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
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 m² 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 approximately 43.2 m² 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).
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.

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.
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, UF1, MF retentate, whole milk, standardized milk samples, drained whey, and pressed whey were analyzed for TS, protein, fat, ash, vitamins/elements (iron, Ca, and Na), minerals/elements (iron, Ca, and Na), and moisture were measured in all the cheeses from 2 wk of ripening. 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, fat, protein, vitamin A, minerals/elements (iron, Ca, and Na), and ash, and moisture were measured in all the cheeses from 2 trials.

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).

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

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 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 αs-CN and β-CN during ripening was monitored with urea-PAGE gels, which were prepared as described by Özturk 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 ≤ 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-
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.

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RESULTS AND DISCUSSION

Composition and Particle Size Analyses of Fluids

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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
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
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
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

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

<table>
<thead>
<tr>
<th>Component recovery</th>
<th>Control</th>
<th>89:100 CN:TP</th>
<th>95:100 CN:TP</th>
<th>SEM</th>
<th>P-value&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat recovery (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>88.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Drain whey</td>
<td>9.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.30</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Press whey</td>
<td>0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>98.68</td>
<td>99.35</td>
<td>99.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nitrogen recovery (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>76.54&lt;sup&gt;f&lt;/sup&gt;</td>
<td>82.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>88.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.65</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Drain whey</td>
<td>21.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Press whey</td>
<td>0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total</td>
<td>101.01</td>
<td>101.68</td>
<td>101.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Solid recovery (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>63.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>65.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>69.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Drain whey</td>
<td>35.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Press whey</td>
<td>2.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>Total</td>
<td>101.25</td>
<td>101.87</td>
<td>102.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Whey protein recovery in cheese (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>6.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Drain whey&lt;sup&gt;4&lt;/sup&gt; (%, mass)</td>
<td>78.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Press whey&lt;sup&gt;4&lt;/sup&gt; (%, mass)</td>
<td>2.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Means within the same row not sharing a common superscript differ (P < 0.05).
<sup>1</sup>Values represent the means of 4 replicates for each treatment.
<sup>2</sup>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.
<sup>3</sup>Value for full statistical model that incorporated all 3 treatments and 4 blocks (4 replicate cheese-making days).
<sup>4</sup>Amount of drain whey and press whey obtained from 100 kg of cheese milk.
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 4. Actual and calculated cheese yield values for Cheddar cheeses that had various levels of whey protein depletion

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>89:100 CN:TP</th>
<th>95:100 CN:TP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF value</td>
<td>0.886</td>
<td>0.887</td>
<td>0.908</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>RC value</td>
<td>0.930</td>
<td>0.940</td>
<td>0.940</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>RS value</td>
<td>1.084</td>
<td>1.084</td>
<td>1.087</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Actual yield (%)</td>
<td>18.15</td>
<td>18.74</td>
<td>19.02</td>
<td>0.19</td>
<td>0.04</td>
</tr>
<tr>
<td>Van Slyke cheese yield (%)</td>
<td>18.14</td>
<td>18.74</td>
<td>19.02</td>
<td>0.19</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Means within the same row not sharing a common superscript differ (P < 0.05).
Values represent the means of 4 replicates for each treatment.

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.

RF is the fat recovered in cheese, determined experimentally from cheese trials.
ND = not determined.

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.

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

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

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

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

<table>
<thead>
<tr>
<th>Nutritional component</th>
<th>Control</th>
<th>89:100 CN:TP</th>
<th>95:100 CN:TP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (cal/100 g of cheese)</td>
<td>405.0a</td>
<td>406.0a</td>
<td>414.0a</td>
<td>4.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Calories from fat (cal/100 g of cheese)</td>
<td>295.0a</td>
<td>297.0a</td>
<td>306.0a</td>
<td>3.5</td>
<td>0.36</td>
</tr>
<tr>
<td>Fat by acid hydrolysis (%)</td>
<td>32.9a</td>
<td>32.9a</td>
<td>34.0a</td>
<td>0.38</td>
<td>0.34</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>25.6a</td>
<td>25.5a</td>
<td>25.1a</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.93a</td>
<td>3.73b</td>
<td>3.57c</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>35.9a</td>
<td>36.2a</td>
<td>35.5c</td>
<td>0.53</td>
<td>0.83</td>
</tr>
<tr>
<td>Vitamin A as retinol (IU/100 g of cheese)</td>
<td>753.0b</td>
<td>774.0a</td>
<td>831.0a</td>
<td>14.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vitamin C (mg/100 g of cheese)</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Calcium (mg/100 g of cheese)</td>
<td>762.0a</td>
<td>779.0a</td>
<td>773.0a</td>
<td>8.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Iron (mg/100 g of cheese)</td>
<td>&lt;0.39</td>
<td>&lt;0.39</td>
<td>&lt;0.38</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sodium (mg/100 g of cheese)</td>
<td>79.0b</td>
<td>678.0a</td>
<td>630.0b</td>
<td>10.2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Means within the same row not sharing a common superscript differ (P < 0.05).
Values represent the means of 4 replicates for each treatment.
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.

Total % N × 6.38.
ND = not determined.
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>
<thead>
<tr>
<th>Item</th>
<th>Time (d)</th>
<th>Control</th>
<th>89:100 CN:TP</th>
<th>95:100 CN:TP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1</td>
<td>5.28</td>
<td>5.28</td>
<td>5.26</td>
<td>0.012</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.21</td>
<td>5.19</td>
<td>5.18</td>
<td>0.026</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>5.26</td>
<td>5.25</td>
<td>5.24</td>
<td>0.021</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5.29</td>
<td>5.26</td>
<td>5.24</td>
<td>0.025</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>5.30</td>
<td>5.25</td>
<td>5.23</td>
<td>0.035</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>5.31</td>
<td>5.27</td>
<td>5.27</td>
<td>0.025</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>5.29</td>
<td>5.26</td>
<td>5.29</td>
<td>0.030</td>
<td>0.75</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>1</td>
<td>0.61</td>
<td>0.50</td>
<td>0.42</td>
<td>0.021</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.52</td>
<td>0.38</td>
<td>0.32</td>
<td>0.034</td>
<td>0.02</td>
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<td>14</td>
<td>0.45</td>
<td>0.35</td>
<td>0.27</td>
<td>0.031</td>
<td>0.009</td>
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<td>30</td>
<td>0.44</td>
<td>0.31</td>
<td>0.23</td>
<td>0.031</td>
<td>0.009</td>
</tr>
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<td>0.30</td>
<td>0.23</td>
<td>0.16</td>
<td>0.029</td>
<td>0.03</td>
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<td>0.12</td>
<td>0.040</td>
<td>0.04</td>
</tr>
<tr>
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<td>270</td>
<td>0.34</td>
<td>0.26</td>
<td>0.13</td>
<td>0.029</td>
<td>0.007</td>
</tr>
<tr>
<td>Lactic acid (%)</td>
<td>1</td>
<td>0.72</td>
<td>0.74</td>
<td>0.75</td>
<td>0.023</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.81</td>
<td>0.88</td>
<td>0.84</td>
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</tr>
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<td>0.82</td>
<td>0.89</td>
<td>0.88</td>
<td>0.031</td>
<td>0.32</td>
</tr>
<tr>
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<td>30</td>
<td>0.86</td>
<td>0.93</td>
<td>0.92</td>
<td>0.028</td>
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</tr>
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<td>0.51</td>
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<td>270</td>
<td>1.01</td>
<td>1.04</td>
<td>1.13</td>
<td>0.046</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*Means within the same row not sharing a common superscript differ (P < 0.05).

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

---

[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.]

<table>
<thead>
<tr>
<th>Item</th>
<th>Time (d)</th>
<th>Control</th>
<th>89:100 CN:TP</th>
<th>95:100 CN:TP</th>
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<td>0.82</td>
<td>0.89</td>
<td>0.88</td>
<td>0.031</td>
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<td>0.86</td>
<td>0.93</td>
<td>0.92</td>
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<td>1.00</td>
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<td>1.08</td>
<td>1.10</td>
<td>0.049</td>
<td>0.51</td>
</tr>
<tr>
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<td>1.01</td>
<td>1.04</td>
<td>1.13</td>
<td>0.046</td>
<td>0.28</td>
</tr>
</tbody>
</table>

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

2Split-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.

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

4Amount of intact αS1-CN as a percentage of the total amount at 1 d.

5Amount of intact β-CN as a percentage of the total amount at 1 d.
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, αS1-CN was hydrolyzed into αS1-CN (f102–199) and αS1-CN (f24–199) after 1 d of ripening (results not shown). The primary site of chymosin action on αS1-CN is Phe23-Phe24, which produces the peptides αS1-CN (f24–199) and αS1-CN (f1–23) (Bansal et al., 2009). However, the peptide αS1-CN (f1–23) is quickly hydrolyzed by proteinases from starter microorganisms and therefore does not accumulate in cheese. Generally, chymosin hydrolyzes αS1-CN in several places during the early stages of ripening,

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).
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 αS1-CN decreased with age for all 3 cheeses (Figure 3b). Treatment had a significant impact on the amount of intact αS1-CN remaining (Table 7). After 1 mo of ripening, the control cheese had a significantly higher level of intact αS1-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 αS1-CN between the cheeses, since most intact αS1-CN had been hydrolyzed (<20% intact αS1-CN remained). Harper et al. (1989) reported a decrease in the rate of αS1-CN hydrolysis in Cheddar cheese slurries prepared with higher WP content. Creamer et al. (1987) also saw slower αS1-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-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 (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 7).
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.

### Table 8. Degrees of freedom, statistical significance (P-values), and R² values for changes in textural, rheological, and sensory properties for Cheddar cheeses that had various levels of whey protein depletion during ripening (n = 4)

<table>
<thead>
<tr>
<th>Factor</th>
<th>df²</th>
<th>TPA hardness³</th>
<th>LTmax⁴</th>
<th>Crossover point⁵</th>
<th>df</th>
<th>Sensory firmness</th>
<th>Sensory bitterness</th>
<th>Sensory sulfur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole plot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>2</td>
<td>&lt;0.01</td>
<td>0.15</td>
<td>0.02</td>
<td>2</td>
<td>0.02</td>
<td>0.07</td>
<td>0.26</td>
</tr>
<tr>
<td>Day of cheese-making (D)</td>
<td>3</td>
<td>&lt;0.01</td>
<td>0.10</td>
<td>0.62</td>
<td>3</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Error (T × D)</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Split plot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (A)</td>
<td>4</td>
<td>&lt;0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>2</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.46</td>
</tr>
<tr>
<td>A × T</td>
<td>8</td>
<td>0.98</td>
<td>0.51</td>
<td>0.98</td>
<td>4</td>
<td>0.99</td>
<td>0.03</td>
<td>1.00</td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>0.89</td>
<td>0.68</td>
<td>0.94</td>
<td></td>
<td>0.62</td>
<td>0.72</td>
<td>0.21</td>
</tr>
</tbody>
</table>

¹Split-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.

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

³TPA = texture profile analyses.

⁴Maximum loss tangent.

⁵Temperature at which loss tangent = 1.

![Figure 4](https://example.com/figure4.png)

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.
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 αS1-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 αS1-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
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 differ-
ences were mainly attributed to lower primary pro-
etolysis 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 pro-
cedure based on casein concentration, satisfactory flavor
development was achieved in all cheeses. Likewise, Neo-
cleous 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 con-
tributors 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 deg-
radation 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

### 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)

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

*Means within the same row not sharing a common superscript differ (P < 0.05).
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 Slinger, 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 $\alpha_{s1}$-CN was observed in cheeses with higher WP levels, suggesting some rennet inhibition. At long aging times, greater breakdown of $\beta$-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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