L of rennet/g of casein. Single strength rennet (Chymax, Chrs. Hansen, Milwaukee, WI) was diluted 1:40 in deionized water and added to the MFM that was immediately weighed and transferred into an Alcurd continuous cheese coagulator (Alfa-Laval). The setting tube tank of the unit was previously set at 36°C with tap water. All the MFM remaining in the balance tank and in the lines was collected to determine the net amount of MFM that was let into the setting tube. Based on previous work (Brandsma and Rizvi, 2001b), the cutting time chosen for each of the 4 MFM was 35 min from the time of rennet addition, which allows for sufficient hydrolysis of κ -casein to occur.

Cutting, whey draining, and cooking. The coagulum was cut by setting the cutting mill to 10 rpm, which produced 1.2-cm cubes. Curds were allowed to heal for 5 min and then transferred into a vat and heated to 38°C with gentle agitation until the pH of the whey reached 5.8. At this point the whey was drained and the temperature of the curd maintained at 38°C until it reached a pH of 5.5 at which point it was ready for salting. Salt was added at a rate of 2.2% wt/wt of the curd in 3 additions at 5 min intervals for a total period of 20 min. The curd reached a pH of 5.3 by the end of the salting and was ready for stretching.

Cooking, stretching, and packaging. The salted curd was continuously stretched using a twin-screw, pilot scale Mozzarella mixer (model 640, Stainless Steel Fabricating Co., Columbus, WI), with a 6% wt/wt circulating salt brine at 57°C, cooled in ice water and vacuum

packed (MultiVac model 160, Koch Ind., Kansas City, MO) in plastic bags (Cryovac, Duncan, SC) and stored at 4°C.

Sampling. Pasteurized skim milk was sampled at 4°C from the holding tank immediately prior to MF. Cream was sampled the day prior to the MF process, and the analysis of fat was conducted on the fresh sample. Microfiltration retentates were sampled at 50°C and diluted with UF permeate at 50°C in a proportion that would render the protein content in the normal range of conventional skim milk. The samples were immediately cooled in ice water, frozen in liquid nitrogen after the MF process, and stored at -40°C until chemical analyses were conducted.

Each MFM was sampled at 36°C prior to GDL addition. As explained above, the sample was diluted with UF permeate. Whey samples were taken after all the whey and salt whey were collected at 36°C in a single container, mixed, and cooled in ice water. The same procedure was applied to stretchwaters but at 57°C. Samples were frozen in liquid nitrogen immediately after cheese making and stored at -40°C until chemical analyses were conducted. Cheeses were sampled on d 1 by cutting a 2-cm thick slice from the center of the block, ground, and stored at -40°C.

Compositional Analyses

Liquid samples. All the liquid samples were analyzed in duplicate for composition with the exception of total solids, which were done in quadruplicate. Total solids were determined using forced oven air drying for 4 h at 100°C (AOAC, 2000). Total N and nonprotein N were determined by Kjeldahl (AOAC, 2000) as well as noncasein N (IDF, 1964). The N conversion factor used was 6.38. Fat was determined by Mojonnier ether extraction (AOAC, 2000). For the diluted samples, the same analyses were conducted on the UF stream used as a diluent. Ash was determined by placing the sample dish in a muffle furnace oven for 20 h at 550°C. Calcium was determined using atomic absorption spectroscopy (Metzger et al., 2000). The salt content of the whey was determined in duplicate using the Volhard procedure (Marshall, 1992).

Cheese composition. Moisture was determined in quadruplicate by forced oven air drying for 24 h at 100°C (AOAC, 2000). Total N, fat, and salt were determined in duplicate by the Kjeldahl, Babcock, and Volhard methods (AOAC, 2000), respectively. Calcium was determined using atomic absorption spectroscopy (Metzger et al., 2000). The WP content was calculated by mass balances, because the WP content was determined for MFM, whey, and stretchwater. This determination was conducted previously by Iyer and Lelievre (1987).

It was considered that 60% of the nitrogen in the whey and stretchwater was glycomacropeptide from additional determinations (data not shown). The following formula was used to determine the WP content in cheese: WP in cheese = WP in MFM - $0.4 \times (\text{WP in whey} + \text{WP in stretchwater})$. The percentage of WP in cheese was obtained by dividing the amount of WP in the cheese by the weight of the cheese. Casein was determined by subtracting the WP from the total protein in cheese.

Cheese proteolysis. pH 4.6 soluble N, 12% TCA soluble N (Bynum and Barbano, 1985) methods were selected to measure cheese proteolysis at 1, 7, 30, and 60 d of age. The soluble N as a percentage of total N was reported for both methods. Proteolysis data for CM from Brandsma and Rizvi (2001b) were used to compare it to the MF cheeses. The samples studied were obtained from Great Lakes Cheese Co. (Cuba, NY).

Functional Tests

Meltability. Cheese meltability was determined using a modified Schreiber test following the procedure described by Brandsma and Rizvi (2001a) at 7, 14, 30, 45, and 60 d of age. For each cheese, 4 cylindrical disks (10 mm height $\times$ 36.7 mm diameter) were cut and placed in Petri dishes, tempered to 20°C, heated in an oven at 100°C for 7 min, and subsequently cooled at room temperature for 30 min. Two diameter measurements were taken per sample, and the average of the 4 samples was recorded and used to compute the ratio of melted to unmelted disk diameters squared (D_m/D_{um})². Data for CM meltability from Brandsma and Rizvi (2001b) were used to compare it to the MF cheeses.

Stretchability. Cheese stretchability was measured in triplicate by a modified Ring-and-Ball Method (Hicasmaz et al., 2004). The vertical length at which all the strands broke was reported as cheese stretchability. Data for CM stretchability from Juneja (2002) were used to compare it to MF cheese stretchability.

Statistical Analyses

Composition of skim milk, MFR, MFM, and cheeses were compared against CF by ANOVA at a significance level of $P = 0.05$. The ANOVA for changes in proteolysis (pH 4.6 and 12% TCA soluble nitrogen) as well as for functional properties (meltability and stretchability) was done using a split-plot design (see Table 6). For the whole plot factor, CF was analyzed as class variable. For the subplot factor, age and the quadratic form of age (age $\times$ age) were analyzed as continuous variables. The interaction term of CF $\times$ replicate was used as the error term for the treatment effect. Differences in the

means among treatments were determined by comparisons using least-squares means. Cheese age was transformed as follows: age = day of storage - [(last testing day - first testing day)/2]. The transformation made the data set orthogonal with respect to age. The statistical analyses were performed using the PROC GLM procedure of SAS.

RESULTS AND DISCUSSION

Retentate Composition

Mean composition values of retentates for each CF are shown in Table 1. The concentrations of all components increased ($P < 0.01$) when compared with normal skim milk, with the exception of lactose and NPN, which are fully permeable to MF and UF membranes.

The percentage of casein of total protein, WP content, and WP depletion values for the MFR are shown in Table 1. The casein as a percentage of true protein increased with increasing CF because more WP were removed from the MFR. Whey proteins are small molecules compared with caseins and pass through the 0.1- μm pore size. However, the fact that the WP concentration increased with retentate CF ($P < 0.01$) could indicate that high molecular weight WP may be retained by the membrane (Jost et al., 1999; Neocleous et al., 2002a), whereas smaller molecular weight WP proteins are not rejected by the membrane. Mass balances indicate that WP depletion values increased with increasing CF ($P < 0.01$) and ranged from 66.97% in the CF 6 MFR to 71.72% in the CF 9 MFR. The remnant WP constituted 6 to 7% of the true protein content in the MFR. Residual WP have implications for increased cheese yields, but their substantial reduction should allow the production of Mozzarella cheese from MFR with better meltability than the one manufactured from UF retentates, since their presence has been associated with reduced proteolysis and impaired melting properties (Madsen and Qvist, 1998).

The use of GDL was effective in attaining the desired final pH level (6.0) in the MFR in a controlled manner without inducing localized coagulation as reported previously by Brandsma and Rizvi (1999). The pH of the retentates decreased with CF ($P < 0.05$) for the 6-, 7-, and 8-times retentates, where it reached a value of 6.01, which remained constant when the 9-times CF was reached. The pH values for the MFR of CF 6, 7, 8, and 9 were 6.16 ± 0.03 , 6.07 ± 0.03 , 6.01 ± 0.02 , and 6.01 ± 0.01 , respectively. The pH attained in the MFR of CF 8 and 9 was significantly different from the other 2 CF retentates, since additional time is required for the hydrolysis of GDL into the amount gluconic acid that produces a pH of 6.0.

Table 1. Mean composition of microfiltration retentates and microfiltration cheesemilks (n = 2).

Component (%)	Microfiltration retentate					Microfiltration retentate + cream								
	Concentration factor					Concentration factor								
	Skim milk	6×	7×	8×	9×	SEM	P value ⁴	Cream	6×	7×	8×	9×	SEM	P value ⁴
Total solids	9.19	22.84	25.09	27.34	30.27	0.128	<0.01	45.73	29.34	30.88	32.14	33.94	0.118	<0.01
Crude protein	3.24	16.81	19.34	21.53	24.53	0.014	<0.01	1.75	13.47	14.27	14.97	15.95	0.022	<0.01
True protein	3.05	15.58	19.18	21.33	24.34	0.218	<0.01	1.64	13.28	14.12	14.84	15.82	0.215	<0.01
Casein ¹	2.56	15.40	17.84	19.96	22.87	0.126	<0.01	1.46	12.37	13.14	13.81	14.74	0.096	<0.01
Casein, % of CP	78.93	91.61	92.24	92.71	93.23	0.012	<0.01	83.76	91.80	92.07	92.25	92.41	0.029	<0.01
Whey protein ¹	0.60	1.18	1.34	1.37	1.47	0.022	<0.01	0.17	0.91	0.98	1.04	1.11	0.023	<0.01
Whey protein depletion (%)	0.00	66.97	68.32	69.93	71.72	0.371	<0.01	...	...	...	...	...	...	...
Fat	0.05	0.30	0.34	0.39	0.44	0.011	<0.01	38.60	11.10	11.82	12.38	13.24	0.103	<0.01
Lactose ³	5.17	3.96	3.43	3.27	3.04	0.032	<0.01	...	3.28	3.19	3.11	3.03	0.006	<0.01
Ash	0.73	1.772	1.982	2.148	2.263	0.023	<0.01	...	1.492	1.606	1.676	1.718	0.003	<0.01
Calcium	0.11	0.58	0.62	0.65	0.71	0.004	<0.01	0.05	0.42	0.44	0.45	0.48	0.002	<0.01
Calcium depletion (%)	0.00	14.94	15.25	15.60	16.01	0.011	<0.01	...	...	...	...	...	...	...
Calcium protein ²	3.51	3.45	3.21	3.02	2.89	0.009	<0.01	3.12	3.16 ^a	3.12 ^b	3.03 ^c	3.03 ^c	0.009	<0.01
Calcium casein ²	4.44	3.77	3.48	3.26	3.10	0.026	<0.01	3.49	3.40 ^a	3.36 ^b	3.26 ^b	3.26 ^b	0.023	0.016

¹N × 6.38.²Multiplied by 100.³Calculated by difference.⁴Means within the same row with statistically significant probabilities ($P < 0.05$) are different.

As the pH of the retentates decreased with increasing CF, more bound calcium became soluble and passed through the membrane. As the calcium was reduced and the casein content increased, both the final Ca:protein and Ca:casein ratios in the MFR decreased with increasing CF ($P < 0.01$). The value of the calcium:casein ratio at CF 6 was 3.77, and it decreased to 3.10 for CF 9. A normalized Ca:casein ratio is essential to produce Mozzarella cheese with good melt and stretch properties (Lawrence, 1989). From the Ca depletion values shown in Table 1, the percentage of depletion was observed to increase with increasing CF ($P < 0.01$) and ranged from 14.94% in the CF 6 MFR to 16.01% in the CF 9 retentate.

MF Cheesemilk (MFM) Composition

The composition for the MFM obtained from the MFR after the addition of cream (40% fat) at 4 different CF is also shown in Table 1. The same trends are observed for the MFM as for the MFR in terms of compositional values. Total solids, crude protein, true protein, casein content fat, and calcium content increased with increasing CF ($P < 0.01$). Casein as a percentage of true protein increased with increasing CF as well as total WP. However, the ratio of WP to casein remained constant in the 4 MFM due to the addition of cream, which contains casein and WP in a ratio similar compared with skim milk. Lactose decreased with CF, and NPN remained relatively constant for the 4 treatments. The casein:fat ratio was the same for all CF, it was adjusted to 1.11 with the addition of cream, and it is in the range of conventional cheesemilk. The Ca:casein ratio and Ca:protein ratio in MF cheesemilk were lowered by the reduction of pH during the MF process. After the standardization with cream, the Ca:casein ratio in the MFM of concentration factor 6 was statistically different from the ratio in the other 3 CF cheesemilks. The addition of cream dilutes the calcium and the casein contents and the differences in Ca:casein ratios observed in the MFR are no longer evident in the MFM. The difference in the Ca:casein ratio between the CF 6 retentate and the other 3 CF was statistically significant due to the difference in MFR pH at this CF. Furthermore, the addition of cream did not change the ratio in its corresponding MFM to a point of no statistical difference compared with the other 3 MFM.

Vatless Cheese Manufacture

The cutting time chosen for each of the 4 MFM was 35 min based on previous work (Brandsma and Rizvi, 2001b), which allows for sufficient hydrolysis of κ -casein. The time chosen for the 4 MFM was kept constant

Table 2. Microfiltration low-moisture, part-skim Mozzarella cheese percentage mass balances (n = 2).

	Concentration factor				SEM	P value ⁴	CM ¹
	6×	7×	8×	9×			
Cheesemilk	100.00	100.00	100.00	100.00	...	...	100.00
Salt	1.52	1.60	1.65	1.71	...	...	0.28
Whey ²	28.35	25.91	24.64	22.55	0.05	<0.01	87.43
Salt whey	17.99	15.02	12.71	10.25	0.04	<0.01	2.79
Total whey	46.34	40.93	37.35	32.80	0.04	<0.01	90.22
Cheese yield ³	55.20	60.69	63.35	67.21	0.12	<0.01	10.06
Theoretical cheesemilk equivalent (2.4% casein)	515.42	547.50	575.42	614.17	...	...	...
Theoretical cheese whey	463.57	492.42	517.53	552.38	...	...	...
Reduction in whey (%)	90.01	91.68	92.78	94.06	0.12	<0.01	...

¹CM = Commercial Mozzarella cheese.²Whey drained prior to salting.³Based on curd weight prior to stretching.⁴Means within the same row with statistically significant probabilities ($P < 0.05$) are different.

because the rennet:casein ratio was kept constant for all 4 CF at 0.596 $\mu\text{L/g}$ of casein.

The use of GDL allowed the reduction of pH from 6.2 in the MFM in the beginning to 6.0 at cutting and 5.8 at whey draw. The gradual hydrolysis of GDL into gluconic acid attained a curd pH of 5.5 for salting only 20 min after cutting the coagulum and 5.3 before stretching in an additional 20-min period. Cooking and salting times were kept constant to standardize the moisture content in the curd in the 4 CF treatments, yielding a total cheese making time of 75 min from rennet addition to the end of salting for the 4 treatments.

The mass balances on the cheese made at each concentration factor are shown in Table 2. The MFM used in each treatment was normalized to 20 kg for comparison purposes. The quantity and percentage of whey drained decreased with increased CF of the MFM ($P < 0.01$). Drainage values were 46.35% for CF 6 MFM, 40.93% from CF 7, 37.35% from CF 8, and 32.80% from CF 9.

The theoretical cheese whey drainage values in Table 2 were calculated to compare the reduction in whey drainage from the concentrated MFM with conventional LMPS Mozzarella cheese production. It was assumed that for every 100 kg of cheesemilk used, 90.2 kg of whey would have been drained in the conventional process and this was called the theoretical cheese whey. The percentage reduction in whey was calculated by estimating the quantity of whey minimized by using the MFM at each CF. The reduction in whey values increased with increasing CF ($P < 0.01$) and were greater than 90% for the 4 treatments studied. The whey reduction is due to its removal as permeate in the MF process.

Cheese Composition

Composition of cheeses made from 4 concentration factor MFM is shown in Table 3 and met US legal standards of identity for LMPS Mozzarella (FDA, 2004). These were also within the compositional range of commercial LMPS Mozzarella cheeses.

Statistical analysis of cheese composition showed no significant differences in fat, protein, and moisture contents among the 4 CF cheeses. Although protein content and protein on a dry basis decreased with cheese CF, the differences were not significant. This decrease may be attributed to a slight increasing trend in the moisture content with increasing cheese CF. Whereas casein followed the same trend as the total protein, the WP content increased with cheese CF, but the differences were not significant. Moisture content and moisture in nonfat substance in MF cheeses tended to increase with increasing cheese CF, but this increase was not statistically significant. Salt contents and salt in moisture values did not vary for the 4 CF studied. In general the salt contents were lower than the commercial Mozzarella samples (Table 4).

Despite the fact that the MFM of concentration factor 6 had a different Ca content, there were no significant differences in Ca content in the final cheeses. The mean values were slightly lower than the range of literature values (0.65 to 0.85 wt %) reported for LMPS Mozzarella (Barbano et al., 1994) and lower than the commercial sample analyzed.

The Ca content and the Ca:casein and Ca:protein ratios were lower than the values for commercial sample used for comparison purposes due to the addition of GDL to the skim milk in the MF process. It was found, however, that the ratios were within the normal values for LMPS Mozzarella cheese reported in the lit-

Table 3. Mean microfiltration low-moisture, part-skim Mozzarella cheese composition (n = 2).

Component (%)	Concentration factor				SEM	P value	CM ⁶	Code of federal regulations ⁷
	6×	7×	8×	9×				
Fat	20.17	20.05	20.15	20.24	0.297	0.78	21.43	14.4–24.7
FDB ¹	39.31	39.20	39.44	39.67	1.130	0.84	41.15	30–45
Protein	25.03	24.59	24.31	24.12	0.743	0.31	25.36	...
PDB ²	48.78	48.07	47.58	47.28	1.290	0.30	48.69	...
Casein ³	23.52	23.09	22.82	22.66	0.749	0.33	25.06	...
Whey protein ³	1.51	1.49	1.49	1.46	0.079	0.10	0.30	...
Moisture	48.69	48.85	48.91	48.99	0.581	0.89	47.92	45–52
MNFS ⁴	60.99	61.10	61.25	61.42	0.650	0.91	60.99	...
Salt	1.14	1.70	1.60	1.56	0.215	0.19	1.58	...
Salt:moisture ⁵	2.338	3.477	3.275	3.181	0.453	0.20	3.297	...
Calcium	0.635	0.611	0.634	0.611	0.040	0.87	0.79	...
Calcium:casein ⁵	2.70	2.64	2.78	2.70	0.100	0.59	3.15	...
pH day 1	5.26	5.29	5.25	5.28	0.039	0.73	5.18	...

¹FDB = Fat on a dry basis.²PDB = Protein on a dry basis [(Protein/100 – moisture)].³Determined by mass balances.⁴MNFS = Moisture in nonfat substance.⁵Multiplied by 100.⁶CM = Commercial Mozzarella (Brandsma and Rizvi, 2001b).⁷CFR 133. 155–158, 4-1-2004, US Dept. of Health and Human Services, Food and Drug Administration, Washington, DC.

erature. Neocleous et al. (2002b) and St-Gelais et al. (1995) showed the same reduction in the Ca:casein for Cheddar cheese made from MF even without a preacidification step.

Composition of Whey and Stretchwater

Table 4 shows the mean compositional values for whey and stretchwater obtained from each concentration factor MFM.

Whey. Significant differences were observed in total solids, WP, fat, and salt contents in the whey ($P < 0.01$), which tend to increase with increasing whey CF. Total protein, true protein, and casein did not show statistical differences, although the protein content is lower in the

CF 9 whey when compared with the other 3 CF. This difference is reversed in the stretchwater, where an increase in the same components ($P < 0.01$) is observed in the CF 9. These results suggest that as the CF increased, these components are retained to a higher extent by the curd during whey draining and then released in the stretchwater (Table 4).

No significant differences were found in Ca content among the treatments. It was found that salt content was variable, significantly different, and increased with CF. The salt content of the CF 7, 8, and 9 whey is higher than CF 6, and the difference may be due to differences in the amount of salt added to the curd. The salt contents are in agreement with the fact that a higher CF MFM will produce a greater quantity of curd, and since

Table 4. Mean composition of whey and stretchwater (n = 2).

Component (%)	Whey					Stretchwater				
	Concentration factor				SEM	Concentration factor				SEM
	6×	7×	8×	9×		6×	7×	8×	9×	
Total solids	10.95 ^a	11.04 ^a	11.04 ^a	11.50 ^b	0.070	5.57 ^a	6.11 ^b	6.31 ^{b,c}	6.66 ^c	0.115
Total protein ¹	1.82	2.05	2.13	2.04	0.124	0.08 ^a	0.15 ^b	0.14 ^b	0.27 ^c	0.015
True protein ¹	1.43	1.62	1.68	1.61	0.089	0.05 ^a	0.01 ^a	0.11 ^a	0.20 ^b	0.017
Casein ¹	0.17	0.19	0.17	0.14	0.045	0.02	0.04	0.04	0.04	0.020
Whey protein ¹	1.26 ^a	1.43 ^b	1.51 ^b	1.47 ^b	0.063	0.04 ^a	0.06 ^a	0.07 ^a	0.15 ^b	0.016
Fat	1.65 ^a	1.42 ^b	1.80 ^a	1.56 ^b	0.076	0.20 ^a	0.24 ^a	0.38 ^b	0.65 ^c	0.030
Calcium	0.1110	0.1113	0.1178	0.1151	0.005	0.0108 ^a	0.0139 ^b	0.0147 ^b	0.0269 ^c	0.001
Salt	0.45 ^a	0.79 ^b	0.54 ^b	0.68 ^b	0.086	4.71 ^a	5.06 ^b	5.18 ^b	4.62 ^a	0.053

^{a,b,c,d}Means within same row not sharing common superscripts are different ($P < 0.05$).¹N × 6.38.

Table 5. Mean ($n = 2$) true protein, casein, whey protein, fat, and calcium recoveries in microfiltration low-moisture, part-skim Mozzarella cheese, whey, and stretchwater.

Component	CF				SEM	P value	CM
	6×	7×	8×	9×			
True protein							
Cheese	97.07	96.99	97.06	97.44	0.35	NS	80.70
Whey	2.34	2.14	2.38	1.86	0.24	NS	18.89
Stretchwater	0.27 ^a	0.44 ^b	0.42 ^b	0.81 ^c	0.18	<0.01	0.41
Total	99.68	99.57	99.86	100.12	...	...	100.00
Casein							
Cheese	97.95	97.87	97.94	98.49	0.29	NS	...
Whey	0.76 ^a	0.68 ^a	0.66 ^a	0.43 ^b	0.09	0.03	...
Stretchwater	0.20 ^a	0.45 ^b	0.42 ^b	0.47 ^b	0.08	0.02	...
Total	98.90	99.00	99.02	99.39	...	...	...
Whey protein							
Cheese	85.15	84.72	84.51	84.15	0.53	NS	...
Whey	12.00 ^a	11.56 ^a	10.60 ^b	9.74 ^c	0.19	<0.01	...
Stretchwater	2.84 ^a	3.72 ^b	4.89 ^c	6.11 ^d	0.23	<0.01	...
Total	100.00	100.00	100.00	100.00	...	...	...
Fat							
Cheese	88.78 ^a	89.41 ^a	88.32 ^a	86.72 ^a	0.61	0.04	85.00
Whey	8.15 ^a	7.52 ^b	7.05 ^b	6.57 ^c	0.16	0.02	13.20
Stretchwater	3.12 ^a	3.21 ^a	4.57 ^b	6.83 ^c	0.14	0.01	1.80
Total	100.06	100.14	99.95	100.12	...	...	100.00
Calcium							
Cheese	79.84	80.89	80.32	77.38	0.68	NS	...
Whey	14.58 ^a	13.67 ^b	13.18 ^c	12.99 ^c	0.11	0.01	...
Stretchwater	5.38 ^a	5.41 ^a	6.57 ^b	9.57 ^c	0.06	0.03	...
Total	99.80	99.97	100.07	99.95	...	...	...
Total solids							
Cheese	75.64	77.48	78.10	76.78	0.62	NS	46.00
Whey	20.21 ^a	17.10 ^b	16.78 ^b	15.48 ^c	0.23	0.02	53.66
Stretchwater	4.99 ^a	5.34 ^a	5.18 ^a	6.79 ^b	0.14	0.04	0.34
Total	100.84	99.92	100.06	99.05	...	...	100

^{a,b,c,d}Means within same row not sharing common superscripts are different ($P < 0.05$).

the salt is calculated on a curd basis, it will increase accordingly. Furthermore, a decrease in the quantity of whey drained with CF suggests that more salt was drawn with the whey at lower CF because it remains soluble in the water phase.

Stretchwater. Significant increases with increasing CF were observed in total solids, true protein, casein, WP Ca, and fat content ($P < 0.01$) of stretchwater. No significant differences were found in the casein content among the treatments. It was found that salt contents in stretchwater CF 6 and 9 were significantly different from the salt content in CF 7 and 8. Total protein, true protein WP, and fat contents are much higher for CF 9 stretchwater than for the rest of the treatments, possibly because the curd releases compounds into the stretchwater that were not removed with the whey due to the small quantity of it produced.

Component Recovery

True protein recovery. Table 5 shows the total protein distribution among the cheese, whey, and stretchwater for the 4 CF treatments. Recoveries of true protein in the curd were 97.07, 96.99, 97.06, and 97.44%

for the CF 6, 7, 8, and 9 cheeses, respectively, and there were no significant differences among them. The percentage of true protein that is drawn in the whey did not change significantly with CF, but the percentage in the stretchwater increased significantly ($P < 0.01$) with increasing CF, balancing the recoveries in the cheese. These observations seem to indicate that as whey drainage volumes decrease with increasing CF, the curd tends to retain more proteins that are then lost in the stretchwater.

Casein. Table 5 shows the casein distribution in the cheese, whey, and stretchwater for the 4 CF treatments. Casein recoveries were 97.95%, 97.87%, 97.94%, and 98.49% and were not significantly different ($P > 0.05$). The percentage casein recovered in the whey decreased with increasing CF ($P < 0.05$) and increased in the stretchwater, although the differences were not significant. However, the distribution of the remaining casein in the whey and stretchwater showed significant differences among the 4 CF treatments.

Whey proteins. Table 5 shows the distribution of whey proteins in cheese, whey, and stretchwater for the CF treatments. The percentages of WP from the MFM retained in the MF cheese were 85.15%, 84.72%,

84.51%, and 84.15% for CF 6, 7, 8, and 9, respectively, and were not significantly different ($P > 0.05$). The recoveries decrease significantly with whey CF and increase with stretchwater CF. The retention of WP in the cheese represents an opportunity to increase cheese yield.

Fat. Table 5 shows the distribution of fat in cheese, whey, and stretchwater. The recovery values in the cheese were 88.78%, 89.41%, 88.32%, and 86.72% for CF 6, 7, 8, and 9, respectively. The value for fat recovery in the CF 9 cheese was significantly different from the other 3 cheeses ($P < 0.05$). The fat recovery in conventional LMPS Mozzarella cheese is 85%. Therefore, the manufacture of cheese from MFR has the potential to increase this recovery. Fat recovery values in the whey decreased significantly with increasing whey CF ($P < 0.05$) and increased with increasing CF in the stretchwater. The percentage of fat released to the stretchwater by the CF 9 cheese was twice that of the other 3 CF stretchwaters, which may explain the lower fat recovery in the cheese.

Calcium and total solids. The distribution of calcium and total solids showed similar trends with no differences in the cheeses with retentate CF. However, there was a significant decrease in both total solids and calcium recoveries, with increasing CF in the whey CF and a significant increase with CF in the stretchwater (Table 5). These results suggest that as the CF increased, the solids and calcium drained in the whey decreased due to the decreasing quantity of whey, suggesting that more calcium and solids were retained in the curd with increasing CF. However, the subsequent release of calcium and solids into the stretchwater increased with CF, rendering the recoveries in the cheese not statistically different.

Cheese Proteolysis

Primary proteolysis: pH 4.6 soluble nitrogen.

Figure 3 shows primary proteolysis at d 1, 7, 30, 45, and 60 for the MF cheeses and CM. The pH 4.6 acetate buffer soluble nitrogen (SN) expressed as a percentage of total protein was influenced by age and retentate CF ($P < 0.01$). Further analysis by least-squares means revealed that proteolysis rates were similar in the 4 MF cheeses but significantly different than the commercial sample. Because residual coagulant is responsible for the initial proteolysis in Mozzarella cheese during aging (Barbano et al., 1996) and the ratio of chymosin to casein was kept constant in the 4 MF cheeses, coupled with the fact that moisture and MNFS were not different with retentate CF, primary proteolysis was expected to be similar. The 4 MF cheeses had the same values of pH 4.6 SN at d 1 (6 to 7%), which are greater

Figure 3. Mean content of pH 4.6 soluble N as a percent of total N for commercial low-moisture, part-skim Mozzarella cheese. Commercial Mozzarella = ■. Low-moisture, part-skim microfiltration Mozzarella cheese CF 6 = ●; CF 7 = ○; CF 8 = ▲; CF 9 = ◻.

than the value in the commercial samples (2%). This difference is believed to be caused by the retention of WP or glycomacropetide in the cheese, which remain soluble at pH 4.6. However, the rate of increase of pH 4.6 SN was greater in the commercial sample due to a greater quantity of chymosin used and the absence of whey proteins in the curd.

One of the factors causing the difference in the rate of proteolysis between the commercial sample and the MF cheeses is the smaller quantity of chymosin used for the latter. It has been reported that the proportion of retained chymosin in cheese made from concentrated retentates is greater than for control in UF cheeses (Creamer et al., 1987). However, the fact that the increase in pH 4.6 SN was smaller for the MF cheeses suggests that the amount of rennet per casein retained in the curd is lower than that found in CM. A second factor is the possible presence of inhibitors. It has been reported (Mc Lean and Ellis, 1975) that high molecular weight WP such as $\alpha 2$ -macroglobulin can retard the action of rennet in coagulating milks. Since the molecular weight of $\alpha 2$ -macroglobulin is about 800 kDa, it is possible that it was preferentially retained in the MFR and incorporated in the cheese curd, where it retarded the proteolysis of casein by possibly binding to chymosin.

Secondary proteolysis: 12% TCA soluble nitrogen. Figure 4 shows the secondary proteolysis, expressed as 12% TCA SN. It was found that these values were dependent on cheese CF and age. The significant interaction factor (Table 6) showed that there are differences in the rate of increase in SN among treatments. Least-squares means comparisons analysis revealed that the rate of increase in the commercial sample was different from the MF cheeses. The difference is caused

Figure 4. Mean content of 12% TCA soluble N as a percent of total N for commercial low-moisture, part-skim Mozzarella cheese. Commercial Mozzarella = ■. Low-moisture, part-skim microfiltration Mozzarella cheese CF 6 = ●; CF 7 = ○; CF 8 = ▲; CF 9 = □.

by the absence of starter culture in the latter group, which showed increases in the 12% TCA SN with time due to the presence of nonstarter bacteria (in the range of 1×10^3 cfu/g at d 1). However, the higher bacterial counts from starter culture present in the CM (in the range of 1×10^6 cfu/g at d 1) were responsible for the higher rate of 12% TCA SN production. It was also found that there was a significant difference in secondary proteolysis between the cheese made from the CF 6 retentate and the other 3 treatments. At d 1, there was no difference among the 4 MF cheeses and the commercial samples analyzed, showing that the WP that may have caused d 1 differences in the pH 4.6 SN method are not soluble in 12% TCA.

Measurement of proteolysis by 12% TCA soluble nitrogen represents very small peptides and aminoacids produced by the release of peptidases and proteases upon lysis of starter culture cells at approximately d 30 to 60. (Fox, 1989), and it is referred to as secondary proteolysis in cheese. Since there was no starter culture used in the MF cheeses, secondary proteolysis was slower when compared with commercial LMPS Mozzarella samples, which is in agreement with the work of Brandsma and Rizvi (2001b) and other studies, where Mozzarella cheese was made using direct acidification processes (Dave, 2003).

Functional Properties

Meltability. Figure 5 shows the evolution of meltability with time for the MF cheeses and the commercial samples. The meltability of the MF cheeses increased significantly with CF and age (Table 6). The meltability values for commercial sample were significantly higher than the MF cheeses, especially in the beginning of the aging period. Comparisons by least-squares means showed that there were no differences in the development of meltability among the 4 MF cheeses, but they were significantly different from the commercial sample, which is in agreement with the development of proteolysis. Cheese age had an important effect on the increase of meltability, which is consistent with the increase of soluble nitrogen observed in the analysis of proteolysis. As the casein matrix is broken down by the activity of chymosin and nonstarter lactic bacteria, the cheese loses its ability to maintain its structure during heating (Tunick et al., 1993).

Table 6. Mean squares, probabilities (in parentheses), and degrees of freedom for proteolysis, meltability, and stretchability of low-moisture, part-skim Mozzarella cheese during 6 d of storage at 4°C (n = 2).

Factor	df	pH 4.6 soluble nitrogen	12% TCA soluble nitrogen	Meltability	Stretchability
Whole-plot					
Treatment	4	4.27*** (<i>P</i> < 0.01)	0.35** (<i>P</i> < 0.01)	0.62** (<i>P</i> < 0.01)	1.42** (<i>P</i> < 0.01)
Concentration factor (CF)					
Replicates	1	1.99** (<i>P</i> = 0.01)	0.14** (<i>P</i> < 0.01)	0.003 (<i>P</i> = 0.66)	0.21 (<i>P</i> = 0.68)
Error (CF × replicate)	4	0.18	0.01	0.02	1.29
Subplot					
Age (A)	1	154.74** (<i>P</i> < 0.01)	5.42** (<i>P</i> < 0.01)	4.87** (<i>P</i> < 0.01)	1508.75* (<i>P</i> < 0.01)
A × A	1	0.03 (<i>P</i> = 0.73)	0.16** (<i>P</i> < 0.01)	0.26** (<i>P</i> < 0.01)	6.62* (<i>P</i> = 0.03)
Interaction CF × A	4	0.02 (<i>P</i> = 0.98)	0.84** (<i>P</i> < 0.01)	0.01 (<i>P</i> = 0.80)	55.78* (<i>P</i> < 0.01)
Interaction CF × A × A	4	0.415* (<i>P</i> = 0.03)	0.01 (<i>P</i> = 0.41)	0.01 (<i>P</i> = 0.84)	5.53** (<i>P</i> < 0.01)
Error	20	0.23	0.01	0.02	1.21
R ²		0.99	0.98	0.98	0.98

*Statistically significant (*P* < 0.05).

**Statistically significant (*P* < 0.01).

Figure 5. Mean meltability of commercial low-moisture, part-skim Mozzarella cheese. Commercial Mozzarella = ■ (Brandsma and Rizvi, 2001a). Low-moisture, part-skim microfiltration Mozzarella cheese CF 6 = ●; CF 7 = ○; CF 8 = ▲; CF 9 = □.

The differences in meltability between the MF cheeses and the commercial samples were present until the end of the aging period. It was observed that the meltability achieved in the MF cheeses at d 60 is equivalent to commercial samples at d 1, which showed a significant delay in the development of this important property.

Stretchability. Stretchability of MF cheeses and commercial samples is shown in Figure 6. The values of stretchability decreased significantly with age for all treatments (Table 6). Concentration factor had a significant effect on stretchability, and the interaction between CF and age showed a significant difference in the rate of decrease of this property with time. Further comparisons by least-squares means revealed that the rates of decrease did not differ with CF among the MF cheeses, but there was a significant difference between

Figure 6. Mean stretchability of commercial low-moisture, part-skim Mozzarella cheese. Commercial Mozzarella = ■ (Juneja, 2002). Low-moisture, part-skim microfiltration Mozzarella cheese CF 6 = ●; CF 7 = ○; CF 8 = ▲; CF 9 = □.

the stretchability of the commercial samples and the cheeses made from MFM. The rate of decrease was greater for the commercial samples after d 14, yielding lower stretchability values than the MF cheeses after d 30. The stretchability depends on proteolysis and arrangement of the protein network (Apostolopoulos, 1994), thus, the commercial samples seemed to have lost their stretchability more quickly, since the proteolytic activity was higher in them.

CONCLUSIONS

The use of milk MFR for the production of LMPS Mozzarella cheese in a vatless process rendered cheese with the same compositional characteristics for the 4 CF studied. Furthermore, the composition of MF cheeses was similar to commercial samples and was within the ranges defined by the FDA for LMPS Mozzarella cheese. The use of GDL during microfiltration of skim milk achieved a pH of 6.0 in the CF 7 to 9 retentates, which produced a Ca:casein ratio in MFR and MF cheesemilks in the range found in commercial LMPS Mozzarella cheese, which is consistent with previous studies in our laboratory (Brandsma and Rizvi, 1999). Important reductions in whey drainage can be realized by this process (over 90% for the 4 CF studied) with the use of MF to remove a permeate stream that is very similar in composition to cheese whey but devoid of any of the additives used in cheese making. Microfiltration was found to achieve a 66 to 71% depletion of whey proteins in the retentates at the CF studied. The whey proteins recovered from this process in the permeate stream are in a native state and have the potential for improved functionality over the whey proteins recovered from conventional cheese making.

Proteolysis rates measured as soluble nitrogen in pH 4.6 and 12% TCA were found to be similar in the 4 MF cheeses but significantly slower than the rates in commercial samples. These results are related to the slower development of the functional characteristics tested (stretchability and meltability) of the MF cheeses when compared with commercial Mozzarella cheese samples. Strategies that may be used to normalize the development of proteolysis and functional properties are the use of chymosin in sufficient levels and the use of starter culture to induce hydrolysis of proteins and peptides. Considering prior research (Brandsma and Rizvi, 2001b) and the results from this study, the addition of starter cultures is probably required to increase the secondary proteolysis levels and improve the functional characteristics of the MF LMPS Mozzarella cheese. This process presents additional advantages that include reducing the size of the cheese making operation by replacing vats with a semicontinuous coag-

ulation system, which leads to plant size reduction. Finally, important savings in rennet use (93%) are achieved by this proposed cheese making process.

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