Effects of the depletion of whey proteins from unconcentrated milk using microfiltration on the yield, functionality, and nutritional profile of Cheddar cheese

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ABSTRACT

Some European dairies use low concentration factor microfiltration (MF) in their cheese plants. Removal of whey protein (WP) from milk before cheesemaking using microfiltration without concentration provides the opportunity to produce a value-added by-product, milk-derived whey. However, few studies have focused on the effects on cheese properties caused by the depletion of WP from cheese milk. Most studies have concentrated cheese milk using MF in addition to depletion of WP. In our approach, cheese milk was not concentrated during WP depletion using MF. We wanted to quantify residual WP levels in cheese made from MF milk and to explore whether WP depletion from milk would influence functionality, nutritional profile, and cheese quality during ripening. Casein (CN) contents for all milks were kept at ~2.5%, to eliminate the confounding factor of concentration of CN, which was observed in some previous MF studies. Cheese milks had similar ratios of CN to fat. Three standardized milks were produced with various CN: true protein (TP) ratios: (a) control with a CN:TP ratio of 83:100, (b) 35% WP depletion, 89:100 CN:TP, and (c) 70% WP depletion, 95:100 CN:TP. Cheddar cheeses were made from MF milk with various WP depletion levels and aged for 9 mo, and their functionality was evaluated during ripening. We found no major differences in cheese composition or pH values between samples. Cheese yield, solids recovery, and nitrogen recovery were slightly higher in the 95:100 CN:TP cheeses compared with the control. These enhanced recoveries reflect that MF-treated milk started with a higher fraction of CN-based protein solids, rather than WP solids. The standardized milk from the 95:100 CN:TP treatment also had a slightly higher fat content compared with the control, likely

helping to increase cheese yield. Rheological properties of cheeses during heating were similar between treatments. Hardness initially decreased with age for all cheeses due to proteolysis or solubilization, or both, of calcium phosphate. Maximum loss tangent (LT), an index of cheese meltability, was slightly lower for the control cheese until 30 d of ripening, but after 30 d, all treatments exhibited similar maximum LT values. The temperature where LT = 1 (crossover temperature), an index of softening point during heating, was slightly lower for MF cheese compared with the control cheeses during ripening. Microfiltration treatment had no significant influence on proteolysis. Sensory properties were similar between the cheeses, except for bitterness. Bitterness intensity was slightly lower in the MF cheeses than in the control cheeses and increased in all cheeses during ripening. We detected no major differences in the concentrations of key nutrients or vitamins between the various cheeses. Depletion of WP in cheese milk by MF did not negatively affect cheese quality, or its nutritional profile, and resulted in similar cheesemaking yields.

Key words: membrane filtration, functionality, cheese quality, cheese ripening

INTRODUCTION

The commercialization of membranes for separation purposes, specifically the purification of water, began in the 1930s (Pouliot, 2008). Since then, the applications for membrane filtration have been greatly expanded upon. Membrane filtration, specifically microfiltration (MF), initially gained popularity in the dairy industry in the 1980s with the development of ceramic membranes. Microfiltration has become industrially feasible due to technological advancements, such as uniform and low transmembrane pressure (Saboya and Maubois, 2000), as well as the development of polymeric membrane materials with spiral-wound configurations (Govindasamy-Lucey et al., 2007). These advancements have pushed forward the valorization of MF in the

Received April 14, 2020.

Accepted June 28, 2020.

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dairy industry. Microfiltration can be used for various purposes, such as removal of bacteria, preconcentration of cheese milk, fractionation of macronutrients, separation of proteins, and concentration of whey for further processing.

An MF membrane with a pore size of approximately 0.1 to 0.2 μm is small enough to retain most CN and nearly all fat, yet wide enough to allow most whey proteins (**WP**), lactose, and water to permeate (Ardisson-Korat and Rizvi, 2004). For this reason, MF is a useful tool for the separation of WP from CN. The MF permeate, or milk-derived whey, is valuable because it contains a high concentration of native WP, is virtually sterile, and has consistent composition (not variable due to differences in cheesemaking conditions). Additionally, milk-derived whey does not contain residual cheesemaking ingredients or glycomacropeptide (because milk has not been renneted), whereas traditional cheese whey does (Coppola et al., 2014). Traditional cheese whey can have some limitations on its functionality for further applications due to the presence of residual cheesemaking ingredients. These cheese ingredients (e.g., residual fats, rennet, starter culture, color) may cause limitations, such as the presence of off-flavors, reduced functionality (Coppola et al., 2014), and the necessity for bleaching colored whey if white whey products are desired from colored cheese variants (Campbell et al., 2013). Several studies (Heino et al., 2007; Coppola et al., 2014) compared WP concentrate made from milk-derived whey to that made from traditional cheese whey and reported that the milk-derived whey produced WP concentrate with superior sensory, foaming, and storage properties.

Some studies have examined the use of MF to produce cheese milks, with both high and low concentration factors. Brandsma and Rizvi (1999) used MF to concentrate skim milk to 8–9 $\times$ (without diafiltration, **DF**), and although permeation of WP occurred during MF, the final retentate still contained 2.2% WP. Brandsma and Rizvi (1999) indicated that this level of residual WP could significantly influence cheesemaking properties and quality. Low concentration factor MF milk can result in milks with WP contents similar to or higher than that of a control milk, due to the concentration of milk solids, even though some WP depletion occurs. For example, Neocleous et al. (2002a) studied the effects of low concentration factor (1.26 to 1.82 $\times$) MF milk on the yield and quality of Cheddar cheese. The WP concentration in the control and 1.82 $\times$ concentrated MF milks were 0.52 and 0.59%, respectively (Neocleous et al., 2002a). They also found that MF concentrated cheese milks produced cheeses with slower proteolysis compared with the control (Neocleous et al.,

2002b). Cheddar cheese made using UF-concentrated milks also tend to ripen more slowly than those made from unconcentrated milks (Green et al., 1981). This reduced rate of proteolysis in cheese made from concentrated milk could be due to the increased residual WP concentrations inhibiting chymosin (Creamer et al., 1987; Lelievre and Lawrence, 1988; Harper et al., 1989) or reducing plasmin activity (Bech, 1993).

Nelson and Barbano (2005a) described an MF process to maximize the removal of WP from skim milk. This process involved MF of milk, UF of MF permeate, and DF of MF retentate with UF permeate. This process successfully removed most of the WP originally found in the skim milk. Nelson and Barbano (2005b) manufactured Cheddar cheese from MF milks with various levels of WP but similar low CN levels. They reported that, although the composition of the cheeses made from the 3 treatments was similar, the percentage recoveries of milk solids, protein, and fat increased in the low-WP treatment. The increased solids and protein recoveries in the low-WP treatment was expected because CN made up a higher proportion of the solids in this treatment (instead of WP, which are mostly lost during cheesemaking). The reason for the higher fat recovery in the low-WP treatment was unclear (Nelson and Barbano, 2005b). They also found that cheese made from milk containing low WP had greater proteolysis than the control or high-WP treatments.

However, Nelson and Barbano (2005b) did not evaluate the influence of WP depletion on the texture, sensory, or rheological behavior of Cheddar cheeses. Additionally, Nelson and Barbano (2005b) did not directly measure the WP recovery content in their cheeses. The FDA (2005) has raised questions about whether the use of MF processing of cheese milk would alter the composition of cheese (presumably believing that MF would result in less retention of WP as well as loss of nutrients such as minerals and vitamins). Water-soluble vitamins and small components may permeate the MF membrane, but it is unclear whether these materials would be mostly lost in the cheese whey anyway during the normal drainage step. Little data is available on the effects of MF treatment of cheese milk on the nutritional profile of cheese.

The objective of this study was to use MF to deplete WP from cheese milk without concentrating it, to eliminate concentration factor as a confounding variable. To prevent changes in the soluble phase of milk, DF was carried out during the MF process; the MF retentate was subjected to DF with UF permeate. We explored the influence of WP-depleted milks on the quality, texture, functionality, sensory, and nutritional profiles of Cheddar cheese.

MATERIALS AND METHODS

Membrane Processing and Milk Standardization

A total of 5 trials were performed to remove WP via MF from the cheese milk without concentrating the CN content. For each trial, whole milk was collected from the University of Wisconsin-Madison Dairy Plant. Whole milk was pasteurized at 73°C for 19 s and cooled to 23°C, and then MF was used with extensive DF to significantly deplete the WP. Each trial yielded 1 control milk and 2 experimental milks of differing CN-to-true protein (TP) ratios (89:100 CN:TP and 95:100 CN:TP, respectively). The control milk had a CN-to-TP ratio of 83:100, which is similar to standard cheese milks used for Cheddar cheese manufacture.

Two Synder Filtration MF elements (model V0.2-2B-8038, Vacaville, CA) were used in parallel. The membranes were polyvinylidene fluoride-based materials with a spiral-wound configuration. They were 203.2 mm in diameter and 965.2 mm long, yielding approximately 68.4 m² total membrane area. The feed spacer was 0.8 mm thick. The nominal pore sizes were around 0.2 microns. The MF system was run at about 23°C with an average flux of about 14 L/min and inlet and outlet pressures of around 110.3 and 25.5 kPa, respectively.

Six Synder Filtration UF elements (model ST-3B-4338) were used within 3 parallel vessels, each containing 2 elements. The membranes were polyethersulfone-based material with a spiral-wound configuration. They were 109.2 mm in diameter and 965.2 mm long, yielding 43.2 m² total membrane area. The feed spacer was 1.17 mm in thickness, and the molecular weight cutoff was 10 kDa. The UF system was run at about 23°C with a flux of about 14 L/min and inlet and outlet pressures of around 303.4 and 96.5 kPa, respectively.

Each trial employed batch MF and batch UF loops operating in series. The MF permeate containing the WP flowed into the batch UF loop, and the UF permeate flowed into the batch MF loop as a DF fluid. This routine allowed for the small materials in the UF permeate, such as water, vitamins, lactose, and soluble minerals to keep the MF retentate dilute, which only minimally concentrated the milk fat and CN. The UF membranes were used because the smaller pore size allowed for the retention of WP, whereas other smaller molecules may pass through. Diafiltering the MF retentate (milk) with UF permeate also served to keep the MF feed volume and flux rates constant, and therefore to keep WP removal efficient. Diafiltering the MF retentate with UF permeate was continued until most WP depletion was achieved. The total solids (TS) content of each type of permeate was measured via a

handheld refractometer to monitor the WP depletion. The filtration process took about 3 h total.

The control milk consisted of only the whole milk. The 89:100 CN:TP milk was a blend of $\sim 50.5 \pm 8.1\%$ whole milk, $\sim 42.5 \pm 4.1\%$ MF retentate, and $\sim 7.0 \pm 5.3\%$ UF permeate. The 95:100 CN:TP milk was a blend of $\sim 83.6 \pm 7.6\%$ MF retentate and $\sim 16.4 \pm 7.6\%$ UF permeate. All 3 milks were standardized to approximately 2.3 to 2.4% CN and 0.68:1 CN:fat. Standardized milks were pasteurized a second time using the same protocol as mentioned previously.

Cheese Manufacture

Licensed Wisconsin cheesemakers manufactured 5 batches of milled-curd, full-fat Cheddar cheese at the University of Wisconsin-Madison Dairy Plant over a period of 3 mo. Pasteurized milks were cooled to 32°C and inoculated with direct-vat-set mesophilic blended cultures (Danisco MA19, Copenhagen, Denmark) containing *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* at a rate of 12 g/250 kg of milk. After 60 min of ripening, single-strength recombinant calf chymosin (Chy-max Extra, Chr. Hansen, Milwaukee, WI) was added at a rate of 24 g/250 kg of milk (constant volume of milk). Coagulum was cut at similar firmness, as determined by experienced cheesemakers, with 1.27-cm knives, and then agitated for 20 min. Whey and curd were then heated to 39°C over a period of 30 min. Whey was drained when the mixture reached a pH of approximately 6.35, and the curd was cut into 6 slabs. Slabs were stacked 2 high and turned every 20 min. When the curd pH reached 5.4, it was milled and then stirred for 15 min. Curd was then salted at a rate of 770 g/250 kg of milk. The salted curd was stirred out until a digital moisture analyzer (CEM Corporation, Matthews, NC) determined that the moisture content had reached about 38%. Curd was split into 2 9-kg Wilson hoops and pressed at 414 kPa for 4 h. Cheese blocks were then removed from the hoops and left overnight at ambient temperature ($\sim 25^\circ\text{C}$) to simulate the slow cooling regimen used by some in the industry to help with fermentation of residual lactose by the cultures. Cheese blocks were vacuum-packaged and stored at 4°C until the following morning.

Compositional Analysis

All compositional analyses for each sample were carried out in duplicate. Pasteurized whole milk, MF retentate, UF permeate, UF retentate, drain whey, press whey, and standardized milk samples were analyzed for TS content (Green and Park, 1980), total protein (total

% of N $\times$ 6.35; Kjeldahl method; AOAC International, 2000), CN (AOAC International, 2000), nonprotein nitrogen (AOAC International, 2000), fat (Mojonnier method; AOAC International, 2000), lactose content (high-performance ion-exchange chromatography; Dionex ICS-5000 RFIC-EGTM Dual System, Thermo Fisher Scientific Inc., Waltham, MA; Møller et al., 2012), and total calcium (inductively coupled argon plasma emission spectroscopy; **ICP-OES**; Govindasamy-Lucey et al., 2007). The proportion of insoluble calcium (**INSOL Ca**) of milks was measured by analyzing the calcium content of rennet whey to estimate the content of soluble Ca, by subtracting soluble Ca from the total Ca content of milk (Hassan et al., 2004).

For cheeses, a slab 2.5 cm thick was cut off the block, and the outer edges were discarded. This slab was further sampled for each analysis. The composition of ground cheese samples was analyzed at 2 wk for moisture (Marshall, 1992), fat (Mojonnier method; AOAC International, 2000), protein (Kjeldahl method; AOAC International, 2000), salt (chloride electrode method; Johnson and Olson, 1985), and total calcium, sodium, and potassium via ICP-OES (Govindasamy-Lucey et al., 2007). Total coliforms in cheese were also measured at 2 wk using the standard plate count method on petrifilm agar (AOAC International, 2000), and pH was measured by inserting a spear-tip pH electrode (AB15, Thermo Fisher Scientific) into a cheese block at 1, 14, 30, 90, and 180 d of ripening. The concentrations of lactose, galactose, and lactic acid in cheese were measured by high-performance ion-exchange chromatography (Møller et al., 2012) at 1, 14, 30, 90, and 180 d of ripening. Cheese extracts used for chromatographic analyses were prepared according to the method described by Zeppa et al. (2001). The proportion of **INSOL Ca** in cheese was determined by the acid-base titration method (Hassan et al., 2004) at 1, 14, 30, 90, and 180 d of ripening.

Mass Balance and Recoveries

A mass balance was carried out for each vat of cheese according to Govindasamy-Lucey et al. (2006). The starting milk was weighed with a Mars scale (Mars Scale Manufacturing, ISG Series, Ontario, Canada), and the drain and press wheys were weighed on a Rice Lake scale (Rice Lake Weighing Systems, IQ Plus 255, Rice Lake, WI). Cheeses were weighed on a Cream City scale (Cream City Stateline Scale, CW-80, Lake Mills, WI) for each treatment. The percentages of nitrogen, fat, or TS recovered in the cheese, drain whey, and press whey were calculated as the total amount of nitrogen, fat, or TS in each component divided by the total amount of nitrogen, fat, or TS in the original standardized milk multiplied by 100.

Actual yield was calculated for each vat of cheese as the weight of cheese divided by the weight of the original cheese milk (including the amount of cultures added during cheese manufacture), multiplied by 100. Yield equations were also calculated for each vat, using the method described by Govindasamy-Lucey et al. (2006). Predictive cheese yields were calculated for each vat using the Van Slyke cheese yield (Equation [1]) as shown (Van Slyke and Price, 1936):

Van Slyke cheese yield

$$= \frac{[(RF \times \% \text{fat in milk}) + (RC \times \% \text{casein in milk})] \times RS}{(100 - \% \text{moisture in cheese})}$$

$\times 100$,

[1]

where RF is the fraction of fat recovered in cheese, RC is the fraction of CN recovered in cheese, and RS reflects the proportion of other milk solids and salt recovered in cheese in relation to the amount of CN and fat in cheese.

Nutritional Analysis of Cheeses

After 1 d of ripening, cheese samples were sent to Covance Laboratories Inc. (Madison, WI) for nutritional analysis. The total calories were calculated using general conversion factors of 4, 4, and 9 calories per gram of protein, total carbohydrate, and total fat, respectively (Code of Federal Regulations, 2018). Total fat was analyzed by acid hydrolysis (AOAC International, 2005), carbohydrates by the difference method (United States Department of Agriculture, 1973), protein by the Dumas Method (AOAC International, 2005), vitamin A as retinol (AOAC International, 2005), minerals and elements by ICP-AES (calcium, iron, and sodium; AOAC International, 2005), and vitamin C (AOAC International, 2005), ash (AOAC International, 2005), and moisture (AOAC International, 2005) were evaluated for all cheese treatments from the 3 trials. Potassium content in the cheeses was measured for all trials at the Center for Dairy Research, using ICP-OES (Govindasamy-Lucey et al., 2007).

Particle Size Analysis

The particle size distribution of the whole milk and MF retentate was determined via laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Samples were diluted in deionized water. Measurements were performed in duplicate at an obscuration value between 12 and 13%. The particle size distribution was calculated from the light scattering patternw, using Mie theory. A refractive index

of 1.47 and absorption of 0.01 for milk fat were used. Water was used as the dispersant (refractive index of 1.33; Michalski et al., 2001). Analysis was performed in triplicate.

Rheological Analysis

Dynamic small-amplitude oscillatory rheology was used to measure the rheological properties of the cheeses. An Anton Paar MCR 301 rheometer (Graz, Austria) with a 50-mm serrated parallel plate geometry was used as described by Lee et al. (2005) and Lucey et al. (2005). Cheese samples 3 mm in thickness and 50 mm in diameter were heated from 5 to 85°C at the rate of 1°C per min. A frequency of 0.08 Hz and a constant strain of 0.5% were applied. The storage modulus (G'), loss modulus (G''), and loss tangent ($LT = G''/G'$) were recorded every minute. The G' value represents the elastic properties, the G'' value represents the viscous properties, and the LT represents the ratio of viscous to elastic moduli. Tests were performed in at least duplicate throughout ripening. Rheological analysis was carried out at ripening times of 2 wk, 1, 3, 6 and 9 mo.

Texture Profile Analysis

Texture profile analysis (TPA) of cheese was measured using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) according to the method of Bourne (1978). Cheese samples were sliced to 17.5 mm in thickness and cut with a 16-mm-diameter cork borer. The test compressed the cheese sample twice by 30% using a 50-mm aluminum cylinder probe. The probe measured the force required to compress the cheese sample at a constant speed of 0.8 mm/s. At least 9 replicates per sample were measured at 5°C. Texture profile analysis was carried out at ripening times of 4 d, 2 wk, and 1, 3, 6, and 9 mo.

Whey Protein Content of Milk and Recovery in Cheese

The WP contents in milk and cheese were determined using reverse-phase (RP)-HPLC. For identification and quantification of α -LA, β -LG, and BSA in milk and cheese, standard curves were prepared using purified α -LA, β -LG, and BSA from bovine milk (Sigma-Aldrich, St. Louis, MO). Concentrated standard solutions were prepared in solution A, which contained 0.1 M BisTris buffer (pH 6.8), 6 M guanidine hydrochloride (GdnHCl), 5.36 mM sodium citrate, and 19.5 mM DL-dithiothreitol (pH 7). The concentrated

standard solutions were further diluted at a ratio of 1:3 to concentration of 0.1, 0.25, 0.5, 1.0, 1.5, 2, and 4 mg/mL using solution B, which contained 4.5 M GdnHCl in a solution of acetonitrile, water, and trifluoroacetic acid in a ratio of 100:900:1 (vol:vol:vol, pH 2). The diluted standard solutions were then filtered through a 0.22- μ m syringe filter (Restek, Bellefonte, PA) before RP-HPLC. An injection volume of 25 μ L was used. Standards were prepared in duplicate. Standard curves were plotted using the concentration of the standard protein and its corresponding peak area.

To prepare rennet whey, rennet (Chymax Extra, double strength, Chr. Hansen) was diluted 10 times using deionized water. We weighed 30 g of milk into a 50-mL centrifuge tube, and 16.5 μ L of the diluted rennet was added to the milk and mixed before incubating at 32°C for ~1 h to coagulate. The coagulant was then cut using a spatula and centrifuged at $1,378 \times g$ for 10 min. The supernatant was filtered through Whatman No. 1 filter paper (GE Healthcare UK Ltd., Little Chalfont, UK), and the filtrate was collected. We transferred 500 μ L of the filtrate into a 1.5-mL Eppendorf centrifuge tube and stored it at -20°C until analysis. Samples were prepared in triplicate.

Rennet whey samples for RP-HPLC were prepared according to the method of Bobe et al. (1998). First 500 μ L of rennet whey was mixed with an equal amount of solution A [0.1 M BisTris buffer (pH 6.8), 6 M GdnHCl, 5.36 mM sodium citrate, and 19.5 mM DL-dithiothreitol (pH 7)]. The mixture was then incubated at room temperature for 1 h before centrifugation at $14,000 \times g$ for 10 min at 4°C. The fat layer was removed, and 500 μ L of the bottom layer was mixed with 1.5 mL of solution B [4.5 M GdnHCl in a solution of acetonitrile, water, and trifluoroacetate acid at a ratio of 100:900:1 (vol:vol:vol, pH 2)]. The mixture was then filtered through a 0.22- μ m syringe filter (Restek). Samples were prepared in triplicate. An injection volume of 25 μ L was used for RP-HPLC. Whey protein content in rennet whey was calculated using the standard curves. Whey protein content in milk was calculated based on the WP content in its rennet whey, and a correction factor was used. The correction factor was calculated based on solids contents in milk and the rennet whey (Davies and White, 1960).

Water-soluble extract (WSE) was prepared from cheeses using the method of Kuchroo and Fox (1982). Young cheese was used to reduce the number of peptide peaks (which could make the identification and analysis of individual WP peaks more difficult) in the chromatogram that would be generated during proteolysis (ripening). After 1 d of ripening, 20 g of cheese and 40 g of deionized water were mixed in a

stomacher bag and stomached at 260 rpm for 10 min at room temperature using a Stomacher 400 Circulator (Seward, Islandia, NY). The cheese slurries were then incubated at 40°C for 1 h, followed by centrifugation at $3,000 \times g$ for 30 min. The supernatant was filtered through Whatman No. 1 filter paper, and the filtrate was collected as WSE of cheese. We mixed 500 μL of the WSE with 500 μL of solution A and vortexed for 10 s. This mixture was held at room temperature for 1 h before centrifuging at $14,000 \times g$ for 10 min at 4°C. The fat layer was removed, and 500 μL of the bottom layer was mixed with 1.5 mL of solution B. The mixture was then filtered through a 0.22- μm syringe filter (Restek) before RP-HPLC injection. Samples were prepared in triplicate. An injection volume of 100 μL was used for RP-HPLC because of the low concentration of WP in cheese. Whey protein content in WSE was calculated using the prepared standard curves (α -LA, β -LG, and BSA). Based on Kuchroo and Fox (1982), the recovery rate of this single-extraction method was 70%, which was used for calculating WP content in cheese.

The determination of WP in milk and cheese via RP-HPLC was based on the method of Bonfatti et al. (2008) with slight modifications. The chromatographic system used to perform the analysis consisted of a Waters Alliance e2695 Separations Module equipped with a Waters 2998 photodiode array detector (Waters, Milford, MA). Separation was performed on a reversed-phase analytical column C8 (Zorbax 300SB-C8 RP, 3.5 μm , 150×4.6 internal diameter; Agilent Technologies Inc., Santa Clara, CA). Gradient elution was carried out with a mixture of 2 solvents. Solvent A consisted of 0.1% trifluoroacetic acid in water, and solvent B consisted of 0.1% trifluoroacetic acid in acetonitrile. Separation were performed using the following program: linear gradient from 25 to 35% of solvent B in 5 min, from 35 to 37% solvent B in 4 min, from 37 to 40% solvent B in 9 min, from 40 to 41% solvent B in 4 min, followed by an isocratic elution at 41% solvent B for 5.5 min, then linear gradient elution from 41 to 43% solvent B in 0.5 min, from 43 to 45% solvent B in 8 min, from 45 to 33% solvent B in 1 min, followed by an isocratic elution at 33% solvent B for 3 min, then from 33% to 90% solvent B in 0.1 min, and return to the starting condition, 25% B, in 1.9 min, followed by an isocratic elution at 25% B for 14 min. The total separation time was 56 min. The flow rate was 0.5 mL/min, the column temperature was kept at 45°C, and detection was made at wavelength of 214 nm. Injection volume was 25 μL for rennet whey samples and 100 μL for WSE of cheese. The percentage of residual WP recovered in the cheese was calculated using the amount found in the initial milks and their respective cheeses, as determined via RP-HPLC analysis.

Proteolysis

Proteolysis was monitored during ripening by preparing a pH 4.6 soluble extract according to the method reported by Kuchroo and Fox (1982). Total nitrogen in cheese extracts was measured via the Kjeldahl method (AOAC International, 2000) and expressed as a percentage of the total nitrogen in the cheese. These measurements were performed in triplicate at 4 d, 2 wk, and 1, 3, 6, and 9 mo.

Urea-PAGE was carried out to monitor the breakdown of β - and α_{SI} -CN. Urea-PAGE gels were prepared according to the method reported by Andrews (1983), as modified by Shalabi and Fox (1987). We dissolved 100 mg of ground cheese in 900 μL of sample buffer containing 0.06 *M* Tris, 8.0 *M* urea, 5% (vol/vol) 2-mercaptoethanol, and 0.01% (wt/vol) bromophenol blue, pH 7.6. The mixture of cheese sample and buffer was warmed to 55°C for ~ 8 min, until the cheese particles dissolved completely. The dissolved sample was diluted 10 times using sample buffer and mixed thoroughly before loading to the gel. A standard dual-cooled vertical slab gel electrophoresis unit SE 600 (Hoefer Scientific Instruments, San Francisco, CA) was used for electrophoresis. Separating gel (10% acrylamide) was prepared by dissolving 0.20 g of N,N-methylene bisacrylamide in a mixture of 10 mL of 40% acrylamide solution and 30 mL of Tris-HCl buffer containing 0.33 *M* Tris and 4.33 *M* urea, pH 8.9. TEMED (N,N,N',N'-tetramethylethane-1,2-diamine; 20 μL) and 10% ammonium persulphate solution (150 μL) were added to the separating gel solution before gel casting to catalyze polymerization. Stacking gel was prepared by dissolving 0.028 g of N,N-methylene bisacrylamide in a mixture of 1.38 mL of 40% acrylamide solution and 12.5 mL of Tris-HCl buffer containing 0.06 *M* Tris and 4.33 *M* urea, pH 7.6. Then TEMED (6.9 μL) and 10% ammonium persulphate solution (83 μL) were added into the separating gel solution before gel casting to catalyze polymerization. Twenty micrograms of protein of each cheese sample (7–9 μL of the 10 $\times$ diluted sample solution, based on the protein content in cheese) was loaded onto the gel, and electrophoresis was performed in electrode buffer containing 0.025 *M* Tris and 0.19 *M* glycine. Electrophoresis was carried out at a constant voltage of 300 V for ~ 3.5 h. During electrophoresis, water at $\sim 22^\circ\text{C}$ was circulated to remove the heat generated by electrophoresis. Gel was simultaneously fixed and stained with 0.04% Coomassie brilliant blue R-250 staining solution (Bio-Safe Coomassie Premixed Staining Solution, Bio-Rad, Hercules, CA) for ~ 1 h before destaining with distilled water. Gels were photographed and analyzed using densitometric analysis software (Gel Analyzer 2010a, developed by Istvan Lazar; <http://>

[//www.gelalyzer.com/](http://www.gelalyzer.com/)). Urea-PAGE was performed in duplicate.

Sensory Analysis

Quantitative descriptive analyses of cheese texture and flavors were evaluated by sensory panelists ($n \leq 9$) who had at least 40 h of training according to the method by Meilgaard et al. (1999). The cheese was evaluated in the form of uniform cubes at 11°C for the following attributes: firmness, cohesiveness, chewiness, adhesiveness, sweetness, saltiness, bitterness, acidity, milkfat, butteriness, brothiness, sourness, sulfur, rancidity, cardboard, burn, and astringency. Firmness is the amount of force required to compress the sample between thumb and forefinger by about 30%. Cohesiveness is the degree to which a sample chewed 5 to 7 times holds together on the palette. Chewiness is the amount of total energy required to masticate the sample to a state ready for swallowing. Adhesiveness is the degree to which a mass chewed 12 to 15 times sticks to the teeth and palette surface. Basic tastes (sweet, salty, bitter, and acid) were evaluated as compared with references of water mixed with varying amounts of sugar, salt, caffeine, and lactic acid, respectively. All flavor attributes were scored on a 15-point scale, 0 being absence of the flavor and 15 being overwhelming presence of the flavor. These analyses were performed at 3, 6, and 9 mo for each cheese.

Experimental Design and Statistical Analysis

Five replicate cheesemaking trials were carried out over a period of 3 mo. In each trial, 3 standardized milks (83:100 CN:TP or control, 89:100 CN:TP, and 95:100 CN:TP) were used to make Cheddar cheese. A 3×5 completely randomized block design, which incorporated all 3 treatments and all 5 replicate trials, was used for analysis of the response variables related to milk, cheese, and whey composition. Using SAS version 9.4 (SAS Institute Inc., Cary, NC) ANOVA was performed. The 3 different standardized milks (treatments) were analyzed as discontinuous variables where the 5 cheesemaking trials were blocked. Duncan's multiple-comparison test was carried out to evaluate differences in treatment means. A significance level of $P < 0.05$ was used.

A split-plot design was used to monitor the effects of both treatment and ripening time and their interactions on pH, LT_{max} , LT_{max} temperature, crossover point temperature, hardness, proteolysis, INSOL Ca, urea-PAGE, and sensory attributes during ripening. For the whole-plot factor, the treatment was analyzed as a discontinuous variable, and the cheesemaking trial

was blocked. For the subplot factor analysis, age was treated as a continuous variable. The interactive term treatment $\times$ cheesemaking trial was treated as the error term for treatment effect. Analysis of variance for the split-plot design was carried out using SAS. Duncan's multiple-comparisons test was carried out to evaluate differences in the treatment means at each ripening time at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Composition of Fluids

The chemical compositions of whole milk, MF retentate, UF retentate, and UF permeate can be found in Table 1. The UF permeate had only about 0.17% total protein, which was likely mostly nonprotein nitrogen rather than actual protein. This showed that almost all of the proteins from the starting whole milk (3.02%) were retained by either MF (mostly CN) or UF (mostly WP). Furthermore, the UF retentate contained 0.11% CN, which demonstrated that the MF retained most of the CN but allowed some soluble CN to permeate. The CN found in the UF retentate was most likely soluble CN rather than micellar CN. Particle size analysis indicated that whole milk had smaller particle size ($P < 0.05$) compared with the MF retentate. The volume mean diameter (D[4,3]) values were 3.81 and 3.33 μm in whole milk and MF retentate, respectively. These results indicated that the MF process did not homogenize fat globules due to processing operations such as pumping and filtration. This is in agreement with the results of Michalski et al. (2006) who found a larger milk fat globule retained in MF retentate compared with that in whole milk. Because the MF setup used in the present study did not homogenize the milk fat globule, the MF retentate displayed an increase in the milk fat globule D[4,3] values, likely due to the loss of small fat globules ($<0.1 \mu\text{m}$) into the MF permeate.

Composition of Standardized Cheese Milks

The compositional analysis of the standardized milks is shown in Table 2. Some small differences in fat and CN contents were detectable between the milk treatments, which could have affected cheese yields. The TS content slightly decreased with increasing WP depletion. The WP contributed to the solids content in the control milk (0.56%), and WP were highly depleted (0.16%) in the 95:100 CN:TP milk. The total protein and TP content decreased with MF treatment (Table 2). This was because the WP were increasingly depleted with MF. The MF processing successfully removed around 75% of the original WP from the starting milk. The CN:total

protein and CN:TP subsequently increased with MF treatment because of the decrease in WP.

Composition of Cheeses

The composition of each type of cheese is shown in Table 2. The moisture, protein, and moisture in the nonfat substance content of cheese did not significantly differ between the 3 cheese treatments. The residual WP content in the cheeses made from MF milk were lower than the control (Table 2) due to the depletion of WP during MF processing. The total protein contents of the various cheese milks were different; however, the total protein contents in the cheeses were similar between treatments (Table 2). This was because the starting milk had been standardized to a similar casein content (~2.4%) as well as to the same casein:fat ratio (Table 2). Although the WP content of milks decreased with MF treatment (Table 2), the WP are mostly lost in the whey and therefore do not usually contribute much to the total protein content of cheese. The WP contents in cheeses were 0.11, 0.06, and 0.03% in the control, 89:100 CN:TP, and 95:100 CN:TP treatments, respectively (Table 2). Lelievre and Lawrence (1988) estimated (calculation) around 0.3% residual WP in Cheddar cheese, which was higher than the levels we determined in the control cheese. We used RP-HPLC to determine the WP of cheese, and we were not able to measure the very low levels of minor proteins such as lactoferrin in cheese. The WP contribution to the total protein in cheese was 0.4, 0.2, and 0.1% in the control, 89:100 CN:TP, and 95:100 CN:TP treatments, respectively.

The contents of fat and fat in the DM slightly increased with MF treatment (Table 2). This can be attributed to the MF-treated cheese milks having been standardized to a slightly higher fat content (Table 2).

The salt content also slightly decreased with MF treatment. The calcium and potassium levels were similar in all cheeses (Table 2).

Detailed results for pH and lactic acid contents for the cheeses during ripening are shown in Table 3. The pH values were not significantly affected by treatment or ripening time (Table 4). The pH values for all treatments and time points ranged between 5.20 and 5.27. We detected a small increase in lactic acid levels during ripening for all cheeses (Table 3). Residual lactose was slowly fermented in the control cheese, possibly due to its higher salt-in-moisture level (Table 2). Treatment did not significantly influence lactose or lactic acid levels (Table 4). Variations in pH during ripening could be due to fermentation of residual lactose into lactic acid, thus decreasing the pH, or to solubilization of INSOL Ca phosphate releasing phosphate ions, which bind H^+ ions, resulting in buffering that can cause an increase in pH (Hassan et al., 2004). These 2 opposing trends tend to reduce the likelihood of large pH changes in many Cheddar cheeses.

Composition of Cheese Whey

The composition of each cheese whey is shown in Table 2. The TS and protein content in drain whey decreased with treatment, because WP were depleted from the cheese milks via MF. The fat content in the drain whey very slightly (but not significantly) increased with treatment, possibly because slightly more fat was present in the MF milks. This trend was in agreement with the findings of Guinee et al. (2007), who also observed an increased fat content in drain whey when milks with a higher fat content were used for Cheddar cheese manufacture. Minor differences were detectable in the composition of press wheys, apart from lower protein levels in the MF-treated samples.

Table 1. Average composition of pasteurized whole milk, microfiltration (MF) retentate, ultrafiltration (UF) retentate, and UF permeate¹

Item	Whole milk	MF retentate	UF retentate	UF permeate
Solids (%)	11.95 ± 0.17	12.87 ± 0.72	7.14 ± 0.21	5.24 ± 0.29
Fat (%)	3.44 ± 0.05	4.44 ± 0.40	ND ²	ND
Total protein ³ (%)	3.17 ± 0.09	3.41 ± 0.28	1.52 ± 0.18	0.17 ± 0.01
True protein ⁴ (%)	3.02 ± 0.08	3.23 ± 0.28	1.33 ± 0.18	ND
Casein ⁵ (%)	2.47 ± 0.06	3.08 ± 0.27	0.11 ± 0.05	ND
Casein:total protein (%)	77.97 ± 0.37	90.35 ± 0.75	6.97 ± 2.45	ND
Casein:true protein (%)	81.80 ± 1.35	95.48 ± 0.36	8.01 ± 2.69	ND
Casein:fat	0.72 ± 0.02	0.70 ± 0.01	ND	ND

¹Values represent the means and SD of 5 replicates for each treatment.

²ND = not determined.

³Total % N × 6.35.

⁴(Total % N - % nonprotein N) × 6.35.

⁵(Total % N - % noncasein N) × 6.36.

Mass, Nitrogen, Fat, Solids, and WP Recoveries

The recoveries of mass and milk components are shown in Table 5. We detected a significant increase in cheese mass with increasing WP depletion. Small increases were also detectable in actual cheese yield and

Van Slyke cheese yield in the 95:100 CN:TP sample compared with the control (Table 6). The increase in cheese mass recovery was due to a significant increase in nitrogen and solids recovery in the MF cheeses (Table 5). The slightly higher (not significant) fat and casein levels in the 95:100 CN:TP milk compared with the

Table 2. Compositions of standardized cheese milks that had various levels of whey protein depletion, and the derived drain whey, press whey, and Cheddar cheese¹

Component	Treatment ²			SEM	P-value ³
	Control	89:100 CN:TP	95:100 CN:TP		
Standardized cheese milk					
Lactose (%)	4.26 ^a	4.36 ^a	4.23 ^a	0.07	<0.05
Solids (%)	11.56 ^a	11.56 ^a	11.25 ^b	0.03	<0.05
Fat (%)	3.24 ^b	3.40 ^a	3.49 ^a	0.02	<0.01
Total protein ⁴ (%)	2.98 ^a	2.90 ^a	2.71 ^b	0.02	<0.01
True protein ⁵ (%)	2.80 ^a	2.73 ^a	2.54 ^b	0.02	<0.01
Casein ⁶ (%)	2.34 ^a	2.43 ^a	2.42 ^a	0.30	0.05
Casein:total protein (%)	78.58 ^c	83.91 ^b	89.22 ^a	0.39	<0.01
Casein:true protein (%)	83.64 ^c	89.08 ^b	95.28 ^a	0.004	<0.01
Casein:fat	0.72 ^a	0.71 ^a	0.69 ^b	0.02	<0.01
Whey protein ⁷ (%)	0.56 ^a	0.37 ^b	0.18 ^c	0.05	<0.01
Total calcium ⁸ (mg/100 g of milk)	113 ^a	115 ^a	115 ^a	1.01	0.08
Insoluble calcium (mg/100 g of milk)	73 ^a	75 ^a	77 ^a	1.61	NS
Calcium (mg/g of protein)	37.86 ^c	39.66 ^b	42.55 ^a	0.23	<0.01
Calcium (mg/g of casein)	48.49 ^a	47.28 ^a	47.69 ^a	0.23	<0.05
Insoluble calcium (mg/g of protein)	24.52 ^b	26.41 ^a	27.56 ^a	0.56	<0.05
Insoluble calcium (mg/g of casein)	31.20 ^a	31.48 ^a	30.88 ^a	0.68	NS
Drain whey					
Solids (%)	6.56 ^a	6.23 ^b	5.89 ^c	0.03	<0.01
Fat (%)	0.35 ^a	0.36 ^a	0.37 ^a	0.01	<0.01
Total protein (%)	0.83 ^a	0.65 ^b	0.44 ^c	0.01	<0.01
True protein (%)	0.58 ^a	0.41 ^b	0.20 ^c	0.01	<0.01
Whey protein (%)	0.54 ^a	0.37 ^b	0.17 ^c	0.79	<0.01
Press whey					
Solids (%)	17.98 ^a	16.91 ^a	18.45 ^a	0.27	0.05
Fat (%)	2.62 ^a	2.34 ^a	2.85 ^a	0.27	0.26
Total protein (%)	1.13 ^a	0.88 ^b	0.72 ^c	0.02	<0.01
True protein (%)	0.80 ^a	0.58 ^b	0.43 ^c	0.01	<0.01
Cheese (at 14 d)					
Moisture (%)	36.27 ^a	35.99 ^a	36.03 ^a	0.23	NS
Fat (%)	32.75 ^b	33.13 ^{ba}	33.52 ^a	0.11	<0.01
Salt (%)	1.87 ^a	1.75 ^{ba}	1.59 ^b	0.05	<0.05
Potassium ⁸ (mg/100 g of cheese)	101 ^a	99 ^a	99 ^a	1.68	NS
Calcium (mg/100 g of cheese)	776 ^a	770 ^a	774 ^a	10.1	NS
Protein ⁹ (%)	25.39 ^a	25.29 ^a	25.37 ^a	0.09	NS
Moisture in nonfat substance (%)	53.93 ^a	53.81 ^a	54.20 ^a	0.32	NS
Fat in DM (% dry weight basis)	51.39 ^b	51.75 ^{ba}	52.40 ^a	0.19	<0.05
Salt in moisture phase (%)	5.17 ^a	4.87 ^{ba}	4.42 ^b	0.14	<0.05
Whey protein (%)	0.10 ^a	0.06 ^b	0.02 ^c	0.01	<0.01

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

¹Values represent the means of 5 replicates for each treatment.

²The means of the 3 main treatments [different ratios of casein to true protein (TP): control, 89:100 CN:TP, 95:100 CN:TP] were analyzed using ANOVA of PROC GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC). Duncan's multiple-comparison test was used to evaluate differences in the treatments at a significance level of $P < 0.05$.

³Value for full statistical model that incorporated all 3 treatments and 5 blocks (5 replicate cheesemaking days).

⁴Total % N $\times$ 6.35.

⁵(Total % N - % nonprotein N) $\times$ 6.35.

⁶(Total % N - % noncasein N) $\times$ 6.36.

⁷Measured by reverse-phase HPLC.

⁸Measured by inductively coupled argon plasma emission spectroscopy.

⁹Total % N $\times$ 6.31.

Table 3. pH values, lactose (%), and lactic acid contents (%) of Cheddar cheeses that had various levels of whey protein depletion during ripening¹

Item	Ripening time (d)	Treatment ²		
		Control	89:100 CN:TP	95:100 CN:TP
pH	4	5.23 ^a	5.26 ^a	5.21 ^a
	14	5.27 ^a	5.23 ^a	5.24 ^a
	30	5.26 ^a	5.21 ^a	5.26 ^a
	90	5.24 ^a	5.23 ^a	5.24 ^a
	180	5.21 ^a	5.20 ^a	5.28 ^a
	270	5.20 ^a	5.22 ^a	5.24 ^a
Lactose (%)	4	0.40 ^a	0.31 ^a	0.26 ^a
	14	0.38 ^a	0.23 ^a	0.12 ^a
	30	0.32 ^a	0.16 ^a	0.11 ^a
	90	0.23 ^a	0.11 ^a	0.05 ^a
	180	0.19 ^a	0.09 ^a	0.04 ^a
	270	0.15 ^a	0.08 ^a	0.05 ^a
Lactic acid (%)	4	0.85 ^a	0.92 ^a	0.88 ^a
	14	0.92 ^a	0.97 ^a	1.02 ^a
	30	0.99 ^a	1.10 ^a	1.11 ^a
	90	1.08 ^a	1.15 ^a	1.17 ^a
	180	1.18 ^a	1.23 ^a	1.23 ^a
	270	1.27 ^a	1.29 ^a	1.27 ^a

^aMeans within the same row not sharing a common superscript differ ($P < 0.05$).

¹Values represent the means of 5 replicates for each treatment.

²Treatments represent different ratios of casein to true protein (TP).

control probably contributed to the small increase in cheese yield observed for the MF treatment (because the fat and CN recoveries were similar in all treatments; Table 6).

Nitrogen recovery in cheese increased with WP depletion (Table 5). This was in agreement with results reported by Govindasamy-Lucey et al. (2007), Neocleous et al. (2002a), and Nelson and Barbano (2005b). Nitrogen in bovine milk occurs in the form of CN, WP, and nonprotein nitrogen such as urea, amino acids, and

creatine. The amount of WP in milk decreased with MF (Table 2), which resulted in a lower proportion of WP in the nitrogen fraction for the 89:100 CN:TP and 95:100 CN:TP cheese milks compared with the control milk. Caseins are effectively recovered in cheesemaking, so increasing the proportion of CN in cheese milk results in a more efficient cheesemaking process, in terms of protein recovery. This likely explained why nitrogen recovery in the 95:100 CN:TP cheese was the highest, because it contained highest proportion of CN as a function of the nitrogen in the milk. The same explanation may apply to the higher TS recovery in the MF cheeses (Table 5). Because the 89:100 CN:TP and 95:100 CN:TP milks had less WP contributing to their starting TS content, they lost fewer solids to the whey, thus increasing the effective solids recovery in the cheese.

Fat recovery in cheese was not affected by treatment (Table 5). Previous studies (Govindasamy-Lucey et al., 2007; Brandsma and Rizvi, 2001) have reported increases in fat recovery in cheese made from MF concentrated milk. However, the milks in the present study were not concentrated and were manufactured by identical cheesemaking processes, so the similar fat recovery between treatments was expected. Homogenization of milk fat globules can lead to the adsorption of CN and WP onto the fat surface and, thus, greater retention in the cheese protein matrix. However, our MF process had no homogenization effect (i.e., no significant change in the fat particle size).

The non-CN, nonfat solids recovery was also lower in the 95:100 CN:TP cheese (Table 6), possibly due to poorer salt recovery. Levels of WP recovery in the control, 89:100 CN:TP, and 95:100 CN:TP cheeses were 1.56, 1.55, and 1.12%, respectively. The WP recovery was significantly lower in the 95:100 CN:TP cheeses.

Table 4. Degrees of freedom, statistical significance (P -values), and R^2 values for changes in pH, lactose, lactic acid, hardness, insoluble calcium content, and proteolysis (pH 4.6-soluble nitrogen, expressed as a percentage of total nitrogen) for Cheddar cheeses that had various levels of whey protein depletion during ripening

Factor ¹	df	pH	Lactose	Lactic acid	TPA hardness ²	Insoluble Ca ³	Proteolysis ⁴
Whole-plot							
Treatment (T)	2	0.46	0.12	0.08	0.43	0.34	0.79
Day of cheesemaking (D)	4	0.12	<0.01	<0.01	0.03	0.03	0.99
Error (T × D)	8						
Split-plot							
Age (A)	5	0.80	<0.01	<0.01	<0.01	<0.01	<0.01
A × T	10	0.33	0.26	0.90	0.52	0.64	0.98
R^2		0.38	0.94	0.82	0.81	0.86	0.93

¹Split-plot design with the 3 treatments [different ratios of casein to true protein (TP): control, 89:100 CN:TP, 95:100 CN:TP] analyzed as a discontinuous variable and cheesemaking day blocked (3×5). Subplot included the effect of age of cheese (A) and age × treatment as variables.

²Texture profile analysis (TPA) hardness was measured by texture analyzer.

³Percentage of insoluble calcium as a percentage of total calcium.

⁴pH 4.6-soluble nitrogen as a percentage of total nitrogen.

Table 5. Fat, nitrogen, and solids recoveries in the Cheddar cheeses manufactured from milks that had various levels of whey protein depletion¹

Component recovery	Treatment ²			SEM	P-value ³
	Control	89:100 CN:TP	95:100 CN:TP		
Fat recovery (%)					
Cheese	87.95 ^a	88.34 ^a	87.30 ^a	0.87	0.26
Drain whey	11.45 ^a	11.42 ^a	11.09 ^a	0.49	0.17
Press whey	1.19 ^a	1.06 ^a	1.17 ^a	0.14	0.48
Total	100.59	100.82	99.56		
Nitrogen recovery (%)					
Cheese	74.79 ^c	79.64 ^b	85.57 ^a	0.75	<0.01
Drain whey	25.67 ^a	20.32 ^b	14.48 ^c	0.35	<0.01
Press whey	0.62 ^a	0.57 ^{ba}	0.38 ^b	0.05	<0.05
Total	101.08	100.53	100.43		
Solid recovery (%)					
Cheese	47.97 ^b	50.18 ^a	51.57 ^a	0.42	<0.01
Drain whey	51.40 ^a	48.66 ^b	47.21 ^c	0.24	<0.01
Press whey	2.29 ^a	2.23 ^a	2.31 ^a	0.05	0.08
Total	101.66	101.07	101.09		
Drain whey ⁴ (% mass)	89.42 ^a	89.26 ^a	89.15 ^a	0.11	0.16
Press whey ⁴ (% mass)	1.48 ^a	1.53 ^a	1.41 ^a	0.05	0.36

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

¹Values represent the means of 5 replicates for each treatment.

²The means of the 3 main treatments [different ratios of casein to true protein (TP): control, 89:100 CN:TP, 95:100 CN:TP] were analyzed using ANOVA of PROC GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC). Duncan's multiple-comparison test was used to evaluate differences in the treatments at a significance level of $P < 0.05$.

³Value for full statistical model that incorporated all 3 treatments and 5 blocks (5 replicate cheesemaking days).

⁴Amount of drain whey and press whey obtained from 100 kg of cheese milk.

Table 6. Actual and calculated cheese yield values for Cheddar cheeses that had various levels of whey protein depletion¹

Item	Treatment ²			SEM	P-value ³
	Control	89:100 CN:TP	95:100 CN:TP		
RF value ⁴	0.880	0.881	0.873	ND ⁵	ND
RC value ⁶	0.950	0.950	0.950	ND	ND
RS value ⁷	1.093	1.092	1.086	ND	ND
Actual yield ⁸ (%)	8.71 ^b	9.06 ^{ba}	9.07 ^a	0.10	0.05
Van Slyke cheese yield ⁹ (%) using RF, RC, and RS values	8.70 ^b	9.03 ^{ba}	9.07 ^a	0.01	0.05

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

¹Values represent the means of 5 replicates for each treatment.

²The means of the 3 main treatments [different ratios of casein to true protein (TP): control, 89:100 CN:TP, 95:100 CN:TP] were analyzed using ANOVA of PROC GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC). Duncan's multiple-comparison test was used to evaluate differences in the treatments at a significance level of $P < 0.05$.

³Value for full statistical model that incorporated all 3 treatments and 5 blocks (5 replicate cheesemaking days).

⁴RF = fat recovered in cheese, determined experimentally from cheese trials.

⁵ND = not determined.

⁶RC = casein recovered in cheese, calculated as described by Govindasamy-Lucey et al. (2006). The calculated RC values for control, 89:100 CN:TP, and 95:100 CN:TP cheeses were 0.947, 0.942, and 0.953, respectively. Thus, all calculations were carried out using an average RC value of 0.95 for all the cheeses.

⁷RS = recovery of non-CN, nonfat solids in cheese, calculated as described in Govindasamy-Lucey et al. (2006).

⁸Actual yield, determined experimentally from cheese trials, was calculated for each vat of cheese as the weight of cheese divided by the weight of the original cheese milk (including the amount of cultures added during cheese manufacture), multiplied by 100.

⁹Van Slyke cheese yield was calculated using Equation [1], using milk and cheese composition data given in Table 2.

Nutritional Analysis of Cheese

The nutritional analysis of the 3 cheese treatments is shown in Table 7. Cheeses made with MF treatment had slightly higher fat contents and calories from fat, in agreement with the cheese compositional results (Table 2). An increase in vitamin A content with MF treatment was detectable, also likely due to the slightly higher fat content in this cheese, as vitamin A is a fat-soluble vitamin. The potassium content was similar (0.10%) between all 3 cheese treatments (Table 2). The sodium content was slightly lower in the 95:100 CN:TP cheese. One possible explanation is that the MF treatment resulted in a slight increase in cheese yield, but because all curds were salted at a constant milk volume, the MF cheeses probably received less salt compared with the control. No other significant differences were detectable in the nutritional quality of the 3 cheese treatments, indicating that MF did not cause a detrimental change in the nutritional properties of cheese.

Insoluble Calcium Content in Cheeses

Figure 1 shows the changes in the amount of INSOL Ca in the cheeses during ripening. The INSOL Ca content did not significantly differ between treatments but did slightly decrease over time (Table 4), in agreement with previous studies on Cheddar cheese (Hassan

et al., 2004). A close relationship exists between the pH of the cheese and the INSOL Ca content. A low pH value in cheese causes INSOL Ca to solubilize. All cheeses were manufactured with an identical process, so the rate of acidification was similar between cheeses. The pH values and lactic acid contents of the cheeses were not significantly influenced by treatment (Table 4) and were similar throughout ripening (Table 3), which helped to produce comparable rates of dissolution of calcium phosphate.

Proteolysis

Changes in pH 4.6-soluble nitrogen during ripening are shown in Figure 2. No significant difference occurred in the amount of pH 4.6-soluble nitrogen between the 3 cheese treatments (Table 4). The amount of pH 4.6-soluble nitrogen as a percentage of total nitrogen increased with ripening (Figure 2), as intact CN was broken down. Primary proteolysis is the degradation of CN by proteolytic enzymes, such as chymosin and the indigenous milk protease plasmin (Ivens et al., 2017). Proteolysis was further analyzed after 6 mo of ripening time, using urea-PAGE (Figure 3). No visual difference in the degradation profile of CN was detectable between treatments. Generally, chymosin hydrolyzes α_{S1} -CN during primary proteolysis. In Cheddar cheese, β -CN is relatively resistant to chymosin hydrolysis but

Table 7. Nutritional analysis (performed at 1 d of ripening) of Cheddar cheeses that had various levels of whey protein depletion¹

Nutritional component	Treatment ²			SEM	P-value ³
	Control	89:100 CN:TP	95:100 CN:TP		
Calories (cal/100 g of cheese)	403 ^a	405 ^a	408 ^a	1.54	0.22
Calories from fat (cal/100 g of cheese)	294 ^b	298 ^a	301 ^a	1.07	<0.05
Fat by acid hydrolysis (%)	32.7 ^b	33.1 ^{ba}	33.4 ^a	0.13	<0.05
Carbohydrates (%)	2.03 ^a	1.57 ^a	1.47 ^a	0.26	0.29
Protein ⁴ (%)	25.4 ^a	25.2 ^a	25.3 ^a	0.14	<0.05
Ash (%)	4.00 ^a	3.92 ^a	3.71 ^a	0.08	0.13
Moisture (%)	35.9 ^a	36.2 ^a	36.1 ^a	0.31	0.38
Vitamin A as retinol (IU/100 g of cheese)	875 ^b	892 ^{ba}	923 ^a	10.68	<0.01
Vitamin C (mg/100 g of cheese)	<1 ^a	<1 ^a	<1 ^a	ND ⁵	ND
Calcium (mg/100 g of cheese)	806 ^a	795 ^a	802 ^a	4.44	0.18
Iron (mg/100 g of cheese)	<0.38 ^a	<0.38 ^a	<0.39 ^a	ND	ND
Sodium (mg/100 g of cheese)	764 ^a	709 ^{ba}	628 ^b	21.17	<0.05

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

¹Values represent the means of 3 replicates for each treatment.

²The means of the 3 main treatments [different ratios of casein to true protein (TP): control, 89:100 CN:TP, 95:100 CN:TP] were analyzed using ANOVA of PROC GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC). Duncan's multiple-comparison test was used to evaluate differences in the treatments at a significance level of $P < 0.05$.

³Values for full statistical model that incorporated all 3 treatments and 3 blocks (3 replicate cheesemaking days).

⁴Total % N $\times$ 6.38.

⁵ND = not determined.

is hydrolyzed by plasmin (Ivens et al., 2017). In all 3 cheeses, β -CN was hydrolyzed by plasmin into β -CN f(29-209), β -CN f(106-209), β -CN f(108-209), and β -CN f(1-189/192). β -CN f(1-189/192) is very hydrophobic and contributes to bitterness in cheese (Bansal et al., 2009). Previous studies have concluded that cheeses made from UF concentrated milk had slower proteolysis, likely due to some type of inhibition of rennet or plasmin activity (Lelievre and Lawrence, 1988; Bech, 1993). Concentration of milk by MF could also potentially concentrate proteinase-peptidase inhibitors (Govindasamy-Lucey et al., 2007). Aaltonen and Ollikainen (2011) reported that MF/DF of milk enhanced plasmin activity in milk, due to the reduction in the β -LG concentration (which inhibits plasmin). The similar proteolysis levels in our cheeses suggests that any potential inhibition of rennet or plasmin might occur only at higher residual WP levels in cheese (our cheeses had WP levels $\leq 0.11\%$).

Textural and Rheological Properties

The textural properties (hardness) of cheeses during ripening are shown in Figure 4. Treatment did not significantly affect TPA hardness (Table 4). However, TPA hardness did significantly decrease within the first 90 d of ripening but hardly changed thereafter (Figure 4). This decrease in hardness over ripening time was expected, as it is typical for Cheddar cheese (Lucey et al., 2003). Similarly, Neocleous et al. (2002b) reported a

Figure 1. Insoluble calcium content, expressed as a percentage of total calcium, for Cheddar cheeses made with various levels of whey protein depletion; control (●), 89:100 CN:true protein (TP; ▽), and 95:100 CN:TP (■) Cheddar cheeses during ripening. Error bars represent SD ($n = 5$).

Figure 2. pH 4.6-soluble nitrogen as a percentage of total nitrogen for Cheddar cheeses made with various levels of whey protein depletion; control (●), 89:100 CN:true protein (TP; ▽), and 95:100 CN:TP (■) Cheddar cheeses during ripening. Error bars represent SD ($n = 5$).

slight decrease in the hardness of Cheddar cheese up to 30 d of ripening; thereafter, hardness hardly changed. The similarity in hardness between cheese treatments can be attributed to samples having similar compositions (Table 2), pH values (Table 3), INSOL Ca contents (Figure 1), and proteolysis (Figure 2).

Minor but significant differences were observed in the LT_{max} and melting point (crossover temperature)

Figure 3. Urea-PAGE electrophoretogram showing the breakdown of casein in Cheddar cheeses made with various levels of whey protein depletion; control, 89:100 CN:true protein (TP), and 95:100 CN:TP Cheddar cheeses after 6 mo of ripening. NaCN is the sodium caseinate standard.

between the control and experimental cheeses (Figure 5; Table 8). The control cheese had slightly lower LT_{max} values than the MF cheeses at 14 d and 1 mo of ripening (Figure 5a). As ripening continued, we observed no significant difference between the LT_{max} values of any of the treatments. The crossover temperature was slightly higher in the control cheese compared with the other cheeses throughout ripening (Figure 5b). The control cheeses had slightly lower fat and higher salt contents (Table 2); these minor compositional differences could have contributed to these small changes in the rheological properties. The LT_{max} values for all cheeses increased during ripening as expected (Figure 5a) up to 3 mo, after which no significant change occurred. This indicated that the meltability of all 3 cheeses increased during ripening. The temperature of the crossover point (Figure 5b) decreased with ripening for all 3 cheeses, indicating that less energy was needed for flow as the cheese matrix underwent aging. This was in agreement with previous reports for Cheddar cheese (Lee et al., 2005; Lucey et al., 2005).

Softening of cheese takes place during ripening due to various different mechanisms, which are determined by the types of interactions between CN that are present in the system (Lucey et al., 2003). During ripening, INSOL Ca phosphate partly dissolves into the serum phase (Figure 1), due to the acidic environment of cheese (Hassan et al., 2004). This reduces the amount of calcium phosphate cross-links between CN particles and thus makes it easier to flow. This trend, along with

Figure 4. Hardness (N) from texture profile analysis for Cheddar cheeses made with various level of whey protein depletion; control (●), 89:100 CN:true protein (TP; ▽), and 95:100 CN:TP (■) Cheddar cheeses during ripening. Error bars represent SD (n = 5).

Figure 5. Rheological properties during heating profile of Cheddar cheese samples during ripening. The maximum loss tangent (LT) values (a) and temperature of the crossover point (where $LT = 1$; b), for Cheddar cheeses made with various levels of whey protein depletion; control (●), 89:100 CN:true protein (TP; ▽), and 95:100 CN:TP (■). Error bars represent SD (n = 5).

ongoing proteolysis, likely explains why all the Cheddar cheeses exhibited an increase in LT_{max} values during ripening (Lucey et al., 2005).

Sensory Properties

The sensory textural and flavor attributes of cheeses are shown in Table 9. No sensory attributes except for bitterness differed between the control and experimental cheeses. Panelists detected no significant difference in any textural attributes, such as firmness, cohesiveness, and chewiness, between treatments or over time. This agrees with the TPA hardness trends during cheese aging (Figure 4). Adhesiveness did increase with ripening time (Table 9). Panelists did detect a significant increase in bitterness with ripening time for

Table 8. Degrees of freedom, statistical significance (*P*-values), and *R*² values for changes in rheological properties and sensory attributes of Cheddar cheeses that had various levels of whey protein depletion during ripening (*n* = 5)

Factor ¹	df ²	LT _{max} ³	Crossover point ⁴ (°C)	df	Sensory bitterness	Sensory sulfur
Whole-plot						
Treatment (T)	2	<0.01	<0.01	2	<0.01	0.10
Day of cheesemaking (D)	4	0.71	0.02	4	0.05	0.05
Error (T × D)	8			8		
Split-plot						
Age (A)	4	<0.01	<0.01	2	<0.01	0.10
A × T	8	0.35	0.87	4	0.98	0.99
<i>R</i> ²		0.69	0.91		0.67	0.60

¹Split-plot design with the 3 treatments analyzed as a discontinuous variable and cheesemaking day blocked (3 × 5). Subplot included the effect of age of cheese (A) and age × treatment as variables.

²Degrees of freedom differed for variable measurements, as time points for the analyses were different.

³Maximum loss tangent.

⁴Temperature at which loss tangent = 1.

all cheeses (Table 8). A main contributor to the evolution of bitterness in aged Cheddar cheese is the degradation of β-CN into its bitter peptides, such as β-CN f(1–189/192; Lemieux and Simard, 1991; Børsting et al., 2012). The production of this peptide, as observed in the urea-PAGE results (Figure 3), was likely why our sensory panelists detected an increase in cheese bitterness as a function of ripening time. The intensity of bitterness in our cheeses was quite low (≤ 2.7 on our 15-point scale; Table 9). The MF cheeses in our study had lower WP and slightly but significantly less bitter intensity compared with the control cheeses throughout ripening (Table 8). No other differences in any other flavor attributes were detected during ripening time. It was previously speculated that a decrease in flavor development in cheeses made from concentrated milk was due to WP inhibiting proteolysis. However, the differences in residual WP content in all our cheeses were small (Table 2); thus, it was not surprising no major differences in the sensory properties occurred.

CONCLUSIONS

Polymeric MF membranes, when used in conjunction with UF membranes for the DF of MF retentate with UF permeate, can be used to deplete WP from cheese milk without concentrating the CN content of milk. Removal of WP from milk before cheesemaking provides the opportunity to produce a value-added by-product, milk-derived whey. When Cheddar cheeses were manufactured from these WP-depleted cheese milks, the composition, texture, and nutritional quality were comparable to those of the control cheese. Because the CN content of MF milk was similar to that of the control milk, no adjustments to the cheesemaking process were necessary to achieve cheeses with similar composition and quality. Depletion of WP from milk by

MF did not affect proteolysis, nutritional properties, or sensory attributes during cheese ripening.

Table 9. Sensory textural and flavor attributes (intensities based on a scale of 0–15 points) for cheeses that had various levels of whey protein depletion [control, 89:100 CN:true protein (TP), and 95:100 CN:TP] at ripening times of 3, 6, and 9 mo (*n* = 5)

Attribute	Treatment		
	Control	89:100 CN:TP	95:100 CN:TP
3 mo			
Firmness	13.6 ^a	13.3 ^a	13.5 ^a
Cohesiveness	10.8 ^a	11.3 ^a	11.3 ^a
Chewiness	5.4 ^a	5.3 ^a	5.4 ^a
Adhesiveness	6.6 ^a	7.0 ^a	6.8 ^a
Salt	5.7 ^a	5.6 ^a	5.5 ^a
Acid	2.1 ^a	2.1 ^a	1.9 ^a
Bitter	1.2 ^a	0.8 ^b	0.6 ^b
Milkfat	2.2 ^a	2.4 ^a	2.5 ^a
Butter	2.0 ^a	1.9 ^a	2.0 ^a
Sulfur	0.9 ^a	0.7 ^a	0.8 ^a
6 mo			
Firmness	13.4 ^a	13.3 ^a	13.3 ^a
Cohesiveness	11.0 ^a	11.3 ^a	11.0 ^a
Chewiness	5.7 ^a	5.8 ^a	5.7 ^a
Adhesiveness	7.6 ^a	8.1 ^a	7.7 ^a
Salt	4.1 ^a	4.3 ^a	4.0 ^b
Acid	3.6 ^a	3.7 ^a	3.5 ^a
Bitter	2.0 ^a	1.8 ^b	1.8 ^b
Milkfat	2.4 ^a	2.5 ^a	2.5 ^a
Butter	1.5 ^a	1.7 ^a	1.6 ^a
Sulfur	0.3 ^a	0.5 ^a	0.4 ^a
9 mo			
Firmness	12.8 ^a	12.7 ^a	12.7 ^a
Cohesiveness	11.4 ^a	11.7 ^a	11.7 ^a
Chewiness	5.7 ^a	5.7 ^a	5.7 ^a
Adhesiveness	8.9 ^a	9.4 ^a	9.2 ^a
Salt	4.1 ^a	4.0 ^a	4.1 ^a
Acid	4.7 ^a	4.6 ^a	4.7 ^a
Bitter	2.7 ^a	2.5 ^b	2.5 ^b
Milkfat	2.4 ^a	2.5 ^a	2.5 ^a
Butter	1.0 ^a	1.0 ^a	1.0 ^a
Sulfur	0.6 ^a	0.5 ^a	0.5 ^a

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

ACKNOWLEDGMENTS

The authors thank the personnel from the Center for Dairy Research and University of Wisconsin Dairy Plant (Madison, WI) for their assistance and support in cheesemaking, analytical work, and sensory analyses. The financial support of the Center for Dairy Research Industry Team and the National Dairy Council (Rosemont, IL) is gratefully acknowledged. The authors have not stated any conflicts of interest.

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