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Slower proteolysis in Cheddar cheese made from high-protein cheese milk is due to an elevated whey protein content

E. Reale,[1] S. Govindasamy-Lucey,[2]* Y. Lu,[2] M. E. Johnson,[2] J. J. Jaeggi,[2] M. Molitor,[2] and J. A. Lucey[2]
[1]Department of Food Science, University of Wisconsin, Madison 53706
[2]Center for Dairy Research, University of Wisconsin, Madison 53706

ABSTRACT

A growing number of companies within the cheese-making industry are now using high-protein (e.g., 4–5%) milks to increase cheese yield. Previous studies have suggested that cheeses made from high-protein (both casein and whey protein; WP) milks may ripen more slowly; one suggested explanation is inhibition of residual rennet activity due to elevated WP levels. We explored the use of microfiltration (MF) to concentrate milk for cheese-making, as that would allow us to concentrate the casein while varying the WP content. Our objective was to determine if reducing the level of WP in concentrated cheese milk had any impact on cheese characteristics, including ripening, texture, and nutritional profile. Three types of 5% casein standardized and pasteurized cheese milks were prepared that had various casein:true protein (CN:TP) ratios: (a) control with CN:TP 83:100, (b) 35% WP reduced, 89:100 CN:TP, and (c) 70% WP reduced, 95:100 CN:TP. Standardized milks were preacidified to pH 6.2 with dilute lactic acid during cheese-making. Composition, proteolysis, textural, rheological, and sensory properties of cheeses were monitored over a 9-mo ripening period. The lactose, total solids, total protein, and WP contents in the 5% casein concentrated milks were reduced with increasing levels of WP removal. All milks had similar casein and total calcium levels. Cheeses had similar compositions, but, as expected, lower WP levels were observed in the cheeses where WP depletion by MF was performed on the cheese milks. Cheese yield and nitrogen recoveries were highest in cheese made with the 95:100 CN:TP milk. These enhanced recoveries were due to the higher fraction of nitrogen being casein-based solids. Microfiltration depletion of WP did not affect pH, sensory attributes, or insoluble calcium content of cheese. Proteolysis (the amount of pH 4.6 soluble nitrogen) was lower in control cheeses compared with WP-reduced cheeses. During ripening, the hardness values and the temperature of the crossover point, an indicator of the melting point of the cheese, were higher in the control cheese. It was thus likely that the higher residual WP content in the control cheese inhibited proteolysis during ripening, and the lower breakdown rate resulted in its higher hardness and melting point. There were no major differences in the concentrations of key nutrients with this WP depletion method. Cheese milk concentration by MF provides the benefit of more typical ripening rates.

Key words: microfiltration, whey protein depletion, Cheddar cheese, melting

INTRODUCTION

Modern cheese plants are increasingly focused on improving the consistency of their production processes and increasing cheese yields. New cheese plants have also become very costly to build, which encourages cheese makers to increase throughput in their existing plants by using concentrated milks. The most common approaches for concentrating cheese milk include the use of skim milk powder, condensed milk, or UF retentates (Johnson, 2017). Creamer et al. (1987) suggested that cheeses with higher whey protein (**WP**) content had slower proteolysis, slower development of characteristic flavors and textures, increased hardness, and altered melting behavior throughout ripening. Previous studies have suggested that cheeses made from UF-concentrated milk had slower proteolysis due to inhibition of rennet or plasmin activity (Lelievre and Lawrence, 1988; Bech, 1993). It has been suggested that WP may contribute to the inhibition of rennet or plasmin (Covacevich and Kosikowski, 1978). The exact mechanism by which WP may contribute to slower proteolysis in cheese is still debated. However, in some past studies, cheeses made from concentrated milk also had different pasteurization temperatures and altered ratios of rennet to casein, and they ended up with different cheese compositions like lower moisture contents. These factors could also have contributed to differences in

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*Corresponding author: rani@cdr.wisc.edu

ripening behavior between the control and the cheeses made from concentrated milk.

Microfiltration (**MF**) is another potential option for concentrating cheese milk (Govindasamy-Lucey et al., 2007). Initially the dairy industry's use of MF focused on removing bacteria, spores, and somatic cells from milk as well as residual milk fat from cheese whey for the manufacture of WP isolate (Maubois, 2002). Microfiltration has been used for the separation of casein micelles from WP in milk, and that casein concentrate can then be used for cheese-making (Pierre et al., 1992). Nelson and Barbano (2005a) optimized an MF process to maximize removal of WP from skim milk that involved MF of milk, UF of the MF permeate, and diafiltration (**DF**) of the MF retentate with UF permeate. An MF membrane with a pore size between 0.1 and 0.2 μm can be used to retain caseins and allow WP to permeate. Previous studies have explored the use of MF milk with both high and low concentration factors (**CF**) for cheese-making. Several studies had indicated that use of MF concentrates for Cheddar cheese-making resulted in cheeses with higher protein levels but lower moisture contents (St-Gelais et al., 1995; Neocleous et al., 2002a; Xia et al., 2020). However, for cheese milk derived from MF concentrates, simple adjustments to the cheese-making procedures (lowering setting temperature, increasing the curd size, and lowering the wash water temperature) can obtain higher (normal) moisture contents (Govindasamy-Lucey et al., 2007).

Ardisson-Korat and Rizvi (2004) found that MF milks with a high CF of 6, 7, 8, or 9 produced mozzarella cheeses with slower proteolysis than commercial samples. Although they were microfiltered, their cheese milks had higher WP contents than the original skim milk. This may have been due to the very high CF used. Even with lower-CF (1.26–1.82×) MF milk, as was produced in the study by Neocleous et al. (2002b), the WP content was still higher in MF milks than in the control, and subsequently MF cheeses had slower proteolysis when compared with the control. Our recent studies in unconcentrated milk showed that there were no differences in the rate of proteolysis in Cheddar cheeses manufactured from milks with various WP depletion levels (Reale et al., 2020). This might suggest that any potential inhibition of rennet/plasmin in cheese might only occur at higher residual WP levels (these cheeses only had WP levels ≤0.11%) (Reale et al., 2020).

The objective of this study was to explore the effect of using concentrated milk (~5% casein) and varying the amount of WP depletion in the cheese milk on the textural, functional, compositional, and ripening properties of Cheddar cheese.

MATERIALS AND METHODS

No animals were used in this study, and ethical approval for the use of animals was thus deemed unnecessary.

Four trials were performed to remove various levels of WP via MF from the cheese milk that had been concentrated 2× for casein (~5% casein; Figure 1). For each trial, whole milk obtained from the University of Wisconsin-Madison Dairy Plant was pasteurized at 73°C for 19 s, cooled to 23°C, and then subjected to membrane filtration mostly as described by Reale et al. (2020). From each trial, we produced one control milk and 2 experimental milks of differing casein-to-true-protein (**CN:TP**) ratios (89:100 CN:TP and 95:100 CN:TP, respectively). The control milk had a CN:TP ratio of 83:100, as is typical in most standardized milk used for Cheddar cheese.

Two MF elements (model V0.2–2B-8038, Synder Filtration) made from polyvinylidene fluoride–based material with a spiral-wound configuration were used in parallel. They were 203.2 mm in diameter and 965.2 mm long, yielding approximately 68.4 m^2 of total membrane area. The feed spacer was 0.8 mm thick, and the pore sizes were around 0.2 μm. The MF system was run at about 23°C with a flux of about 14 L/min, with inlet and outlet pressures of around 110.3 and 25.5 kPa, respectively. We also used 6 UF elements (model ST-3B-4338, Synder Filtration) made of polyethersulfone-based material with a spiral-wound configuration to concentrate the pasteurized control milk. They were 109.2 mm in diameter and 965.2 mm long, yielding 43.2 m^2 of total membrane area. The feed spacer was 1.2 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 11 L/min, with inlet and outlet pressures of around 303.4 and 96.5 kPa, respectively.

Each trial involved 4 processing steps. The first step was to UF some of the pasteurized whole milk to concentrate the casein 2-fold without depleting any WP during the concentration process (control milk; Figure 1). The UF retentate (**UFR1**) and permeate (**UFP1**) streams were later used for standardization of the control milk. The second step was to MF the rest of the pasteurized whole milk to concentrate it and deplete WP. The third step was the UF (using 8 UF elements; model ST-2B-3838, Synder Filtration) of the MF permeate to separate WP from other materials, such as water, lactose, vitamins, and minerals. The UF membranes were used because the smaller pore size allowed for retention of WP, while other smaller molecules could pass through. The final step was the DF of the MF retentate with UF permeate (**UFP2**)

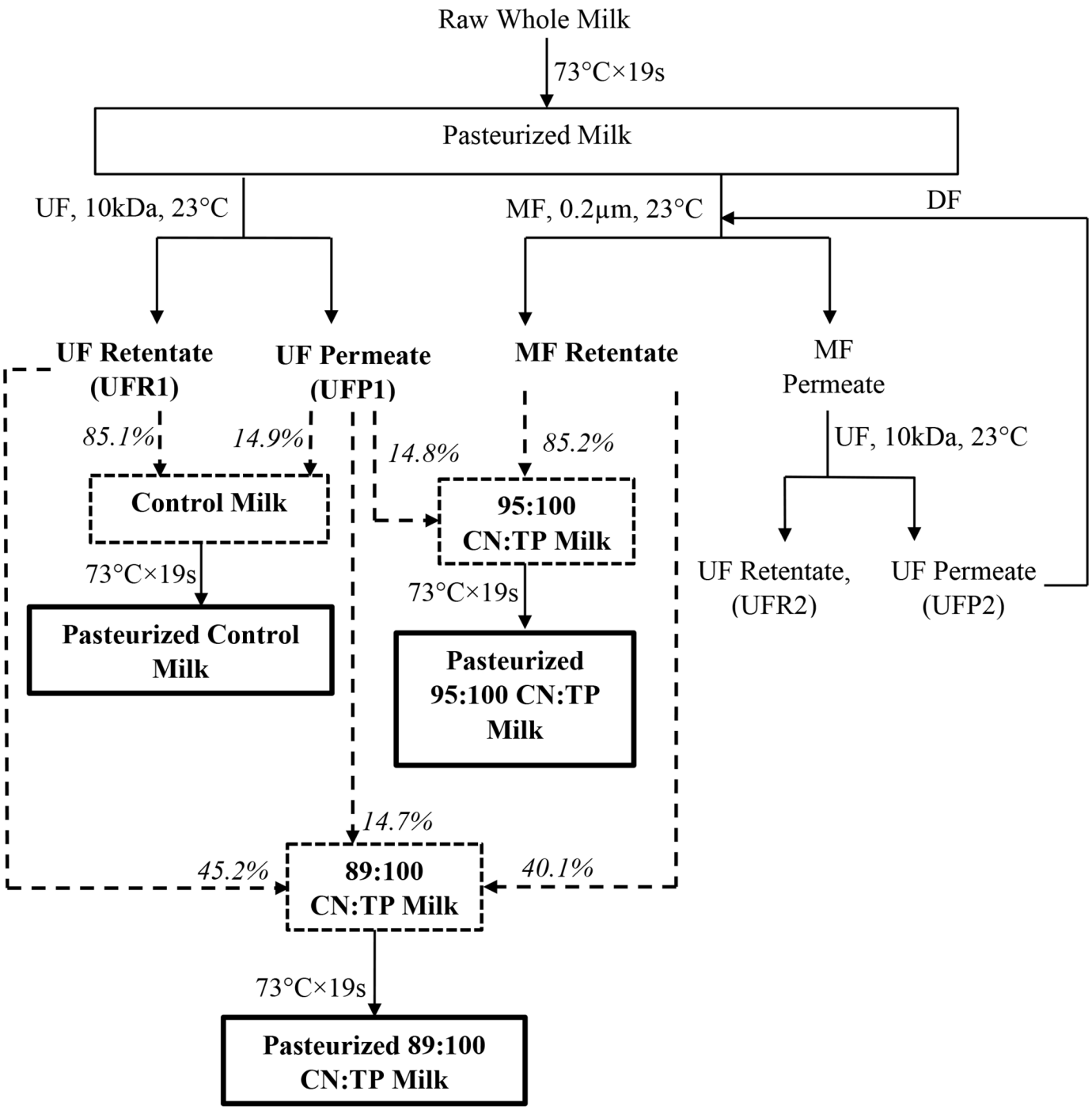

Figure 1. The filtration process [microfiltration (MF) and UF] applied to produce the different milk fraction streams used in the preparation of the standardized cheese milks for the 3 treatments. Average percentage weights of the different milk fraction streams used to prepare the standardized cheese milks are given in italics. CN:TP = casein:true protein ratio; DF = diafiltration.

such that it could be fed back through the MF system to help remove WP (similar to the approach of Nelson and Barbano, 2005a); concentration was continued until we reached ~5% casein. The DF step kept the MF feed volume and rate constant so that the flux, and therefore WP removal efficiency, did not decrease as WP was removed via the MF permeate. This recirculation of MF retentate and UFP2 through the system was continued until the targeted WP depletion level was achieved.

Table 1. Average weights and composition of pasteurized whole milk, microfiltration (MF) retentate, UF retentate, and UF permeate used in preparation of standardized milks for different treatments[1]

Variable	Pasteurized whole milk	MF retentate	UF retentate[2]	UF permeate[3]
Composition				
Solids (%)	12.31 ± 0.05	20.58 ± 2.07	22.06 ± 2.03	5.41 ± 0.27
Fat (%)	3.54 ± 0.04	8.95 ± 1.22	8.82 ± 1.06	ND[4]
Total protein[5] (%)	3.18 ± 0.12	6.66 ± 1.16	7.55 ± 1.04	0.17 ± 0.01
TP[6] (%)	3.00 ± 0.13	6.41 ± 1.09	7.34 ± 1.05	ND
Casein[7] (%)	2.50 ± 0.10	6.16 ± 1.07	6.21 ± 0.87	ND
Casein:total protein (%)	78.48 ± 0.28	92.42 ± 1.27	82.19 ± 0.29	ND
Casein:TP (%)	83.39 ± 0.60	96.10 ± 0.94	84.54 ± 0.21	ND
Casein:fat	0.71 ± 0.03	0.69 ± 0.03	0.70 ± 0.04	ND
Weight (%)				
Control	—	—	85.1 ± 12.2	14.9 ± 12.2
89:100 casein:TP	—	40.1 ± 4.6	45.2 ± 8.9	14.7 ± 12.9
95:100 casein:TP	—	85.2 ± 13.6	—	14.8 ± 13.4

[1]Values represent the means ± SD of 4 replicates for each treatment.
[2]UF retentate obtained from UF of pasteurized whole milk (UFR1).
[3]UF permeate obtained from UF of pasteurized whole milk (UFP1).
[4]ND = not determined.
[5]Total % N × 6.35.
[6]True protein = (total % N – % nonprotein N) × 6.35.
[7](Total % N – % noncasein N) × 6.36.

We produced milks with 3 different WP contents by blending appropriate ingredients to obtain the desired CN:TP ratios (Table 1). All milks were standardized to similar casein concentration (~5%) and a casein-to-fat ratio of ~0.7. The control milk was prepared by blending UFR1 and UFP1. The 89:100 CN:TP milk was standardized by blending MF retentate, UFR1, and UFP1 streams. The 95:100 CN:TP milk was a blend of the MF retentate and UFP1. All 3 standardized milks were then pasteurized a second time at 73°C for 19 s.

Cheese Manufacture

Four batches of milled-curd Cheddar cheese were manufactured by licensed Wisconsin cheese makers at the University of Wisconsin-Madison Dairy Plant over a period of 4 mo. On each cheese-making day, 3 square, jacketed stainless steel open cheese vats (Stoelting LV60), with a maximum capacity of 272 kg of milk, were used to manufacture cheeses. Milk for each individual vat was sampled for chemical analysis and then weighed on a floor scale (model 31-1822-FD, Toledo Scale Co.) before being gravity-fed into each individual vat. Each vat contained 125 kg of cheese milk. Lactic acid (88%, wt/wt; Chr. Hansen) was diluted (wt/wt) at a rate of 4 parts water to 1 part lactic acid, and the diluted acid was added to the cold milk (~5°C). The addition of diluted lactic acid to the standardized milks lowered the initial pH from 6.63 ± 0.04 to 6.20 ± 0.02 in all the milks. Preacidification is often applied to concentrated milks used for cheese-making. The milks were then heated to 28.9°C. Direct vat-set starter culture, containing *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* blend (MA19, DuPont Danisco), was added at the rate of 12 g per 125 kg of milk. After 60 min of ripening, fermentation-produced calf chymosin (Chy-Max Extra, 630 international milk clotting units/mL; Chr. Hansen) was used as coagulant at the rate of 24 g per 125 kg milk (3.89 mL of chymosin/kg of casein). The coagula were cut on similar firmness (~5 min) as evaluated subjectively by an experienced licensed Wisconsin cheese maker. The coagula were cut with 1.27 cm knives at pH 6.12 ± 0.05 (determined by taking the pH of the whey immediately after cutting), healed for 2 min, and then agitated for 20 min. Curd was then heated to 39°C, over 30 min. Whey was drained slowly (~25 min) when the curd reached a pH of about 6.0, and the curd was allowed to mat. The matted curd was cut into 6 equal-sized slabs. Slabs were stacked 2 high and turned every 20 min. When the curd pH reached 5.40, it was milled, and salt was added at a rate of 770 g per 125 kg of milk in 3 equal portions, 5 min apart. Curd was divided into 2 9-kg rectangular Wilson hoops (0.37 m × 0.29 m × 0.08 m) and pressed at 414 kPa for 4 h. Cheese blocks were then removed from the hoops and left overnight at ambient temperature (~22°C) to simulate the slow cooling regimen used by some in the industry to encourage more complete fermentation of lactose by the starter cultures.

The next morning the cheese blocks were vacuum-packaged and stored at 4°C. The cheeses were analyzed at 1, 14, 30, 90, 180, and 270 d.

Compositional Analysis

All compositional analyses for each sample were carried out in duplicate. Ultrafiltered retentate, UFP1, MF retentate, whole milk, standardized milk samples, drained whey, and pressed whey were analyzed for TS (Green and Park, 1980), protein (total percentage N × 6.35, Kjeldahl method; AOAC International, 2000), casein (AOAC International, 2000), nonprotein nitrogen (AOAC International, 2000), fat content (Mojonnier method; AOAC International, 2000), lactose content (high-performance ion-exchange chromatography; Dionex ICS-5000 RFIC-EG Dual System, Thermo Fisher Scientific Inc.; Møller et al., 2012), and total calcium (inductively coupled argon plasma emission spectroscopy; 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, then subtracting soluble Ca from the total Ca content of the milk (Hassan et al., 2004).

The cheeses were sampled after 2 wk for compositional analysis. At the time of sampling, a 2.5-cm-thick slab was cut off the block of cheese, and the outer edges were discarded. This slab was further sampled for each analysis. This cheese sample was completely ground and used for analysis. The ground cheese samples (~200 g) were analyzed for moisture (Marshall, 1992), fat (Mojonnier method; AOAC International, 2000), protein (Kjeldahl method; AOAC International, 2000), lactose (Møller et al., 2012), salt (chloride electrode method; Johnson and Olson, 1985), and total Ca and Na via inductively coupled argon plasma emission spectroscopy (Govindasamy-Lucey et al., 2007). pH was measured by inserting a spear-tip pH electrode (AB15, Fisher Scientific) into a cheese block at 1, 14, 30, 90, 180, and 270 d of ripening. Lactose, galactose, and lactic acid contents in cheese were measured by high-performance ion-exchange chromatography (Møller et al., 2012) at 1, 14, 30, 90, 180, and 270 d of ripening. The cheese extracts used for chromatographic analyses were prepared as 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, 180, and 270 d of ripening.

Fat Globule Particle Size Analysis

The particle size distribution of the whole milk, UF retentate, and MF retentate was measured by laser light scattering (Mastersizer 2000, Malvern Instruments), mainly to determine the fat globule size distribution. Samples were diluted in deionized water, and measurements were performed in duplicate at an obscuration value between 12% and 13%. The particle size distribution was calculated from the light scattering pattern using Mie theory. A refractive index of 1.47 and an absorption of 0.01 for milk fat were used (Michalski et al., 2001). Water was used as the dispersant (refractive index of 1.33), and measurements were carried out in triplicate for each sample.

Mass Balance and Recoveries

A mass balance was carried out for each vat of cheese according to Govindasamy-Lucey et al. (2006). The milk was weighed with a Mars scale (Mars Scale Manufacturing, ISG Series), and the drain and press wheys were weighed on a Rice Lake scale (Rice Lake Weighing Systems, IQ Plus 255). Cheeses were weighed on a Cream City scale (Cream City Stateline Scale, CW-80) for each treatment. The percentages of nitrogen, fat, and 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 the cheese divided by the weight of the original milk (including the amount of culture added during cheese manufacture), multiplied by 100. The approach described by Govindasamy-Lucey et al. (2006) was used to determine the predictive cheese yield and the recoveries of fat, casein, and other solids in cheese. Predictive cheese yields were calculated for each vat using the Van Slyke cheese yield model equation (equation as shown below; Van Slyke and Price, 1936).

$$\text{Van Slyke Cheese Yield} = \frac{\left[\left(\text{RF}\times\%\text{Fat in milk}\right)+\left(\text{RC}\times\%\text{CN in milk}\right)\times\text{RS}\right]}{\left(100-\%\text{Moisture of cheese}\right)}\times 100,$$

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

Nutritional Analysis of Cheeses

After 1 d of ripening, nutritional analyses on the cheese samples were carried out by Covance Laboratories Inc. (Madison, WI), as described by Reale et al. (2020). Total calories, total fat, protein, vitamin A, vitamin C, minerals/elements (iron, Ca, and Na), ash, and moisture were measured in all the cheeses from 2 trials.

Rheological and Textural Analyses

Rheological properties of the cheese samples were measured by dynamic small amplitude oscillatory rheology as described by Reale et al. (2020). Temperature sweep test (from 5°C to 85°C at a heating rate of 1°C/min) was used with a frequency of 0.08 Hz and a strain of 0.5% to measure the storage modulus (**G′**), loss modulus (**G″**), and loss tangent (**LT**, i.e., the ratio between the viscous and the elastic properties of the material; LT = G″/G′) values. The LT values observed during heating were also recorded. We calculated the temperature where LT = 1 (i.e., where G″ = G′), as this indicates the transition from a solid to a liquid-like system (i.e., a crossover point). Texture profile analyses were carried out using a TA.XT2 Texture Analyzer (Texture Technologies Corp.), as described by Reale et al. (2020). Both rheological and textural tests were carried out at 2 wk and 1, 3, 6, and 9 mo.

Whey Protein Content of Milk and Recovery in Cheese

The WP content in milk and cheese was determined using reverse-phase (**RP**)-HPLC as described by Reale et al. (2020). 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). Standards were prepared in duplicate. Standard curves were plotted using the concentration of the standard protein and its corresponding peak area.

Rennet whey samples were prepared by coagulating the milks using a 10-fold diluted rennet (Chymax Extra, double strength; Chr. Hansen), as described by Reale et al. (2020). The coagulum was cut with a spatula and centrifuged, and the supernatant was filtered. Rennet whey samples for RP-HPLC were prepared according to the method of Bobe et al. (1998). 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 the rennet whey, and a correction factor was used. The correction factor was calculated based on the solids contents in the milk and rennet whey (Davies and White, 1960).

Water-soluble extract was prepared from 1-d-old cheeses by the method of Kuchroo and Fox (1982). Young cheese was used to reduce the number of peptide peaks (to facilitate the identification and analysis of individual WP peaks) in the chromatogram that would be generated during proteolysis (ripening). Whey protein content in water-soluble extract was calculated using the prepared standard curves (α-LA, β-LG, BSA). Based on Kuchroo and Fox (1982), recovery of this single extraction method was 70%, which was used for calculating WP content in cheese.

The determination of WP in milk and cheese by RP-HPLC was based on the method of Bonfatti et al. (2008) with slight modifications as described by Reale et al. (2020). The percentage of residual WP recovered in the cheese was calculated using the amount found in the starting milks and their respective cheeses as determined via RP-HPLC analysis.

Proteolysis and Urea-PAGE Gels

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 duplicate at 4 d, 2 wk, and 1, 3, 6, and 9 mo.

The breakdown of α_S-CN and β-CN during ripening was monitored with urea-PAGE gels, which were prepared as described by Ozturk et al. (2013). Photographs of the gels underwent densitometric analysis using image analysis software (GelAnalyzer 2010 version 1.6; Lazar software).

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). Cheese cubes (2 cm × 2 cm × 2 cm) were evaluated at 11°C. Samples were identified with random 3-digit numbers. The attributes of firmness, cohesiveness, chewiness, adhesiveness, sweetness, saltiness, bitterness, acidity, sourness, rancidity, astringency, and tasting burnt, buttery, brothy, milk fat, cardboard, or sulfur were scored on a 15-point scale, 0 being absence of the characteristic and 15 being overwhelming presence of the characteristic. The definitions of the attributes used by the trained panelists to evaluate cheeses were described by Ibáñez et al. (2020).

Experimental Design and Statistical Analysis

Four replicate cheese-making trials were carried out over a period of 4 mo. In each trial, 3 5% casein standardized milks (i.e., 83:100 CN:TP or control, 89:100 CN:TP, and 95:100 CN:TP) were used to make Cheddar cheese. A 3 × 4 completely randomized block design, which incorporated all 3 treatments and all 4 replicate trials, was used for analysis of the response variables relating to milk, cheese, and whey composition. Analy-

sis of variance was performed using SAS (version 9.4; SAS Institute Inc.). The 3 different standardized milks (treatments) were analyzed as discontinuous variables, whereas the 4 cheese-making 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 (Montgomery, 2013) was used to monitor the effects of both treatment and ripening time and their interactions on pH, lactic acid contents, lactose contents, INSOL Ca, proteolysis, and textural, rheological, and sensory properties. For the whole-plot factor, the treatment was analyzed as a discontinuous variable, and the cheese-making trial was blocked. For the subplot factor analysis, age was treated as a continuous variable. The interactive term "treatment × cheese-making trial" was treated as the error term for treatment effect. Analysis of variance for the split-plot design was carried out with SAS. When significant differences ($P < 0.05$) were found, the means of the different treatments were analyzed using Duncan's multiple-comparison test.

RESULTS AND DISCUSSION

Composition and Particle Size Analyses of Fluids

The composition of all the different streams collected during the UF and MF steps is given in Table 1. The true protein (**TP**) contents of the pasteurized whole milk, UFR1, and MF retentate were about 3.00%, 7.34%, and 6.41%, respectively (Table 1). The casein contents of the MF and UF retentates were 6.16 ± 1.07% and 6.21 ± 0.87%, respectively. This indicated that the UF and MF systems successfully concentrated casein more than 2-fold. The UFP1 only contained about 0.17% total protein (and no TP was detected), which was likely to be NPN rather than actual protein. Thus, the CN:TP ratio of the UF retentate was similar to that of the pasteurized whole milk (83:100), as the UF membrane only permeated the NPN. The CN:TP ratio of the MF retentate was much higher at 96:100, as most of the WP had permeated through the MF membrane (into the MF permeate stream).

Fat globule size measurement indicated that there was a small difference in the volume mean diameter (**D[4,3]**) of fat globules in the whole milk, UF, and MF retentates. The D[4,3] values for the MF retentate was 3.49 μm, which was slightly smaller than the values for the whole milk and UF retentates, 3.72 and 3.65 μm, respectively. There was no difference in the D[4,3] values between the whole milk and UF retentates. Harsh mechanical treatment of milk, such as extensive pumping or membrane filtration, could potentially have a homogenization effect on the milk fat globules. However, the decrease in the D[4,3] value of the milk fat globule in the MF retentate compared with the whole milk was very minor (0.23 μm) and did not indicate that fat globules were homogenized since homogenized fat globules would have D[4,3] value of < 1 μm. Previous research (Michalski et al., 2006; Reale et al., 2020) found that larger milk fat globules were retained in MF retentate compared with that for whole milk due to the loss of very small fat globules (<0.1 μm) into the MF permeate.

Composition of Standardized Cheese Milks and Drain Whey

The fat and casein contents were similar for the 3 standardized milks at around 7.0% and ~5.0%, respectively (Table 2). The TS, lactose, total protein, and TP content all slightly decreased with MF. This was in agreement with previous work (Nelson and Barbano, 2005b; Reale et al., 2020), where TS content decreased with WP depletion of milk via MF. Because less, or none, of the UF retentate was used for standardizing the 89:100 and 95:100 CN:TP treatments, respectively, the retained components were not all added back. This decrease in lactose and TP contents contributed to the decrease in TS with MF treatment (Reale et al., 2020). The WP was highly depleted in cheese milk from 1.02% in the control to 0.67% and 0.21% in the 89:100 CN:TP and 95:100 CN:TP cheese milks, respectively (as measured by the RP-HPLC method). Because the casein content was kept consistent (~5%), while the WP was depleted, the CN:total protein and CN:TP ratios significantly increased with MF treatment. Our cheese-making process involved an initial pasteurization of all milks before filtration, and again the standardized milks were pasteurized before cheese-making. The CN:TP ratio was 78.4% in the pasteurized control whole milk (Table 1), indicating that heat treatment caused only a minor level of WP denaturation, which was consistent with prior trends we have observed with these pasteurization conditions.

The TS, casein, total protein, and TP contents in drain whey decreased with increasing WP depletion (Table 2). There were few differences in the composition of the press wheys (Table 2), except for the total proteins and TP, which also decreased when WP was highly depleted.

Composition of Cheeses

There were only minor differences in the composition of the 3 types of cheeses (Table 2). This was in agreement with Nelson and Barbano (2005b), who found that

Table 2. Compositions of standardized milks that had various levels of whey protein removal and the derived drain whey, press whey, and Cheddar cheese[1]

	Treatment[2]				
Component	Control	89:100 CN:TP	95:100 CN:TP	SEM	*P*-value[3]
Standardized cheese milk					
Lactose (%)	4.03[a]	3.86[b]	3.58[c]	0.03	<0.01
Solids (%)	18.41[a]	18.16[a]	17.46[b]	0.12	<0.01
Fat (%)	6.89[a]	7.15[a]	7.23[a]	0.09	NS
Total protein[4] (%)	6.02[a]	5.72[b]	5.28[c]	0.06	<0.01
TP[5] (%)	5.80[a]	5.53[b]	5.10[c]	0.06	<0.01
Casein (CN)[6] (%)	4.95[a]	4.96[a]	4.92[a]	0.05	NS
CN:total protein (%)	82.19[c]	86.83[b]	93.13[a]	0.43	<0.01
CN:TP (%)	85.31[c]	89.82[b]	96.52[a]	0.49	<0.01
CN:fat	0.72[a]	0.70[ba]	0.68[b]	0.01	<0.05
Whey protein[7] (%)	1.02[a]	0.67[b]	0.21[c]	0.02	<0.01
Total calcium[8] (mg/100 g of milk)	189.0[a]	190.0[a]	188.0[a]	1.51	NS
Total calcium (mg/g protein)	31.49[c]	33.29[b]	35.62[a]	0.08	<0.01
Total calcium (mg/g casein)	38.30[a]	38.34[a]	38.25[a]	0.20	NS
Drain whey					
Solids (%)	8.31[a]	7.71[b]	6.68[c]	0.08	<0.01
Fat (%)	0.83[a]	0.87[a]	0.76[a]	0.03	NS
Total protein (%)	1.63[a]	1.22[b]	0.69[c]	0.02	<0.01
True protein (%)	1.34[a]	0.94[b]	0.42[c]	0.02	<0.01
Casein (%)	0.13[a]	0.08[b]	0.04[c]	0.01	<0.01
Whey protein (%)	1.21[a]	0.85[b]	0.38[c]	0.02	<0.01
Press whey					
Solids (%)	20.55[a]	21.05[a]	19.66[a]	0.58	NS
Fat (%)	1.74[a]	2.64[a]	1.85[a]	0.28	NS
Total protein (%)	1.40[a]	1.26[a]	0.92[b]	0.05	<0.01
True protein (%)	1.14[a]	0.99[b]	0.64[c]	0.04	<0.01
Casein (%)	0.11[a]	0.11[a]	0.11[a]	0.04	NS
Whey protein (%)	0.26[a]	0.27[a]	0.29[a]	0.02	NS
Cheese (at 14 d)					
Moisture (%)	36.05[a]	36.32[a]	36.10[a]	0.21	NS
Fat (%)	33.63[a]	33.85[a]	34.50[a]	0.27	NS
Salt (%)	1.91[a]	1.79[a]	1.71[a]	0.05	NS
Protein[8] (%)	25.19[a]	24.91[a]	24.49[b]	0.09	<0.01
Sodium[9] (mg/100 g of cheese)	910.0[a]	834.0[ba]	794.0[b]	18.83	<0.05
Calcium[9] (mg/100 g of cheese)	680.0[a]	673.0[a]	681.0[a]	3.42	NS
Calcium (mg/g protein)	26.97[b]	27.00[b]	27.78[a]	0.16	<0.05
Moisture in nonfat substance (%)	54.33[a]	54.90[a]	55.11[a]	0.20	NS
Fat in DM (%, dry weight basis)	52.59[b]	53.15[ba]	53.98[a]	0.30	0.05
Salt-in-moisture phase (%)	5.31[a]	4.93[ba]	4.73[b]	0.13	<0.05
Whey protein[7] (%)	0.38[a]	0.24[b]	0.07[c]	0.01	<0.01

[a–c]Means within the same row not sharing a common superscript differ ($P < 0.05$).

[1]Values represent the means of 4 replicates for each treatment.

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

[3]Value for full statistical model that incorporated all 3 treatments and 4 blocks (4 replicate cheese-making days).

[4]Total % N × 6.35.

[5](Total % N – % nonprotein N) × 6.35.

[6](Total % N – % noncasein N) × 6.36.

[7]Measured by RP-HPLC.

[8]Total % N × 6.31.

[9]Measured by inductively coupled argon plasma emission spectroscopy.

changing the amount of serum protein in MF cheese milks did not significantly affect cheese composition, as expected, since WP is only a minor component of the solids in cheese. The protein content slightly decreased in the 95:100 CN:TP cheese, although all the casein contents for all milks were similar. This could be due to the decrease in WP content in cheeses made from MF milks compared with the cheeses made with control milk.

The fat, salt, and moisture in nonfat substance were not significantly different between treatments. In pre-

vious studies, the moisture contents in cheeses made from concentrated MF milk were lower than for cheeses made from unconcentrated milks (St-Gelais et al., 1995; Neocleous et al., 2002a). The decreased moisture content of cheeses made from MF-concentrated milk can be corrected by adjusting coagulation and cutting conditions (Govindasamy-Lucey et al., 2007). All cheeses in our study were made from cheese milks with the same casein contents. In practice, in milks with higher protein levels (> 4.0%), the cutting process becomes more difficult because of short gelation times and very rapid gel-firming rates (Sutherland and Jameson, 1981; Guinee et al., 1994). Thus, to reduce the rate of protein aggregation in our cheese milks, as we had used high casein contents (~5%), the renneting temperature was reduced from 32°C to 28.9°C for all the cheese milks (Govindasamy-Lucey et al., 2011). Additionally, all cheese samples were manufactured from milks that were preacidified to pH 6.2, which helped to counteract the effects of casein concentration of milk on the moisture content of the cheeses. Preacidification is often used with milks high in casein content. (Govindasamy-Lucey et al., 2007). The higher casein content and associated insoluble Ca requires more acidification before cutting the coagulum (or in the finished cheese) to obtain a cheese with similar properties compared with cheese made from milk that has less casein (Johnson and Lucey, 2006). Thus, preacidification was carried out to remove some of the colloidal calcium phosphate content in milk at 5°C before adjusting the milk to the ripening temperature and inoculating it with starter.

Fat, Nitrogen, Solids, and WP Recoveries in Cheese

The amount of fat recovered in the cheeses was significantly different between treatments, with highest recoveries in the 95:100 CN:TP cheese (Table 3). In cheese made from unconcentrated milk, depletion of WP using MF had no impact on fat recovery (Reale et al., 2020). Previous studies (Govindasamy-Lucey et al., 2007; Brandsma and Rizvi, 2001; Nelson and Barbano, 2005b) reported that cheeses made from MF-concentrated milk had higher fat recovery than the control (unconcentrated milk). However, all our treatments had milks with similar fat and casein contents, and they were manufactured by identical cheese-making processes. One possibility was that the lower WP in the 95:100 CN:TP milk altered the gelation properties and thereby improved the fat recovery, as there were lower fat losses in the drain whey for the 95:100 CN:TP sample (Table 3).

The amount of nitrogen recovered in the control, 89:100 CN:TP, and 95:100 CN:TP cheeses was 76.5%, 82.1%, and 88.7%, respectively (Table 3). The solids recovery also increased from 63.0% in the control cheese to 65.7% and 69.6% in the 89:100 CN:TP and 95:100 CN:TP cheeses, respectively. Previous studies have also seen an increase in solids and nitrogen recoveries in MF cheeses as compared with the control (Govindasamy-Lucey et al., 2007; Neocleous et al., 2002a; Nelson and Barbano, 2005b; Reale et al., 2020). The amount of WP in milk decreased with its depletion by MF, as expected (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. Caseins are effectively (~95%) recovered in cheese-making, so increasing the proportion of casein in cheese milk results in a more efficient cheese-making process in terms of nitrogen recovery. The increased nitrogen and solids recovery in the present study likely resulted because in the control milk, more of the starting nitrogen and solids content was WP compared with the 89:100 CN:TP and 95:100 CN:TP samples. Thus, cheeses made from milk with less WP and more casein as a function of the nitrogen in the milk would likely have a higher percentage of nitrogen and solids recovery. We also noted that the casein content in the drain whey (Table 2) was significantly lower in the 95:100 CN:TP sample compared with the control. This suggested that casein losses, such as casein fines, were lower in the WP-depleted cheeses. Since the milk compositions were similar, WP depletion seemingly somehow modified the gel structure and decreased cutting losses. We can speculate that in the presence of high amounts of WP in a rennet gel, the curd healing process after cutting could be impaired. Maybe in a system without WP, faster fusion of casein particles might occur after cutting.

The amount of WP recovered in the control, 89:100 CN:TP, and 95:100 CN:TP cheeses was 6.8%, 6.7%, and 6.4%, respectively (Table 3). Although the percentage of WP recovery was slightly lower in the 95:100 CN:TP cheese, the difference was not statistically significant ($P > 0.05$).

Cheese Yield

Actual cheese yield and the Van Slyke cheese yield (Table 4) in the 95:100 CN:TP cheese sample was significantly higher compared with the control cheese, both having been increased with WP depletion. The increase in cheese mass recovery was due to a significant increase in fat, nitrogen, and solids recoveries in the MF cheeses (Table 3). It has been previously reported that WP inclusion in cheese via UF-concentrated cheese milk increases cheese yield (Goudédranche et al., 1980). The higher residual WP content in the control cheese (Table 2) should help increase its yield, but the control cheese

Table 3. Fat, nitrogen, and solids recoveries in the Cheddar cheeses manufactured from milks that had various levels of whey protein depletion[1]

	Treatment[2]				
Component recovery	Control	89:100 CN:TP	95:100 CN:TP	SEM	*P*-value[3]
Fat recovery (%)					
Cheese	88.56[b]	88.72[ba]	90.78[a]	0.60	<0.05
Drain whey	9.54[a]	9.76[a]	8.23[b]	0.30	<0.05
Press whey	0.58[a]	0.87[a]	0.60[a]	0.10	NS
Total	98.68	99.35	99.61		
Nitrogen recovery (%)					
Cheese	76.54[c]	82.17[b]	88.73[a]	0.65	<0.01
Drain whey	21.73[a]	16.72[b]	10.41[c]	0.39	<0.01
Press whey	0.74[a]	0.71[a]	0.39[b]	0.06	<0.05
Total	99.01	99.60	99.53		
Solid recovery (%)					
Cheese	63.02[c]	65.67[b]	69.59[a]	0.33	<0.01
Drain whey	35.86[a]	33.62[b]	30.26[c]	0.29	<0.01
Press whey	2.37[a]	2.58[a]	2.51[a]	0.06	0.13
Total	101.25	101.87	102.36		
Whey protein recovery in cheese (%)	6.76[a]	6.65[a]	6.39[a]	0.34	NS
Drain whey[4] (%, mass)	78.86[a]	78.45[a]	78.42[a]	0.23	NS
Press whey[4] (%, mass)	2.13[a]	2.23[a]	2.25[a]	0.10	NS

[a–c]Means within the same row not sharing a common superscript differ ($P < 0.05$).

[1]Values represent the means of 4 replicates for each treatment.

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

[3]Value for full statistical model that incorporated all 3 treatments and 4 blocks (4 replicate cheese-making days).

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

had a lower yield than the 95:100 CN:TP cheese (Table 4). Govindasamy-Lucey et al. (2007) saw an increased cheese yield in MF cheeses compared with the control, although these MF milks also had higher WP content than the control. The MF-treated milks in the present study had far lower WP content than previous studies (due to the WP depletion approach used here). Even though the cheeses were all made from milks with the same amount of casein, more cheese mass was obtained even when WP was depleted. This is in agreement with Nelson and Barbano (2005b), who saw an increase in cheese yield even with WP depletion, and this increase was attributed to increased fat recovery. This suggests that the increased cheese yield in the present study was likely due to the increased fat, nitrogen, and solids recoveries in the MF-treated cheeses.

Nutritional Analysis of Cheese

The full nutritional profile of cheeses was analyzed to understand if WP depletion also removed key nutrients, which could be a concern for some consumers. However, there were only minimal difference in nutrients between treatments (Table 5). There was also a slight decrease in the ash and sodium content with increasing WP depletion, which was expected because the salt content of the cheeses decreased slightly (although not significantly; Table 2). Similar decreases were seen when MF treatment was used to deplete WP without concentrating the casein in milk (Reale et al., 2020). This decrease was attributed to salting all the curds at a constant milk volume even though MF treatment did cause a slight increase in actual cheese mass (Reale et al., 2020). There were slightly higher levels of vitamin A in the 95:100 CN:TP cheese, which might be due to the slightly higher fat content in that milk sample (Table 2) as vitamin A is a fat-soluble vitamin. There were no other significant differences in the nutritional quality of the 3 cheese treatments, suggesting that use of MF to concentrate milk, even to high casein contents, did not cause any detrimental change in the nutritional properties of Cheddar cheese.

pH, Lactose, and Lactic Acid

The lactose contents in all the cheeses were affected by treatment (Tables 6 and 7), fermenting slowly during ripening, possibly due to the starter cultures being sensitive to this salt-in-moisture level (Table 2). The amount of residual lactose in cheese decreased during

Table 4. Actual and calculated cheese yield values for Cheddar cheeses that had various levels of whey protein depletion[1]

Item	Treatment[2]			SEM	*P*-value
	Control	89:100 CN:TP	95:100 CN:TP		
RF value[3]	0.886	0.887	0.908	ND[4]	ND
RC value[5]	0.930	0.940	0.940	ND	ND
RS value[6]	1.084	1.084	1.087	ND	ND
Actual yield[7] (%)	18.15[b]	18.73[ba]	19.02[a]	0.19	0.04
Van Slyke cheese yield[8] (%) using RF, RC, and RS values	18.14[b]	18.74[ba]	19.02[a]	0.19	0.04

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

[1]Values represent the means of 4 replicates for each treatment.

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

[3]RF is the fat recovered in cheese, determined experimentally from cheese trials.

[4]ND = not determined.

[5]RC was 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 0.926, 0.940, and 0.947, respectively. Thus, all calculations were carried out using an average RC value of 0.930 for control and 0.940 for both 89:100 CN:TP and 95:100 CN:TP cheeses, respectively.

[6]RS is the recovery of noncasein, nonfat solids in cheese; it was calculated as described in Govindasamy-Lucey et al. (2006).

[7]Actual 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.

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

ripening, and lower levels were observed in the 95:100 CN:TP cheese (Table 6), likely due to its lower salt-in-moisture content (Table 2). By 270 d of ripening, there were no major differences in the lactose contents between the cheeses. Although the lactic acid content of the control cheese was slightly lower than that of the 95:100 CN:TP cheese up to 1 mo (Table 6) (in agreement with the higher residual lactose content in

Table 5. Nutritional analysis (performed at 1 d of ripening) of Cheddar cheeses that had various levels of whey protein removal[1]

Nutritional component	Treatment[2]			SEM	*P*-value
	Control	89:100 CN:TP	95:100 CN:TP		
Calories (cal/100 g of cheese)	405.0[a]	406.0[a]	414.0[a]	4.1	0.52
Calories from fat (cal/100 g of cheese)	295.0[a]	297.0[a]	306.0[a]	3.5	0.36
Fat by acid hydrolysis (%)	32.9[a]	32.9[a]	34.0[a]	0.38	0.34
Protein[3] (%)	25.6[a]	25.5[a]	25.1[a]	0.10	0.11
Ash (%)	3.93[a]	3.73[b]	3.57[c]	0.01	<0.01
Moisture (%)	35.9[a]	36.2[a]	35.5[a]	0.53	0.83
Vitamin A as retinol (IU/100 g of cheese)	753.0[b]	774.0[b]	831.0[a]	14.8	<0.01
Vitamin C (mg/100 g of cheese)	<1.0	<1.0	<1.0	ND[4]	ND
Calcium (mg/100 g of cheese)	762.0[a]	779.0[a]	773.0[a]	8.0	0.16
Iron (mg/100 g of cheese)	<0.39	<0.39	<0.38	ND	ND
Sodium (mg/100 g of cheese)	759.0[a]	678.0[ba]	630.0[b]	10.2	<0.05

[a–c]Means within the same row not sharing a common superscript differ ($P < 0.05$).

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

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

[3]Total % N × 6.38.

[4]ND = not determined.

Table 6. pH values, lactose (%), and lactic acid content (%) of control, 89:100 casein-to-true protein (CN:TP), and 95:100 CN:TP cheeses during ripening[1]

Item	Time (d)	Treatment			SEM	*P*-value
		Control	89:100 CN:TP	95:100 CN:TP		
pH	1	5.28[a]	5.28[a]	5.26[a]	0.012	0.58
	7	5.21[a]	5.19[a]	5.18[a]	0.026	0.66
	14	5.26[a]	5.25[a]	5.24[a]	0.021	0.92
	30	5.29[a]	5.26[a]	5.24[a]	0.025	0.35
	90	5.30[a]	5.25[a]	5.23[a]	0.035	0.41
	180	5.31[a]	5.27[a]	5.27[a]	0.025	0.52
	270	5.29[a]	5.26[a]	5.29[a]	0.030	0.75
Lactose (%)	1	0.61[a]	0.50[b]	0.42[c]	0.021	0.002
	7	0.52[a]	0.38[b]	0.32[b]	0.034	0.02
	14	0.48[a]	0.35[b]	0.27[b]	0.031	0.009
	30	0.44[a]	0.31[b]	0.23[b]	0.031	0.009
	90	0.30[a]	0.23[ba]	0.16[b]	0.029	0.03
	180	0.30[a]	0.20[ba]	0.12[b]	0.040	0.04
	270	0.34[a]	0.26[a]	0.13[b]	0.029	0.007
Lactic acid (%)	1	0.72[a]	0.74[a]	0.75[a]	0.023	0.68
	7	0.81[a]	0.88[a]	0.84[a]	0.045	0.55
	14	0.82[a]	0.89[a]	0.88[a]	0.031	0.32
	30	0.86[a]	0.93[a]	0.92[a]	0.028	0.25
	90	0.99[a]	1.02[a]	1.00[a]	0.036	0.80
	180	1.02[a]	1.08[a]	1.10[a]	0.049	0.51
	270	1.01[a]	1.04[a]	1.13[a]	0.046	0.28

[a–c]Means within the same row not sharing a common superscript differ ($P < 0.05$).

[1]Values represent the means of 4 replicates for each treatment.

the control cheese), there were no significant differences between treatments (Table 7).

Likewise, treatment had no significant effect on cheese pH (Table 7). During the 270 d of ripening, the pH values varied between 5.18 and 5.31 (Table 6). Nelson and Barbano (2005b) also did not see any difference in pH values for cheeses manufactured from MF milks with varied WP contents. The small fluctuations in cheese pH during ripening can be attributed to the production of lactic acid as well as to solubilization of INSOL Ca phosphate releasing phosphate ions, which bind H^+ ions, resulting in buffering (Hassan et al., 2004).

Insoluble Calcium Content in Cheeses

Depleting the WP in 5% casein milk using MF had no impact on the amount of INSOL Ca in all 3 cheeses during ripening (Table 7). The amount of INSOL Ca slowly decreased with ripening time (Figure 2), in agreement with previous studies on Cheddar cheese (Has-

Table 7. Degree 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 SN/TN[1]) for Cheddar cheeses that had various levels of whey protein removal during ripening (n = 4)

Factor[2]	df	pH	Lactose	Lactic acid	INSOL Ca[3]	pH 4.6 SN/TN	α_{S1}-CN[4]	β-CN[5]
Whole plot								
Treatment (T)	2	0.39	<0.01	0.40	0.99	<0.0001	<0.01	0.14
Day of cheese-making (D)	3	<0.05	<0.05	<0.05	0.22	0.04	0.46	<0.05
Error (T × D)	6							
Split plot								
Age (A)	6	<0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A × T	12	0.96	0.93	0.51	0.89	0.02	0.17	0.01
R^2		0.71	0.96	0.96	0.83	0.99	0.99	0.97

[1]pH 4.6 soluble nitrogen as a percentage of total nitrogen.

[2]Split-plot design with the 3 treatments [different casein-to-true protein (CN:TP) ratios: control, 89:100 CN:TP, 95:100 CN:TP] analyzed as a discontinuous variable and cheese-making day was blocked (3 × 4). Subplot included the effect of aging of cheese (A) and age × treatment as variables.

[3]Percentage of insoluble calcium as a percentage of total Ca.

[4]Amount of intact α_{S1}-CN as a percentage of the total amount at 1 d.

[5]Amount of intact β-CN as a percentage of the total amount at 1 d.

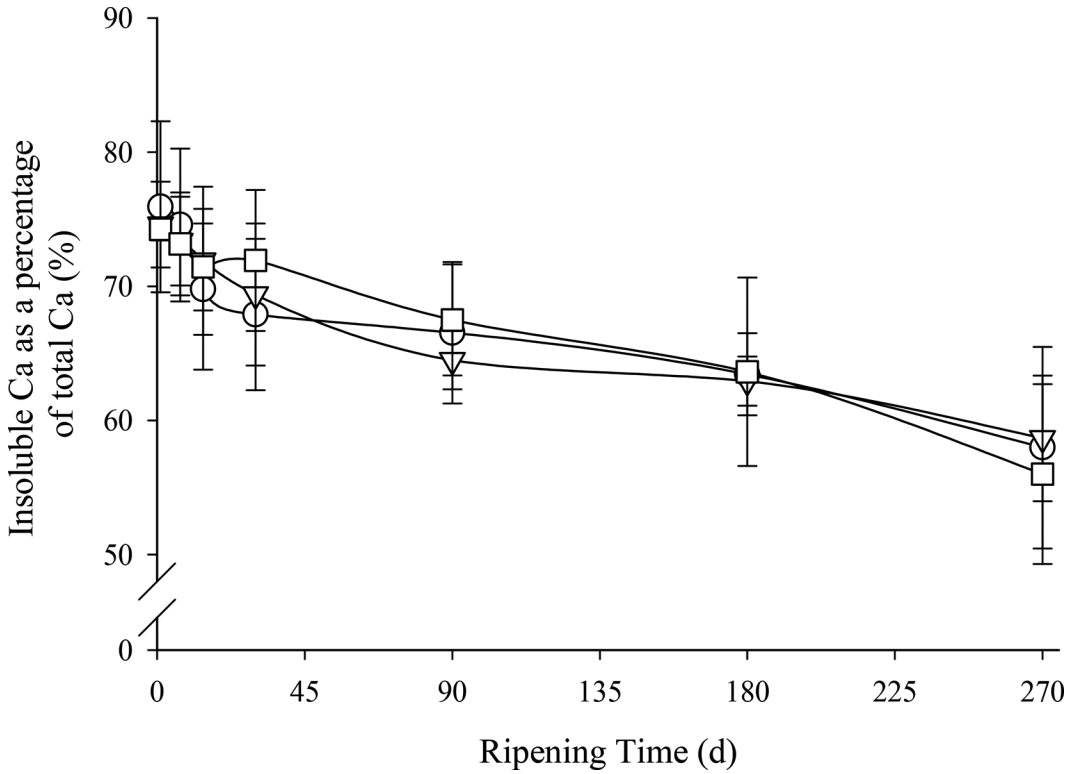

Figure 2. Insoluble calcium content, expressed as a percentage of total calcium, for Cheddar cheeses made with various levels of whey protein removal, control (○), 89:100 casein-to-true protein (CN:TP; ▽), and 95:100 CN:TP (□), during ripening. Vertical bars represent standard deviations (n = 4).

san et al., 2004; Reale et al., 2020). The total calcium contents were similar between cheese treatments (Table 2). Calcium content in cheese affects the structure and texture of cheese (Hassan et al., 2004). The slow dissolution of INSOL Ca throughout ripening contributes to the functionality changes observed in cheese (e.g., meltability; Lucey et al., 2003). In our present study, the INSOL Ca contents in all 3 cheeses were similar as all 3 milks were concentrated by the same amount (~5% casein), were preacidified to the same extent (~pH 6.2), and had similar rates of acidification. Additionally, the pH values and lactic acid content of the cheeses were similar between treatments (Table 7).

Proteolysis

As expected, all 3 treatments saw an increase in the amount of pH 4.6 soluble nitrogen during ripening as intact casein was being degraded (Figure 3a). Treatment had a highly significant effect on proteolysis as indicated by pH 4.6 soluble nitrogen levels (Table 7). The control cheese, which had the highest concentration of WP (Table 2), had a significantly lower amount of pH 4.6 soluble nitrogen after 1 mo of ripening compared with the 95:100 CN:TP cheese (which had the lowest concentration of WP; Figure 3a). These results are consistent with prior studies where cheeses made from MF-concentrated milk had slower proteolysis than cheeses made from unconcentrated milk (Brandsma and Rizvi, 2001; Neocleous et al., 2002b; Ardisson-Korat and Rizvi, 2004; Nelson and Barbano, 2005b). Concentration of milk by MF increases the WP content compared with the level in unconcentrated milk, unless DF is performed to deplete the WP content.

To further investigate the specific type of proteolysis occurring in the cheeses, urea-PAGE was carried out on all 3 cheese treatments (Supplemental Figure S1; http://digital.library.wisc.edu/1793/83609; Govindasamy-Lucey, 2022). In all 3 cheeses, α_{S1}-CN was hydrolyzed into α_{S1}-CN (f102–199) and α_{S1}-CN (f24–199) after 1 d of ripening (results not shown). The primary site of chymosin action on α_{S1}-CN is Phe23-Phe24, which produces the peptides α_{S1}-CN (f24–199) and α_{S1}-CN (f1–23) (Bansal et al., 2009). However, the peptide α_{S1}-CN (f1–23) is quickly hydrolyzed by proteinases from starter microorganisms and therefore does not accumulate in cheese. Generally, chymosin hydrolyzes α_{S1}-CN in several places during the early stages of ripening,

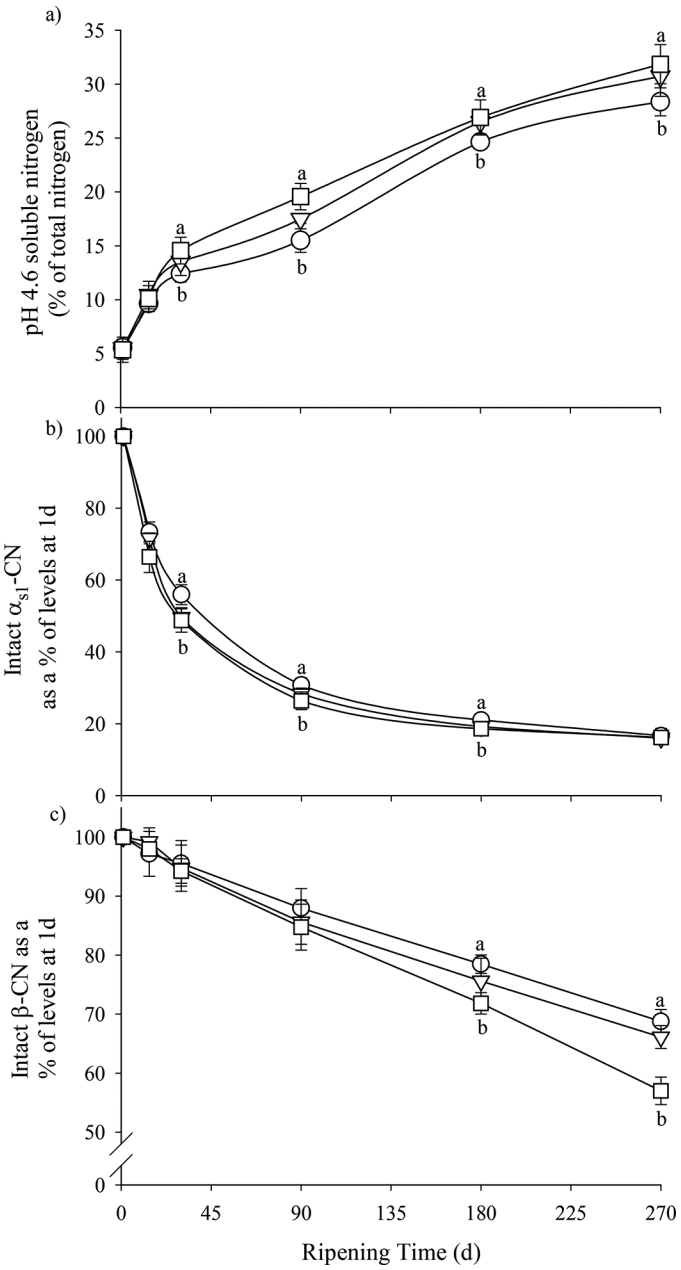

Figure 3. pH 4.6 soluble nitrogen as a percentage of total nitrogen (a), amounts of intact α_{S1}-CN (b), and intact β-CN as a percentage of the level at 1 d (c) for Cheddar cheeses made with various levels of whey protein removal, control (○), 89:100 casein-to-true protein (CN:TP; ▽), and 95:100 CN:TP (□), during ripening. Vertical bars represent standard deviation (n = 4). Different letters (a, b) indicate a significant ($P < 0.05$) difference between the control and the 95:100 CN:TP cheeses at the indicated ripening time.

while plasmin is mainly responsible for degradation of β-CN, as it is largely resistant to chymosin (Ivens et al., 2017).

The amount of intact α_{S1}-CN decreased with age for all 3 cheeses (Figure 3b). Treatment had a significant impact on the amount of intact α_{S1}-CN remaining (Table 7). After 1 mo of ripening, the control cheese had a significantly higher level of intact α_{S1}-CN compared with the 95:100 CN:TP cheese (Figure 3b), and this trend was seen up to 6 mo of ripening. However, by 9 mo of ripening there was no difference in the amount of intact α_{S1}-CN between the cheeses, since most intact α_{S1}-CN had been hydrolyzed (<20% intact α_{S1}-CN remained). Harper et al. (1989) reported a decrease in the rate of α_{S1}-CN hydrolysis in Cheddar cheese slurries prepared with higher WP content. Creamer et al. (1987) also saw slower α_{S1}-CN proteolysis in Cheddar cheeses made from highly concentrated UF milk that had a much higher residual WP content. The higher WP content in the control cheese (0.38%) compared with the 95:100 CN:TP cheese (0.07%) could have reduced proteolysis due to chymosin inhibition.

In all 3 samples, 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) (Supplemental Figure S1). Although, β-CN f(189–192) is not bitter, the presence of this peptide suggests other bitter peptides, such as β-CN f(193–209) and some of its degradation products, could also be present in the cheese (Visser et al., 1983). However, the intensity of this peptide was still very low even after 9 mo of ripening, so it was unlikely to have a major effect on the sensory attributes. Overall, treatment had no significant impact on levels of intact β-CN (Table 7). There was a difference in the intensity of the β-CN band between treatments by 180 d of ripening (Figure 3c), with greater breakdown in the 95:100 CN:TP cheese.

The control cheese in the current study was manufactured from milks that had been concentrated using UF, while the 95:100 CN:TP cheese was made using MF milks. Aaltonen and Ollikainen (2011) reported that MF/DF of milk enhanced plasmin activity in milk, likely due to the reduction in the β-LG concentration (which inhibits plasmin). The lower plasmin activity in UF-concentrated milk has previously been suggested to result from the inhibition of plasmin by elevated WP, presumably due to the competition between WP and the specific plasmin substrate being hydrolyzed by plasmin (Benfeldt, 2006). Alternatively, the decreased plasmin activity in UF cheese could result from an increase in proteinase inhibitors, including plasmin inhibitors, in the cheese curds as a result of the UF process (Christensen et al., 1995). Reale et al. (2020) showed that when the WP levels are ≤0.11%, proteolysis was similar between the control cheeses and WP-depleted cheeses, suggesting that any potential inhibition of rennet or plasmin might occur only at higher residual WP levels in cheese.

There was also a minor difference in the salt-in-moisture level between cheeses (range of 0.58%; Table

Table 8. Degrees of freedom, statistical significance (P-values), and R^2 values for changes in textural, rheological, and sensory properties for Cheddar cheeses that had various levels of whey protein depletion during ripening (n = 4)

Factor[1]	df[2]	TPA hardness[3]	LTmax[4]	Crossover point[5]	df	Sensory firmness	Sensory bitterness	Sensory sulfur
Whole plot								
Treatment (T)	2	<0.01	0.15	0.02	2	0.02	0.07	0.26
Day of cheese-making (D)	3	<0.01	0.10	0.62	3	0.05	0.05	0.05
Error (T × D)	6				6			
Split plot								
Age (A)	4	<0.001	<0.0001	<0.0001	2	<0.01	<0.01	0.46
A × T	8	0.98	0.51	0.98	4	0.99	0.03	1.00
R^2		0.89	0.68	0.94		0.62	0.72	0.21

[1]Split-plot design with the 3 treatments [different casein-to-true protein (CN:TP) ratios: control, 89:100 CN:TP, 95:100 CN:TP] analyzed as a discontinuous variable and cheese-making day was blocked (3 × 4). Subplot included the effect of aging of cheese (A) and age × treatment as variables.

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

[3]TPA = texture profile analyses.

[4]Maximum loss tangent.

[5]Temperature at which loss tangent = 1.

2). Large differences in the salt-in-moisture levels and moisture contents of Cheddar cheese can influence proteolysis (e.g., Kelly et al., 1996); however, no differences in moisture contents were observed in our cheeses (Table 2). Kelly et al. (1996) did not observe any significant differences in proteolysis with cheeses within the range of salt-in-moisture levels we observed in our study. In our previous work (Reale et al., 2020), the salt-in-moisture levels varied ~1.0% between the control and WP-depleted cheeses, and yet there were no significant differences in proteolysis between the treatments.

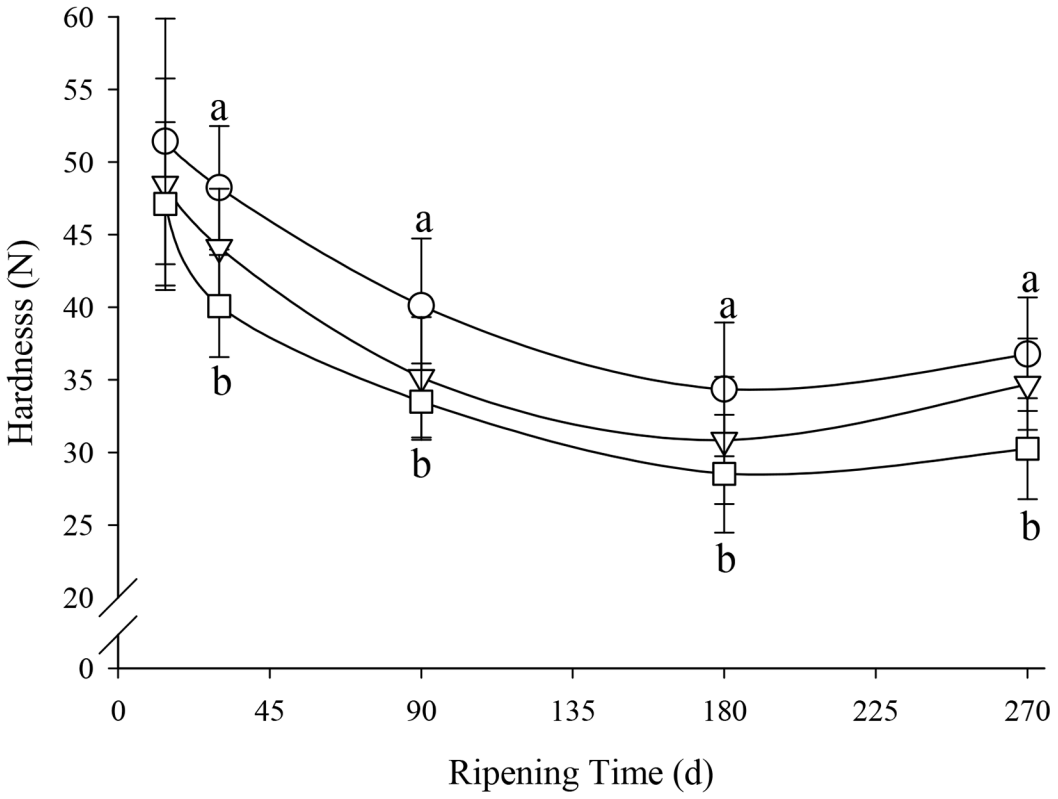

Figure 4. Hardness (N) from texture profile analysis for Cheddar cheeses made with various levels of whey protein removal, control (○), 89:100 casein-to-true protein (CN:TP; ▽), and 95:100 CN:TP (□), during ripening. Vertical bars represent standard deviations (n = 4). Different letters (a, b) indicate a significant ($P < 0.05$) difference between the control and the 95:100 CN:TP cheeses at the indicated ripening time.

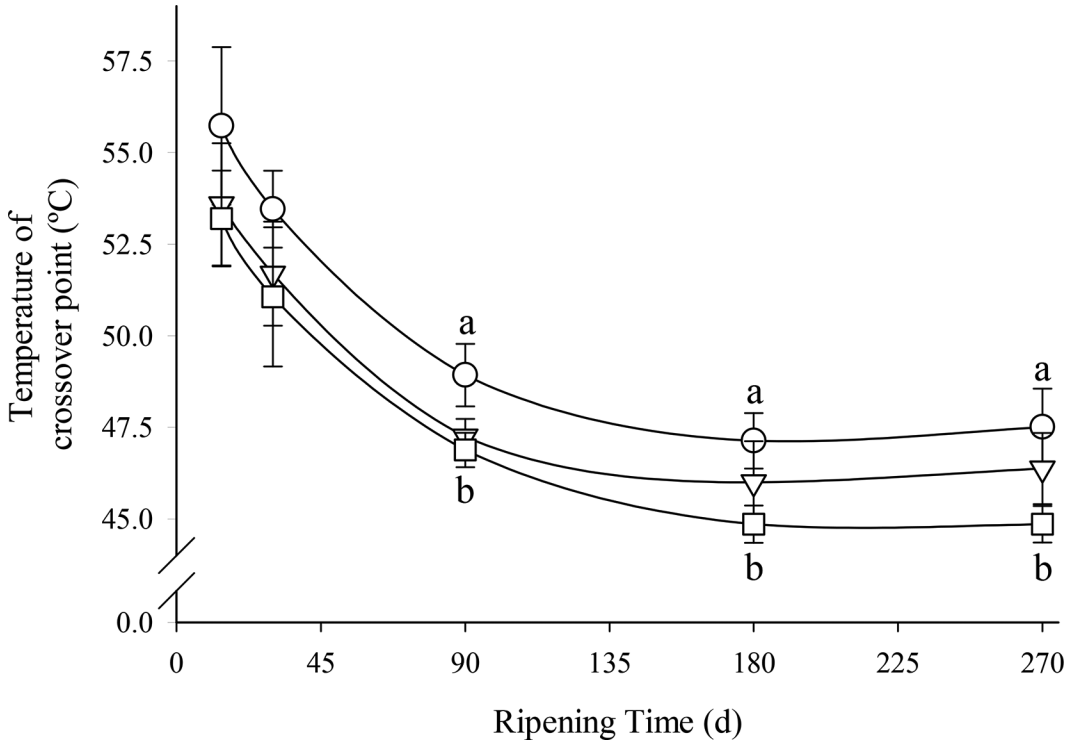

Figure 5. The temperature of the crossover point (where loss tangent = 1) for Cheddar cheeses made with various levels of whey protein removal, control (○), 89:100 casein-to-true protein (CN:TP; ▽), and 95:100 CN:TP (□), during ripening. Vertical bars represent standard deviation (n = 4). Different letters (a, b) indicate a significant ($P < 0.05$) difference between the control and the 95:100 CN:TP cheeses at the indicated ripening time.

Textural and Rheological Properties

Treatment and age significantly influenced cheese hardness values (Table 8). Hardness values of all 3 cheese treatments decreased with ripening time, as expected (Figure 4). After 1 mo of ripening time, the 95:100 CN:TP cheese had lower hardness values than the control cheese. The softening of cheese during ripening is usually attributed to proteolysis, mainly due to degradation of α_{S1}-CN (Creamer and Olson, 1982), as well as to solubilization of INSOL Ca (Lucey et al., 2003; O'Mahony et al., 2005). Cheese composition, specifically moisture content, casein content, and fat content, can also impact the hardness of Cheddar cheese; all of these parameters were similar between the 3 cheese treatments (Table 2). Proteolysis of the control cheese was slightly lower (and had more intact α_{S1}-CN) than for the 95:100 CN:TP cheese (Figure 2), which likely caused the higher hardness in the control sample. Similarly, Neocleous et al. (2002b) reported increased hardness and slower proteolysis in Cheddar cheeses containing higher WP content compared with control cheeses.

During ripening, all cheeses had similar values for maximum loss tangent (LTmax; Table 8), an index of meltability, and these values increased within the first 90 d of ripening but hardly changed thereafter (results not shown). Treatment did impact the crossover point temperature (Table 8); the crossover temperatures (melting points) were slightly higher in the control cheeses compared with the other cheeses (Figure 5). The temperature of the crossover point decreased with ripening for all 3 cheeses, indicating that less energy was needed for flow as the cheese matrix underwent aging; this is consistent with previous reports for Cheddar cheese (Lee et al., 2005; Lucey et al., 2005; Reale et al., 2020). Changes in the rheological properties of cheese during storage are mainly due to ongoing proteolysis and reduction in INSOL Ca cross-linkages (Lucey et al., 2003). Since the INSOL Ca levels were similar between treatments (Table 7), the higher melting point in the control cheese compared with the 95:100 CN:TP cheese was likely due to the slower proteolysis in the control cheese (Figure 3).

Sensory Properties

The sensory textural and flavor attributes of the cheeses are shown in Table 9. There were only a few differences in the sensory textural and flavor attributes

Table 9. Sensory textural and flavor attributes (intensities based on a 0–15-point scale) for cheeses that had various levels of whey protein depletion, control, 89:100 casein-to-true protein (CN:TP), and 95:100 CN:TP, at ripening times of 3, 6, and 9 mo (n = 4)

		Treatment				
Ripening time	Attribute	Control	89:100 CN:TP	95:100 CN:TP	SEM	*P*-value
3 mo	Firmness	13.5[a]	13.1[b]	13.1[b]	0.10	<0.05
	Cohesiveness	11.1[a]	11.5[a]	11.5[a]	0.11	0.10
	Chewiness	5.4[a]	5.4[a]	5.3[a]	0.08	0.72
	Adhesiveness	6.4[a]	6.9[a]	6.4[a]	0.30	0.54
	Salt	3.6[a]	3.5[a]	3.6[a]	0.05	0.39
	Acid	2.6[a]	2.7[a]	2.7[a]	0.08	0.80
	Bitter	1.6[a]	1.7[a]	1.7[a]	0.09	0.49
	Milk fat	4.6[a]	4.5[a]	4.7[a]	0.15	0.70
	Butter	2.0[a]	1.9[a]	2.0[a]	0.05	0.07
	Sulfur	0.9[a]	0.7[a]	0.8[a]	0.03	0.12
6 mo	Firmness	13.2[a]	13.0[b]	12.9[c]	0.04	<0.001
	Cohesiveness	11.4[a]	11.6[a]	11.7[a]	0.12	0.18
	Chewiness	5.4[a]	5.3[a]	5.2[a]	0.13	0.65
	Adhesiveness	7.3[a]	7.8[a]	8.4[a]	0.32	0.13
	Salt	4.1[a]	4.2[a]	4.1[a]	0.11	0.91
	Acid	3.3[a]	3.5[a]	3.4[a]	0.04	0.15
	Bitter	1.9[a]	2.1[a]	2.3[a]	0.12	<0.05
	Milk fat	3.1[a]	3.1[a]	3.2[a]	0.10	0.92
	Butter	1.5[a]	1.7[a]	1.6[a]	0.12	0.36
	Sulfur	0.3[a]	0.5[a]	0.4[a]	0.04	0.07
9 mo	Firmness	12.4[a]	12.0[ba]	11.7[b]	0.14	<0.05
	Cohesiveness	11.8[b]	12.0[ba]	12.2[a]	0.08	<0.05
	Chewiness	5.1[a]	4.9[ba]	4.8[b]	0.05	<0.05
	Adhesiveness	8.4[a]	9.0[a]	9.0[a]	0.22	0.14
	Salt	5.2[a]	5.0[a]	5.1[a]	0.09	0.30
	Acid	3.4[a]	3.6[a]	3.4[a]	0.06	0.19
	Bitter	2.7[a]	2.5[b]	2.5[b]	0.12	<0.05
	Milk fat	3.0[a]	2.9[a]	3.0[a]	0.05	0.65
	Butter	1.0[a]	1.0[a]	1.0[a]	0.04	0.79
	Sulfur	0.6[a]	0.5[a]	0.5[a]	0.11	0.92

[a–c]Means within the same row not sharing a common superscript differ ($P < 0.05$).

of the cheeses (Tables 8 and 9). During ripening the sensory hand firmness values were higher for the control cheeses compared with the MF-treated cheeses (Tables 8 and 9). The hand firmness results agreed with the results obtained using the instrumental texture profile analysis hardness method (Figure 4). Textural differences were mainly attributed to lower primary proteolysis during ripening in the control cheese (Figure 3). Cohesiveness and chewiness attributes were only different between cheeses after 270 d of ripening; the 95:100 CN:TP cheese was slightly more cohesive and less chewy than the control cheeses (Table 9).

Typical flavor development in cheeses made from concentrated milks has been a concern in prior research (e.g., Green et al., 1981; Lelievre and Lawrence, 1988). However, with adjustments in the cheese-making procedure based on casein concentration, satisfactory flavor development was achieved in all cheeses. Likewise, Neocleous et al. (2002b) manufactured cheeses from MF-concentrated milks and were able to achieve what they considered normal Cheddar cheese flavor development after altering the cheese-making procedure to account for the concentration factor.

Sulfur intensity between the 3 cheeses was similar during ripening (Tables 8 and 9). Many typical Cheddar cheeses develop a more intense sulfur flavor over time, and Whetstine et al. (2006) suggested that cheeses with higher WP content may have more intense sulfur notes. The control cheese had slightly higher bitterness values by 9 mo of ripening (Table 9). One of the biggest contributors to the development of bitterness in Cheddar cheese is the degradation of β-CN into bitter peptides, namely, β-CN (f193–209) peptide and some of its degradation products (Visser et al., 1983). Thus, bitterness is likely to increase in proportion to proteolysis because proteolysis leads to a build-up of bitter peptides. It has been previously reported that cheeses made from milks with higher WP content had a lower intensity of bitter flavor (Agrawal and Hassan, 2007; Lemieux and Simard, 1991; Creamer et al., 1987). These authors speculated that WP may inhibit proteolysis and therefore decrease the production of bitter peptides from β-CN. Because the cheese in the present study with the lowest WP content (95:100 CN:TP) had a higher percentage of pH 4.6 soluble nitrogen and β-CN degradation after 6 mo of ripening (Figure 3), it was also expected to have a

higher intensity of bitterness. However, the MF cheeses in our study had lower WP and slightly less bitterness intensity compared with the control cheeses after 180 d of ripening (Table 8). The intensity of bitterness in our cheeses was quite low ($\leq$2.5 on our 15-point scale; Table 9). Perhaps the range in WP concentration in Cheddar cheeses needs to be larger than 0.32% to significantly alter sensory attributes including bitterness. Other factors, like the type of rennet, concentration of rennet, and type of starter culture used, also significantly contribute to bitterness in cheese (Visser and Slanger, 1977).

CONCLUSIONS

Depletion of WP from concentrated milk was achieved with the use of polymeric MF membranes and DF of the MF retentate with UF permeate. The 5% CN milks made with MF had lower total protein, true protein, and WP content because of the depletion of WP. Generally similar compositions and textures were obtained with either UF or MF approaches. Using 5% CN milks made with MF to deplete the WP content resulted in higher fat, nitrogen, and solids recoveries in cheese. These increased recoveries of nitrogen and solids presumably reflect an increased proportion of casein to total nitrogen and TS in these milks. Using MF to remove WP avoided the slower proteolysis in cheese observed in milks concentrated by UF. Slower breakdown of α_{S1}-CN was observed in cheeses with higher WP levels, suggesting some rennet inhibition. At long aging times, greater breakdown of β-CN was observed in the cheese with WP depletion, suggesting that plasmin activity could be slightly impaired in the control cheeses. In conclusion, Cheddar cheeses manufactured from concentrated milk where WP were depleted by MF generally had similar quality, flavor, and nutritional profiles to the control cheese (made from UF-concentrated milk) but higher levels of residual WP in the control cheese reduced proteolysis, and they produced harder, less meltable cheese.

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