ABSTRACT

Process cheese products (PCP) are dairy foods prepared by blending dairy ingredients (such as natural cheese, protein concentrates, butter, nonfat dry milk, whey powder, and permeate) with nondairy ingredients (such as sodium chloride, water, emulsifying salts (ES), color, and flavors) and then heating the mixture to obtain a homogeneous product with an extended shelf life. The ES, such as sodium citrate and disodium phosphate, are critical for the unique microstructure and functional properties of PCP because they improve the emulsification characteristics of casein by displacing the calcium phosphate complexes that are present in the insoluble calcium-paracaseinate-phosphate network in natural cheese. The objectives of this study were to determine the optimum protein content (3, 6, and 9% protein) in micellar casein concentrate (MCC) to produce acid curd and to manufacture PCP using a combination of acid curd cheese and MCC that would provide the desired improvement in the emulsification capacity of caseins without the use of ES. To produce acid curd, MCC was acidified using lactic acid to get a pH of 4.6. In the experimental formulation, the acid curd was blended with MCC to have a 2:1 ratio of protein from acid curd relative to MCC. The PCP was manufactured by blending all ingredients in a KitchenAid blender (Professional 5 Plus, KitchenAid) to produce a homogeneous paste. A 25-g sample of the paste was cooked in the rapid visco analyzer (RVA) for 3 min at 95°C at 1,000 rpm stirring speed during the first 2 min and 160 rpm for the last min. The cooked PCP was then transferred into molds and refrigerated until further analysis. This trial was repeated 3 times using different batches of acid curd. MCC with 9% protein resulted in acid curd with more adjusted yield.

The end apparent viscosity (402.0–483.0 cP), hardness (354.0–384.0 g), melting temperature (48.0–51.0°C), and melting diameter (30.0–31.4 mm) of PCP made from different acid curds were slightly different from the characteristics of typical PCP produced with conventional ingredients and ES (576.6 cP end apparent viscosity, 119.0 g hardness, 59.8°C melting temperature, and 41.2 mm melting diameter) due to the differences in pH of final PCP (5.8 in ES PCP compared with 5.4 in no ES PCP). We concluded that acid curd can be produced from MCC with different protein content. Also, we found that PCP can be made with no ES when the formulation uses a 2:1 ratio of acid curd relative to MCC (on a protein basis).

Key words: process cheese products, micellar casein concentrate, acid curd, functional characteristics

INTRODUCTION

Microfiltration (MF) is a pressure-driven membrane process that is used to fractionate casein and serum protein (SP) from skim milk using a 0.1 µm semipermeable membrane. The retentate stream in this process which holds the casein is called micellar casein concentrate (MCC), which is mostly native casein. The MCC is a high-protein ingredient that is typically manufactured in 3 MF stages using a 3× concentration factor (CF) with diafiltration (DF). Several MF membranes have been used to produce MCC (Hammam et al., 2021), such as spiral-wound membranes (Govindasamy-Lucey et al., 2007; Lawrence et al., 2008; Zulewska et al., 2009; Beckman et al., 2010; Beckman and Barbano, 2013; Marella et al., 2021), isoflux ceramic membranes (Adams and Barbano, 2013; McCarthy et al., 2017), uniform transmembrane pressure ceramic membranes (Vadi and Rizvi, 2001; Hurt et al., 2010), and graded permeability (GP) ceramic membranes (Zulewska and Barbano, 2014; Tremblay-Marchand et al., 2016; Yang et al., 2020; Hammam et al., 2022a).

The MCC has promising applications in some dairy and nondairy products due to its unique physicochemical and functional characteristics (e.g., foam-
The water phase and with the fat phase, respectively, drophobic portions that are now free to interact with similar to any other protein, have hydrophilic and hydrophobic portions that are now free to interact with. The partially dispersed monomers of casein, as disodium phosphate sequester the calcium from the calcium-casein-phosphate network by donating sodium ions. As a result, the major molecular forces (calcium phosphate-based cross-links) that cross-link the various monomers of casein are partially disrupted. This results in the acid curd with low mineral or calcium content. In contrast to the low mineral content of acid curd, MCC has a high level of casein bound calcium due to its pH (6.5–6.7). If acid curd is mixed with MCC (Figure 2), it may be possible to create a partially deaggregated casein network without the use of ES. The ratio of acid curd to MCC will have an effect on the level of deaggregation and the pH of the final PCP. We hypothesize that a ratio of 2 parts of protein from acid curd to 1 part of protein from MCC will create a partially deaggregated casein network similar to a typical PC that utilizes ES (Figure 2).

Acid curd could be produced from skim milk in a process similar to cottage cheese manufacture. There is a possibility of using MCC instead of skim milk to produce acid curd. Making acid curd from MCC has advantages as compared with skim milk, because manufacturing MCC using MF results in milk-derived whey protein as a by-product, which can be utilized in many applications, particularly making whey protein isolate. In contrast, acid curd produced from skim milk results in acid whey as a by-product, which is more difficult to utilize. The typical composition of MCC (3 stages using 3 × CF with DF) is >9% true protein (TP) and >13% TS (Zulewska et al., 2009). This MCC can be used as is to make acid curd or diluted to lower protein levels before making acid curd if required.

Many consumers are perceiving ES in PC as chemicals, which is reducing the consumption of those products. Also, ES increase the level of sodium in PC, which could elevate the blood pressure. As a result, few attempts were performed to produce PC with no ES, using whey proteins (Yee et al., 1998), calcium-reduced ingredients (Smith and Rivera, 2017), and blends of sheared and nonsheared amounts of fat (Kimmel et al., 2014). To date, no study related to manufacture PCP with no ES is available in the literature. Therefore, the objectives of this study were to develop a process to produce acid curd from MCC and to determine if PCP could be produced without ES using a novel method by combining acid curd and MCC at a 2:1 ratio on a protein basis in the formulation.
MATERIALS AND METHODS

Experimental Design

Manufacture of MCC (as described below) was completed in approximately 10 h in one day at the Davis Dairy Plant at South Dakota State University (Brookings, SD). The experiment was repeated 3 times with different lots of skim milk. Part of the final MCC was dried using a spray dryer to produce MCC powder, whereas the rest of the MCC was divided into 3 aliquot solutions and diluted with water to standardize the protein content to 3, 6, and 9% before making acid curd.

Preparation of Skim Milk.

The MCC was manufactured as described in our previous study with some modifications (Hammam et al., 2022a). Approximately 800.0 kg of whole bovine milk was separated (Model MSE 140–48–177 AirTight centrifuge; GEA Co.) at 4°C at the South Dakota State University Davis Dairy Plant and then pasteurized in a plate heat exchanger (model PR02-SH, AGC Engineering) at 76°C for 16 s. The pasteurized skim milk was then kept at ≤4°C until MF was conducted. Tanks and cans were sanitized and covered during processing to minimize airborne contamination.

Microfiltration Operation.

To fractionate skim milk into casein and SP to produce MCC, a pilot scale ceramic GP MF system (TIA, Rond-point des, Portes de Provence, Rue Robert Schumann 84500) was utilized. The GP MF system was equipped with 7 ceramic tubes (19 channels with a diameter of 3.3 mm) mounted in the system vertically. The ceramic GP MF membranes had a 0.1-µm pore size, 1.68-m² surface area, and a 1.02-m membrane length. The GP MF system was also equipped with a feed pump and a retentate recirculation pump (TIA). The MF of skim milk (approximately 730.0 kg) was performed in 3 stages to produce MCC.

Manufacture of MCC

First Stage. The GP MF system was started with soft water at 50°C using 3 × CF (1 kg retentate:2 kg of permeate) in a feed and bleed mode (one-way pass) with 400 kPa retentate pressure inlet (Rpi), 200 kPa retentate pressure outlet (Rpo), and 200 kPa permeate pressure outlet (Ppo). The skim milk with ~10.6% Brix (Misco, Palm Abbe Digital Refractometer #PA201) was heated to 50°C with a plate heat exchanger (SABCO Plate-pro Sanitary Chiller; NP925–41) before processing. When the processing conditions were stable while running with water, the system was transitioned

Figure 1. Emulsifying salts (ES) interaction during the making of process cheese (PC) or process cheese products (PCP).
to skim milk. The skim milk was microfiltered with the GP MF system at a constant flux (71.42 L/m² per hour) using a 3× CF in a feed and bleed mode at 50°C (Figure 3). The water at the beginning of the process was flushed out with skim milk by collecting about 37.5 kg of permeate and 19.5 kg of retentate. The permeate flow rate was set at 120 L/h (flux of 71.42 L/m² per hour) and the retentate flow rate was 60 L/h to produce a 3× retentate. After this startup, retentate and permeate were collected and weighed continuously. During MF of skim milk, Rpi, Rpo, and Ppo were targeted to maintain 400, 200, and 200 kPa, respectively. The CF was calculated every 15 min by collecting permeate and retentate samples. The composition of retentate and permeate during MF was monitored using an infrared spectrophotometer (MilkoScan FT1-Lactoscope FTIR, FOSS Instruments-FOSS Analytical A/S). The collected retentate was kept in tanks during the MF process. The processing time of the first stage was approximately 4 h.

Second Stage. The retentate (~17.7% Brix) from the first stage was diluted with soft water (approximately 219.0 kg of retentate mixed with 438.0 kg of water) to obtain a DF of 3× to get back the original volume of skim milk. After mixing, the diluted retentate (~5.9% Brix) was heated to 50°C and processed with the GP MF system using a 3× CF, as described in the first stage. The water at the beginning of the process was flushed out of the system with the diluted retentate by collecting about 37.0 kg of permeate and 18.0 kg of retentate. The permeate flow rate was 120 L/h (flux of 71.42 L/m² per hour) and the retentate flow rate was 60 L/h. Permeate and retentate were weighed continuously, as described in the first stage. The retentate was collected in sanitized cans. The processing time of the second stage was approximately 4 h.

Third Stage. Approximately 200.0 kg of the retentate (~13.0% Brix) was microfiltered in a recirculation mode. The retentate of the second stage was placed in the tank of MF unit and then proceeded to the third stage using a 3× CF at 50°C. The following conditions were applied: Rpi, Rpo, and Ppo were 400, 200, and 200 kPa, respectively, whereas the permeate flow rate was 120 L/h (flux of 71.42 L/m² per hour) and the retentate flow rate was 60 L/h. The retentate was recirculated, whereas permeate was collected until the TS reached 13.0–14.0% (CEM Smart System5 SL7199) or ~16.0% Brix. Increasing the solids content of MCC during MF.

Figure 2. Acid curd and micellar casein concentrate (MCC) interaction during the making of process cheese products (PCP) with no emulsifying salts (ES).
led to a reduction in Ppo. This reduction is related to the concentration polarization and membrane fouling that accumulated on the membrane during recirculation. The final MCC resulting from the third stage was collected. The processing time for the third stage was around 1 h. The MCC was then pasteurized at 63°C/30 min. Part of the liquid MCC was dried using a spray dryer to produce MCC powder, whereas the rest of the liquid MCC was used in making acid curd. This trial was replicated 3 times using 3 separate lots of raw milk.

**Cleaning the Membrane**

After processing, the GP MF system was flushed with soft water to remove all retentate residues from the system. The initial flux was measured with approximately 60 kg of soft water at 27°C. During the flux measurement, the retentate valves were closed and the permeate valves were completely opened with the feed pump running. Subsequently, 30.0 kg of soft water was added to the system and heated to 74°C, then 900 mL of Ultrasil 110 Alkaline cleaner (Ecolab Inc.) and 200 mL of XY 12 (Ecolab Inc.) was added to get pH of 11 (Accumet, Fisher Scientific). This solution was recirculated for 30 min at a 350 L/h permeate flow rate (flux of 208 L/m² per hour). After cleaning the MF system with the alkaline solution, the membrane was cooled to 50°C (less than 10°C/min). The alkaline solution was flushed out of the MF system with soft water until the pH of outlet water ranged from 8.3 to 8.5. The flux was measured again, as described previously. The system was cleaned with an acid solution (Ultrasil 78 acid cleaner) by adding 30 kg of soft water and heated to 52°C; subsequently, 400 mL of Ultrasil 78 (Ecolab Inc.) was added to get a pH of 2. The recirculation of the acid solution was applied for 20 min at a flux of 208 L/m² per hour. Subsequently, the machine was stopped, and the acid was retained inside the system. Before using the system again, the acid solution was flushed out with soft water until the pH reached 8.3 to 8.5. The flux was measured again after flushing the acid solution. Within each MF stage, membrane was flushed.

![Diagram of manufacturing micellar casein concentrate (MCC) using 3 stages, 3× CF. MF = microfiltration; CF = concentration factor = 3 × = 2 kg of permeate: 1 kg of retentate; DF = diafiltration = 3 × = 2 times of the amount of retentate water added.](image)
using water and flux was measured. The membrane was cleaned within stages using the abovementioned procedures when the flux did not show the original values.

**MCC Drying**

A pilot scale spray dryer at the Davis Dairy Plant at South Dakota State University was used to dry the MCC. The nozzle used to dry the MCC had a core size of 21 and an orifice size of 66. The inlet pressure was set at 2,250.0 psi using high-pressure pump speed, and it was adjusted manually through the fan (30.0%). The supply fan and exhaust fan were set at 80.0 and 90.0%, respectively. The inlet temperature was 175°C, whereas the outlet temperature was 82°C. The dryer was connected to a fluid bed (Dahmes Stainless INC: DSI, Model no 10011–11) that was equipped with sieves. The fluid bed was attached to 3 fans (hot = 71°C and 40.0% speed; warm = 50°C and 50.0% speed; cool = 21°C and 40.0% speed). The liquid MCC was heated to 50°C in a water bath before feeding into the dryer. The powder was collected in an airtight container and stored at room temperature until further analysis.

**Manufacture of Acid Curd**

The liquid MCC was diluted with water to standardize the protein content to 3 (acid curd-3), 6 (acid curd-6), and 9% (acid curd-9). Lactic acid (88% Lactic Acid FCC, product code: 175820, lot number: 1501277028, ADM Inc.) was added to reduce the pH to 4.6 (isoelectric point) at 4°C. Approximately 0.6, 1.2, and 2.0% of lactic acid were added to MCC 3, 6, and 9% protein, respectively to achieve the pH of 4.6. The acidified MCC was then warmed to 25°C, left to set, then cut, and mixed gently during heating to 45°C. The whey was subsequently drained from the curd, and the curd was then pressed for 1 h at 80 psi. After pressing, the curd was kept in the freezer at −20°C until further analyses. The adjusted moisture yield of the acid curd was calculated considering 60% moisture as the desired water content (adjusted moisture). This experiment was repeated 3 times.

**Manufacture of Process Cheese Products**

Techwizard software (Excel-based-formulation software program provided by Owl Software) was used to develop the PCP formulations (Metzger, 2010). The percentage of ingredients used in PCP formulations is shown in Table 1 to produce PCP with 49.0% moisture, 20.0% fat, 18.0% protein, and 2.0% salt. In each formulation [PCP made from acid curd-3 (FR-3), PCP made from acid curd-6 (FR-6), and PCP made from acid curd-9 (FR-9)], the amount of protein from acid curd and MCC was adjusted to have a ratio of 2:1, respectively. The ingredients were natural Cheddar cheese (Kraft Heinz Aged Cheddar), unsalted butter (Land O’Lakes Inc.), acid curd, MCC powder, deproteinized whey (Bongards’ Creameries), and salt (Cargill). Approximately 10% of aged Cheddar cheese was added into PCP formulations (FR-3, FR-6, and FR-9) to get a mild Cheddar flavor in the final PCP. The deproteinized whey was used to standardize the solids content in the PCP formulations. Control PCP was also manufactured using disodium phosphate (Lot 085651, Fisher Scientific) as the ES, commercial MCC powder (CasPro 8500, Lot # NF8109A1, Milk Specialties Global), young and aged Cheddar cheeses, deproteinized whey, and salt. Young and aged Cheddar cheeses (1:1 ratio), as well as commercial MCC, were used in control formulation to have the typical intact casein (unhydrolyzed casein) to produce PCP with typical functionality. Acid curd was not utilized in control PCP formulation. All PCP were manufactured in the rapid visco analyzer (RVA; Perten RVA 4500) using the methodology as described by Kapoor and Metzger (2005). Kapoor and Metzger (2005) have shown in their work that small-scale PC manufacture in RVA can successfully replicate PC manufacture made in a pilot scale twin screw cooker. The PCP formulations were prepared by mixing all ingredients in a KitchenAid blender equipped with a handle (Professional 5 Plus, KitchenAid) at room temperature for 30 min to produce a homogeneous paste. A 25-g sample of the paste was weighed in a canister, and then a paddle was inserted. The canisters were warmed in a water bath at 40°C for 10 min and then cooked in the RVA for 3 min at 95°C. The stirring speed was 1,000 rpm for the first 2 min and 160 rpm for the last min. Each batch (300 g) was divided into 10 canisters. The cooked PCP was then poured into copper molds (diameter = 20 mm; height = 30 mm) to measure the hardness using texture profile analysis. Also, it was poured into plastic molds (diameter = 28.3 mm; height = 25 mm) to measure the melt temperature using dynamic stress rheometry (DSR) and melt diameter using the Schreiber melt test.

**Chemical Analyses**

The TS (AOAC International, 2000; method 990.20; 33.2.44), total protein (TPr = total nitrogen × 6.38; AOAC International, 2000; method 991.20; 33.2.11), ash (AOAC International, 2000; method 945.46; 33.2.10), and pH (Thermo Scientific) of the MCC and acid curd were determined before they were used in...
PCP formulations. Also, the TS and pH of the final PCP were determined.

**Functional Analyses**

*End Apparent Viscosity.* The end apparent viscosity of the PCP was measured at the end of the cooking time using the RVA at 95°C by calculating the mean value of the last 5 values, which is referred to the end apparent viscosity. The end apparent viscosity was measured in all canisters of each batch.

*Texture Profile Analysis.* The hardness of the PCP was measured using the texture profile analysis. The PCP was prepared by pulling the cheese out from the copper cylinders and then cutting the cheese to 20 mm height. The PCP was analyzed for hardness using a TA.XT-Plus Texture Analyzer (TA.XT-Plus) equipped with a 38 mm diameter cylindrical flat probe (TA-4) and using uniaxial double bite 10% compression with 1 mm/s crosshead speed. The maximum force of the first compression was referred to the hardness of the cheese. This test was repeated 6 times for each batch.

*Dynamic Stress Rheometry.* The PCP was prepared by cutting the cheese into slices (2 mm thick and 28.3 mm diameter) using a wire cutter (Salunke, 2013). A stress sweep test of the PCP was performed at a frequency of 1.5 Hz and constant stress of 50 Pa. The temperature at which \( \tan \delta = 1 \) \((G''/G')\) was referred to the cheese melting temperature. A duplicate was performed on each batch.

**Schreiber Melt Test.** The PCP samples were cut into cylinders (diameter = 28.5 mm and height = 7 mm) and placed in glass Petri dishes (95.0 mm diameter). The dishes were transferred to a forced draft oven at 90°C for 7 min (Salunke, 2013). After cooling the dishes, the diameter of the melted PCP samples was measured in 4 different places using a vernier caliper and reported in millimeters. This test was repeated 4 times for each batch.

**Statistical Analysis**

Statistical analysis was performed to study the effect of treatments on the functional properties of PCP. Analysis of variance was done to obtain the mean squares and \( P \)-values using the GLM procedure available in R software (R × 64–3.3.3, R Foundation for Statistical Computing; https://r-project.org). Differences were tested using the least significant difference test when a significant difference was detected at \( P < 0.05 \).

**RESULTS AND DISCUSSION**

**Composition of Ingredients**

The composition of MCC before and after drying is shown in Table 2. The TS, TPr, and ash content were approximately 13.8, 9.5, and 1.0%, respectively, in liquid MCC. It has been reported that the retentate of 3× MF GP membranes had 89.6% CN%TP with 0.92% SP and 15.3% TS (Tremblay-Marchand et al., 2016). Similar results were also found in other studies; however, the TS and protein of MCC produced in other studies were high relative to our study due to the extra DF stages utilized in those studies (Hurt and Barbano, 2010; Hurt et al., 2010; Zulewska and Barbano, 2014). The composition of MCC can vary depending on many factors, such as membrane type, DF, as well as the composition of skim milk (Hurt and Barbano, 2010; Hammam et al., 2021, 2022a). After spray drying of MCC, it resulted in MCC powder with 97.2% TS, 65.4% TPr, and 7.1% ash. The pH of MCC before and after drying was 6.8. The composition of MCC powder was relatively similar to liquid MCC on a dry basis.

The TS, TPr, ash, pH, and moisture-adjusted yield of acid curd made from 3, 6, and 9% protein MCC are shown in Table 3. The mean TS of acid curd made from 3, 6, and 9% protein MCC was around 37.5, 43.8, and 41.6%, respectively. The TPr content was 32.1% in acid curd made from 3% protein MCC, whereas it was 37.9

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Table 1. Mean (n = 3) ingredients used in process cheese product (PCP) formulations

| Ingredient (%)         | Control | FR-3 | FR-6 | FR-9 |
|------------------------|---------|------|------|------|
| Acid curd              | 0.00    | 32.76| 26.64| 30.83|
| MCC powder (produced)  | 0.00    | 7.80 | 7.80 | 7.80 |
| MCC powder (commercial)| 3.40    | 0.00 | 0.00 | 0.00 |
| Butter (unsalted)      | 0.00    | 20.55| 20.55| 20.55|
| Aged Cheddar cheese    | 30.05   | 10.00| 10.00| 10.00|
| Young Cheddar cheese   | 30.05   | 0.00 | 0.00 | 0.00 |
| Deproteinized whey     | 7.20    | 6.44 | 6.65 | 5.80 |
| Salt (sodium chloride)| 1.00    | 2.00 | 2.00 | 2.00 |
| Water                  | 25.80   | 20.45| 26.36| 23.02|
| Disodium phosphate     | 2.50    | 0.00 | 0.00 | 0.00 |

\(^1\)Control = PCP formulations made with emulsifying salts; FR-3 = PCP formulations made from acid curd-3 [acid curd produced from micellar casein concentrate (MCC) with 3% protein]; FR-6 = PCP formulations made from acid curd-6 [acid curd produced from MCC with 6% protein]; FR-9 = PCP formulations made from acid curd-9 (acid curd produced from MCC with 9% protein).
and 34.5% in acid curd made from 6 and 9% protein MCC, respectively. The ash content ranged from 0.7 to 1.3% in acid curd made from MCC with a protein content of 3 to 9%. The moisture-adjusted yields were 6.6, 16.9, and 32.0% for acid curd produced from MCC with 3, 6, and 9% protein, respectively. There was no significant difference ($P > 0.05$) in the compositions of acid curd made from MCC with 3, 6, and 9% protein; however, the moisture-adjusted yield significantly increased ($P < 0.05$) with elevation of the protein content in MCC, as expected. Although there were substantial variations between replicates, we did not find any significant differences in the composition of acid curd made from the 3 treatments. We think that the replicate effect resulted from differences in pressing time and amount of lactic acid used to acidify and set the curd at the pH of 4.6. Because no ash modification was done, we expected that the acid curd made from 3% protein MCC would be lower in ash as compared with 6 and 9% protein MCC. The target pH in acid curd was 4.6; however, the pH of acid curd made from 9% protein MCC (4.00) was lower than other treatments (~4.6). As the protein content increased in MCC, it was more challenging to adjust the pH with lactic acid possibly due to increased buffering leading to an increase in the amount of used lactic acid. Therefore, 0.3% of sodium hydroxide (40%) was used to standardize the pH of acid curd-9 treatment to elevate the pH to 4.6. The composition of acid curd made from MCC in our study was in the range of typical acid curd made from skim milk on a dry basis (Blanchette et al., 1996; Klei et al., 1998; Sarode et al., 2016; Hammam et al., 2021). The adjusted yield of acid curd made from 6 and 9% protein MCC was high as compared with cottage cheese curd made from skim milk (Klei et al., 1998; Hallab et al., 2007). These results demonstrate the efficiency of making acid curd from 6 and 9% protein MCC, which contributed to more yield.

### Composition of PCP

The moisture content and pH of PCP made with ES (control) and without ES (FR-3, FR-6, and FR-9) are shown in Table 4. Mean squares and $P$-values for the moisture and pH of the final PCP are shown in Table 5. The moisture content of PCP made from acid curds (no ES) ranged from 48.1 to 48.5%, and the moisture content of control PCP was approximately 48.5%. Because all formulations were standardized to have the same composition, no significant difference ($P > 0.05$) was detected in the moisture content of all PCP formulations. The target pH in acid curd was 4.6; however, there was some moisture loss during cooking in the RVA, which led to a decrease in the final moisture content of the PCP. This loss could be compensated by the addition of 0.5 g of water in each canister before cooking in the RVA (Purna et al., 2006).

The pH of PCP made from acid curd and MCC was approximately 5.4 and did not show any differences ($P > 0.05$) within treatments made with no ES; however, the pH of control was significantly higher ($P < 0.05$), which was 5.8. The differences in the type and age of natural cheese, type and level of ES can result in differences in the final pH of PCP (Gupta et al., 1984; Shirashoji et al., 2006; Kapoor and Metzger, 2008; Bulut-Solak and Akin, 2019). The differences in the final pH between the control and experimental PCP may be attributed to the use of ES. In addition to calcium chelation, the other important function of ES is pH buffering, which leads to an increase in the final

| Treatment $^1$ | TS (%) | TPr (%) | Ash (%) | pH    | Moisture-adjusted yield (%) $^2$ |
|---------------|--------|---------|---------|-------|-------------------------------|
| Acid curd-3   | 37.46  | 32.11   | 0.72    | 4.68  | 6.60$^a$                     |
| Acid curd-6   | 43.77  | 37.86   | 1.07    | 4.54  | 16.90$^b$                    |
| Acid curd-9   | 41.65  | 34.50   | 1.26    | 4.00  | 32.00$^a$                    |
| SEM           | 1.79   | 1.70    | 0.10    | 0.15  | 4.40                          |

$^1$Acid curd-3 = acid curd produced from micellar casein concentrate (MCC) with 3% protein; acid curd-6 = acid curd produced from MCC with 6% protein; acid curd-9 = acid curd produced from MCC with 9% protein.

$^2$TPr = total protein = total nitrogen × 6.38. Adjusted yield = actual yield × $\frac{100 - \text{actual } \% \text{ moisture}}{100 - \text{desired } \% \text{ moisture}}$. 

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**Table 2.** The composition of liquid and dried micellar casein concentrate (MCC)

| Treatment          | TS (%) | TPr (%) | Ash (%) | pH   |
|--------------------|--------|---------|---------|------|
| Liquid MCC         | 13.79  | 9.54    | 1.03    | 6.80 |
| MCC powder (produced) | 97.23  | 65.38   | 7.13    | 6.80 |

$^1$TPr = total protein = total nitrogen × 6.38.
pH of PC (Kapoor and Metzger, 2008). It has been reported that the pH of a good-quality PC using ES should range from 5.4 to 5.8 (Palmer and Sly, 1943; Marchesseau et al., 1997; Kapoor and Metzger, 2008; Bulut-Solak and Akin, 2019), which is similar to those results in Table 4. Palmer and Sly (1943) stated that the emulsion of PC is low when the pH is lower than 5.4 or higher than 5.8. The differences in pH of control PCP made with ES relative to PCP made with no ES could affect the structure and quality of final PCP and thereby the functional properties due to its effects on the protein interactions in the final PCP emulsion (Palmer and Sly, 1943; Meyer, 1973; Marchesseau et al., 1997). It was found that as the pH of PC drops to 5.2, the protein-protein interaction increases (Marchesseau et al., 1997) because the pH is close to the isoelectric point of caseins (4.6). This induces the aggregation of protein, which in turn, results in poor emulsion of fat in PC. In contrast, the PC had an open structure when the pH elevated to 6.1, which eventually led to weaker emulsification (Marchesseau et al., 1997). Marchesseau et al. (1997) also found that the pH of 5.7 resulted in PC with better uniform fat emulsion with a closely knit protein network.

### Functional Characteristics of PCP

The mean values of end apparent viscosity and hardness of PCP are illustrated in Table 6. Mean squares and P-values for the end apparent viscosity and hardness of the final PCP are shown in Table 7. The end apparent viscosity of PCP in FR-3, FR-6, and FR-9 treatments were approximately 483.2, 402.1, and 474.9 cP, respectively. The end apparent viscosity of control PCP was around 576.6 cP. Although the end apparent viscosity of PCP made with ES (control) was slightly higher relative to PCP made with no ES (FR-3, FR-6, and FR-9), no significant differences (P > 0.05) were detected.

For the pH effect on end apparent viscosity; it was stated that as the pH elevated in PC, the net negative charges on the casein increased (Kapoor, 2007; Mulso et al., 2007; Lu et al., 2008). This induced the calcium to mediate cross-linking of the casein molecules around each other in PC gel network. The calcium induced cross-linking of the casein molecules limits the movement of casein chains and thereby reduces the flowability of PC. When the PC is heated, the hydrophobic interactions increase as the temperature elevates which could decrease the flowability. When pH is low and being close to the isoelectric point, more protein-protein interactions elevate. Increasing the pH elevates the net negative charges on casein molecules and thereby elevates the water holding capacity of casein molecules (Fox et al., 2015). This results in swelling of casein molecules and increasing the viscosity of PC emulsion. This was also proved when the pH of casein solutions was elevated from 5.0 to 7.0, which resulted in higher viscosity (Zoller, 1921). However, the high pH in PCP made with ES (control) did not result in significant
The hardness of the PCP was 383.7, 363.3, and 354.6 g for FR-3, FR-6, and FR-9, respectively (Table 6). No significant difference was found \((P > 0.05)\) in the hardness of PCP among the 3 experimental treatments made with no ES. However, the PCP made using ES (control) had a lower hardness of 119.0 g \((P < 0.05)\) as compared with PCP made without ES using different acid curds at a ratio of 2:1 protein from acid curd to protein from MCC, respectively. In our previous study, we found that the hardness of PCP made using MCC and ES ranged from 100.0 to 212.0 g (Hamman et al., 2022b). In another study, the hardness of PCP made from MCC using ES was 110.0 to 135.0 g (Salunke, 2013), which is similar to control PCP we made in this study using ES. The results indicate that PCP made without ES was firmer than the control PCP.

The hardness of PC is mainly affected by the emulsified-gel network (Marchesseau et al., 1997). Several casein-based interactions are responsible for forming the basis for the emulsified-gel network and stabilizing casein network, such as hydrophobic interactions, hydrogen bonds, and calcium mediated cross-links (Marchesseau et al., 1997). The changes in pH could affect those interactions. Typically, the net negative charges on casein are increased when the pH is elevated, which induces the robust hydrogen bonds and calcium mediated cross-links within the casein molecules in PCP, and this, in turn, strengthens the gel network of PCP and makes it firmer. Some studies reported that increasing the pH in a specific range led to an increase in the hardness, whereas others proved the opposite suggestions. It was reported that the pH affects the hardness (Kapoor, 2007). Kapoor (2007) found that the firmness of PCF elevated as the pH increased from 5.5 to 6.1 with increasing the intact casein. Another study found that the hardness of PC made with sodium hexametaphosphate as an ES increased with elevating the pH from 5.6 to 5.9 (Lu et al., 2008). In a different study, it was found that increasing the pH led to increasing the hardness of fat free PC spreads (Swenson et al., 2000). However, other studies reported that increasing pH resulted in low hardness PC (Cavalier-Salou and Cheftel, 1991; Lee et al., 1996; Lee and Klostermeyer, 2001; Awad et al., 2002). In a different study, it was found that elevating the pH of PC from 5.7 to 6.7, and decreasing pH from 5.7 to 5.2, led to less firmed PC (Marchesseau et al., 1997). The increased firmness of PCP with no ES, when compared with control in this study, might be due to increases protein-protein interactions of caseins at pH 5.4 leading to a firmer product than control (pH 5.8).

The melting characteristics of the control and experimental PCP are illustrated in Table 8. Mean squares and \(P\)-values for the melting properties of the PCP are shown in Table 9. The melting temperature of PCP made from FR-3, FR-6, and FR-9 was 51.3, 48.4, and 50.5°C, respectively. The melting temperature of PCP made with ES (control) was higher with an average of 59.8°C compared with FR-3, FR-6, and FR-9. No significant difference \((P > 0.05)\) was detected in the melting temperature of PCP made from FR-3, FR-6, and FR-9. However, there was a significant difference \((P < 0.05)\) in the melting temperature of PCP made with no ES relative to control.

The differences in the onset of melting (melting temperature) can be explained by the differences in pH of those cheeses. As the pH drops to the isoelectric point, the net negative charges on caseins reduce which increases the protein-protein interactions, and this leads to aggregation of protein and thereby poor emulsification (Kapoor, 2007). The higher pH in control PCP resulted in a uniform fat emulsion with a closely knit protein network. This led to a higher melting tempera-
ture of PCP made with ES relative to other PCP made with no ES.

The melt diameter of FR-3, FR-6, and FR-9 was 29.9, 30.2, and 31.4 mm, respectively; with a melt area of 704.3, 717.9, and 775.1 mm², respectively. The melt diameter of those treatments was not significantly different \((P > 0.05; \text{Table 8})\). The melting diameter of 41.2 mm and melt area of 1,331.2 mm².

The PCP made with ES (control) resulted in increased meltability due to the high pH in this formulation compared with other PCP formulations made with no ES. This is due to the increased protein-protein interactions (high at lower pH) in PC made with no ES.

The storage modulus \((G'\text{)}\) and loss modulus \((G''\text{)}\) of PCP measured during heating from 20 to 90°C at 10°C increments are shown in Tables 10 and 11, respectively. The ANOVA Table with mean squares and \(P\)-values at \(10°C\) interactions (high at lower pH) in PC made with no ES in PCP and during heating from 20 to 90°C is also presented in Figures 4 and 5, respectively.

The \(G'\) (at a temperature range of 20 to 90°C) of PCP made with no ES using different acid curds (made from 3, 6, and 9% protein MCC) was higher than PCP made with ES (Figure 4) from 20 to 40°C. The \(G'\) of control PCP was high from 50 to 70°C compared with FR-3, FR-6, and FR-9, whereas it was lower than those treatments from 80 to 90°C. These differences were not significant \((P > 0.05)\) at many points (Table 10); however, the \(G'\) was significant \((P < 0.05)\) between control and other treatments at 90°C (room temperature), 50 and 60°C (near melting temperatures). More \(G'\) indicates more firmness or hardness for the final PCP (Kapoor and Metzger, 2008), which was noticed with low pH in PCP made with no ES at 20°C and firmness was measured at the same temperature. The differences in pH play a significant role in the melting characteristics of PCP as mentioned as well as the \(G'\)

### Table 9. Mean squares and \(P\)-values (in parentheses) for the melting properties of the process cheese products (PCP)

| Factor      | df | Melt temperature | Melt diameter | Melt area  |
|-------------|----|------------------|---------------|------------|
| Replicate   | 2  | 4.26 (0.45)      | 1.55 (0.30)   | 4.80 (0.26) |
| Treatment¹  | 3  | 76.05 (<0.05)    | 86.08 (<0.05) | 271.730 (<0.05) |
| Error       | 6  | 4.72             | 1.07          | 2.870       |

¹Control = PCP formulations made with emulsifying salts; FR-3 = PCP formulations made from acid curd-3 (acid curd produced from micellar casein concentrate with 3% protein); FR-6 = PCP formulations made from acid curd-6 (acid curd produced from micellar casein concentrate with 6% protein); FR-9 = PCP formulations made from acid curd-9 (acid curd produced from micellar casein concentrate with 9% protein).

### Table 10. Mean elastic modulus \((G';\text{ Pa})\) of process cheese products (PCP) during heating from 20 to 90°C using dynamic rheological analysis

| Temperature \(^°C\) | Control | FR-3 | FR-6 | FR-9 |
|---------------------|---------|------|------|------|
| 20                  | 18,022.1ab | 40,534.2ab | 34,806.5ab | 70,025.2a |
| 30                  | 7,581.4a  | 13,018.4ab | 12,171.8a  | 19,924.9a  |
| 40                  | 2,925.6a  | 5,275.0a  | 4,995.4a  | 5,272.6a  |
| 50                  | 1,227.5a  | 1,331.2a  | 1,060.5ab | 834.6b   |
| 60                  | 326.4a    | 67.4a     | 181.5a    | 111.6a   |
| 70                  | 10.9a     | 30.4a     | 79.8a     | 99.9a    |
| 80                  | 1.4a      | 11.5a     | 27.2a     | 42.8a    |
| 90                  | 4.26 (0.45)| 1.55 (0.30)| 4.80 (0.26) | 2.870 (0.05) |

²Means in the same row not sharing a common superscript are different at \(P < 0.05\).

### Table 11. Mean viscous modulus \((G'';\text{ Pa})\) of process cheese products (PCP) during heating from 20 to 90°C using dynamic rheological analysis

| Temperature \(^°C\) | Control | FR-3 | FR-6 | FR-9 |
|---------------------|---------|------|------|------|
| 20                  | 7,041.1a | 13,048.2ab | 11,395.2ab | 21,466.5a |
| 30                  | 4,159.7a | 5,277.9a  | 4,831.6a  | 7,352.5a  |
| 40                  | 2,054.9a | 3,093.8a  | 3,363.2a  | 2,784.2a  |
| 50                  | 1,228.2a | 462.5a    | 354.4a    | 271.0a    |
| 60                  | 79.5a    | 66.2a     | 99.9a     | 230.5a    |
| 70                  | 1.4a     | 11.5a     | 27.2a     | 42.8a     |
| 80                  | 4.26 (0.45)| 1.55 (0.30)| 4.80 (0.26) | 2.870 (0.05) |

³Means in the same row not sharing a common superscript are different at \(P < 0.05\).
values. This reflected on the $G'$ values of final PCP. It was found that the $G'$ values of PCP with a pH of 5.77 were higher compared with PCP with 5.55 pH (Salunke and Metzger, 2022a). Another study found that the $G'$ values increase as the pH decrease (Lee et al., 1996) which is similar to our study.

The $G''$ (at a temperature range of 20 to 90°C) of PCP made with no ES using different acid curds was higher than control PCP (Figure 5) from 20 to 40°C. Similar to the $G'$ trend, the $G''$ of control PCP was high from 50 to 70°C compared with FR-6, and FR-9, whereas it was lower than those treatments from 80 to 90°C. These differences were not significant ($P > 0.05$) at several points during running the DSR test (Table 11). However, PCP made from FR-9 (acid curd made from MCC with 9% protein) resulted in the highest $G''$ than FR-3, FR-6, and control, which followed the same trend as in $G'$. It was found that the $G''$ values of PCP with a pH of 5.77 were higher compared with PCP with 5.55 pH (Salunke and Metzger, 2022a). Another study found that the $G''$ values increase as the pH decrease (Lee et al., 1996) which is similar to our study.

The $G'$ of PCP before melting was higher than $G''$. This indicates that the PCP has more elastic behavior (gel) than the viscous behavior (liquid). The $G'$ (elastic) and $G''$ (viscous) are decreased during measuring the melting point using the DSR. Both moduli are decreased with increasing the temperature until the cross point, which is the cheese melting point. This is due to the low protein-protein interactions in the casein network while heating, which lead to fat separations (Salunke and Metzger, 2022a). This trend was similar to those found

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**Figure 4.** Elastic modulus ($G'$: Pa) of process cheese products (PCP) made from control (●) = PCP formulations made with emulsifying salts; FR-3 (▼) = PCP formulations made from acid curd-3 [acid curd produced from micellar casein concentrate (MCC) with 3% protein]; FR-6 (■) = PCP formulations made from acid curd-6 (acid curd produced from MCC with 6% protein); FR-9 (♦) = PCP formulations made from acid curd-9 (acid curd produced from MCC with 9% protein) during heating from 20 to 90°C using dynamic rheological analysis.

**Figure 5.** Viscous modulus ($G''$: Pa) of process cheese products (PCP) made from control (●) = PCP formulations made with emulsifying salts; FR-3 (▼) = PCP formulations made from acid curd-3 [acid curd produced from micellar casein concentrate (MCC) with 3% protein]; FR-6 (■) = PCP formulations made from acid curd-6 (acid curd produced from MCC with 6% protein); FR-9 (♦) = PCP formulations made from acid curd-9 (acid curd produced from MCC with 9% protein) during heating from 20 to 90°C using dynamic rheological analysis.

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**Table 12.** Mean squares and $P$-values (in parentheses) for elastic modulus ($G'$: Pa) and viscous modulus ($G''$: Pa) of process cheese products (PCP) during heating from 20 to 90°C using dynamic rheological analysis.

| Factor    | df | 20°C       | 70°C       | 90°C       | 20°C       | 70°C       | 90°C       |
|-----------|----|------------|------------|------------|------------|------------|------------|
| Replicate | 2  | 15,132,092 (0.98) | 66,454 (0.38) | 3,226 (0.61) | 935,479 (0.98) | 192,537 (0.33) | 29,403 (0.49) |
| Treatment  | 3  | 1,408,926,975 (0.19) | 34,636 (0.64) | 7,578 (0.38) | 109,540,943 (0.19) | 52,928 (0.77) | 29,305 (0.53) |
| Error     | 6  | 653,356,947 | 58,295 | 6,145 | 50,746,681 | 142,187 | 36,217 |

1 Control = PCP formulations made with emulsifying salts; FR-3 = PCP formulations made from acid curd-3 [acid curd produced from micellar casein concentrate (MCC) with 3% protein]; FR-6 = PCP formulations made from acid curd-6 (acid curd produced from MCC with 6% protein); FR-9 = PCP formulations made from acid curd-9 (acid curd produced from MCC with 9% protein).
in other studies (Hennelly et al., 2005; Subramanian et al., 2006; Zhong et al., 2007; Guinee and O’Kennedy, 2009; Kommineni et al., 2012; Hosseini-Parvar et al., 2015; Salunke and Metzger, 2022a).

The functional characteristics and composition of PCP can be affected by formulations, ingredients, pH, intact casein, calcium content, cooking time, cooking temperature, stirring speed, type and amount of ES, and cooling speed. The functionality of the PCP made without ES was in the range of typical PCP made with ES. This indicates that PCP can be manufactured using a 2:1 ratio of acid curd protein to MCC protein and has similar functionality as compared with PCP made with ES.

CONCLUSIONS

In this research, we determined that acid curd could be made efficiently from MCC with different protein contents (3, 6, and 9%). The adjusted yield of acid curd increased with increasing the protein content, therefore 9% protein MCC could be a good option to make acid curd commercially as it would generate less acid whey. Acid curds produced from MCC with different protein content were successful in the manufacture of PCP without ES using 2 parts of protein from acid curd to 1 part of protein from MCC. The 2:1 ratio creates a partially deaggregated casein network that results in PCP with functionality similar to PCP produced with ES. Although there were differences observed in the melted and unmelted texture of PCP made without ES when compared with control, these can be explained due to the possible microstructural interactions induced in the final PCP due to their pH differences. However, no differences were detected in the functionality of PCP produced from acid curds with different protein levels. Future studies will focus on devising methodologies to produce PCP using acid curd with similar pH as conventional PCP. The PCP was not tested and validated in large scale although some correlations have been found between PCP made in RVA and large scale. As a result, large scale will be used to produce PCP as one of the future studies.

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ORCIDS

Ahmed R. A. Hammam © https://orcid.org/0000-0002-3882-7220

Lloyd E. Metzger © https://orcid.org/0000-0003-3929-4539