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Short communication: Effects of nanofiltration and evaporation on the physiochemical properties of milk protein during processing of milk protein concentrate

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ABSTRACT

The aim of this work was to evaluate the effects of nanofiltration and evaporation concentration technologies on the physiochemical properties of milk protein concentrate (MPC) during processing. Skim milk, ultrafiltered milk, evaporated milk, nanofiltered milk, evaporated MPC, and nanofiltered MPC samples were collected at different processing stages. Chemical composition, microstructure of casein micelles, free sulfhydryl content, and surface hydrophobicity of the samples were determined. The insolubility index of MPC was also determined. Casein micelles aggregated compactly after evaporation while surface hydrophobicity increased and free sulfhydryl content decreased in evaporated milk compared with skim milk. However, the microstructure of the casein micelles was relatively undisturbed after nanofiltration, with reduced surface hydrophobicity and free sulfhydryl content. No significant difference was found in chemical composition between the 2 MPC preparations: approximately 61.40% protein and 28.49% lactose. In addition, the particulate microstructures of both MPC were similar. However, the insolubility index of evaporated MPC was significantly (0.58 mL) higher than that of nanofiltered MPC. Nanofiltration may be an effective way to improve the solubility of MPC products.
Key words: milk protein concentrate, nanofiltration, evaporation, physiochemical properties

Short Communication

Milk protein concentrate (**MPC**) has become a popular milk-based ingredient in the food industry in recent years and it is used in products such as cheese, yogurt, beverages, and baked goods. Solubility of MPC is the final step of powder dissolution and the determinant of the overall reconstitution quality (Fang et al., 2008). Solubility is also a prerequisite for achieving other functional properties, such as foaming, gelling, and emulsifying, and then affecting its further applications (Fang et al., 2011).

However, MPC has poor solubility upon rehydration or reconstitution (Mimouni et al., 2010b; Mao et al., 2012), which is attributed to casein micelles (Anema et al., 2006; Havea, 2006). During ultrafiltration of MPC, colloidal calcium may decrease while free ions (e.g., serum calcium and phosphate) and lactose run off with the water (Singh, 2007). This process disrupts the ion balance of the milk product, destabilizing the casein micelles. Consequently, heat treatment likely causes changes in the structures of the milk proteins and thus affects the solubility of the final MPC powders. Some efforts have been made to improve the solubility of MPC, such as elevating the rehydration temperature (Mimouni et al., 2009; Ma, 2012), changing the ion environment during manufacturing to facilitate hydration of casein micelles (Bhaskar et al., 2001; Carr et al., 2002; Gualco, 2010; Mao et al., 2012), treating ultrafiltered or diafiltered milk protein by ultrasound (Sun et al., 2014), applying combinations of pressure and heat to the concentrate before spray drying (Udabage et al., 2012), and high shear treatment of ultrafiltered or diafiltered MPC before spray drying (Augustin et al., 2012). Except for elevating the rehydration temperature, most procedures improve MPC solubility in cold water. However, no study has yet examined the concentration process to reduce milk protein alterations and further improve the solubility of MPC in cold water.

Nanofiltration may be able to improve the solubility of MPC products. The nanofiltration membrane is a type of pressure-driven membrane with properties between those of reverse osmosis and ultrafiltration membranes. The current common application of nanofiltration in the dairy industry is for concentrating and desalinating whey (Suárez et al., 2006; Cuartas-Uribe et al., 2007). The advantages of nanofiltration are a relatively low initial investment and low operation and

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maintenance costs, in addition to low operation pressure, high flux, and high retention of multivalent anion salts and organic molecules >300 Da (Hilal et al., 2004). Because nanofiltration allows lower temperatures, the milk protein would be less exposed to heat treatment, which may lead to less denaturation in milk protein and therefore enhanced solubility.

In this study, a low-heat concentration technology—nanofiltration—was used to concentrate UF retentate to produce MPC. The effects of either traditional evaporation or nanofiltration concentration technologies on the microstructure, free sulfhydryl content, and surface hydrophobicity of milk protein were evaluated during MPC processing. The insolubility index of the final MPC products was also investigated.

Milk protein concentrates produced by evaporation or by nanofiltration were manufactured by Yinchuan Jinhe Dairy Industry Co. Ltd. (Ningxia, China). The manufacturing plant was equipped with 10-kDa cutoff, spiral-wound ultrafiltration membranes and 200-Da cutoff, spiral-wound nanofiltration membranes (both from Parker, Cleveland, OH). The milk was concentrated in a three-effect falling film evaporator, and the temperatures of the first, second, and third effects were 71, 60, and 56°C, respectively. The average inlet and outlet temperatures during spray drying were 160 and 85°C, respectively.

Skim milk (**SM**), ultrafiltered milk (**UF**), evaporated milk (**EP**), and nanofiltered milk (**NF**) collected at various processing points were poured into sterile bottles with addition of sodium azide (0.4 g/L) and were stored at −20°C. Evaporated MPC (**EP-MPC**) and nanofiltered MPC (**NF-MPC**) samples were packed in sterile plastic bags and stored at 4°C before analysis.

The contents of protein, lactose, TS, and fat of the milk samples were measured by a MilkoScan FT2 infrared milk analyzer (Foss, Hillerød, Denmark). Mineral content was measured by an inductive coupled plasma emission spectrometer (Jinhengxiang Instrument Co. Ltd., Beijing, China), and the chemical composition of both MPC samples was analyzed according to *Standard Methods for the Examination of Dairy Products* (Wehr and Frank, 2004).

The MPC insolubility index was obtained by measuring the amount of insoluble sediment (volume in mL) after MPC reconstitution under standard conditions according to an International Dairy Federation standard method (IDF, 2005).

Milk protein microstructures were examined by using a JSM-6700 field emission scanning electron microscope (Jeol, Tokyo, Japan) operating at 5 kV. Because of the high protein concentration, milk samples (UF, EP, and NF) were diluted with deionized water to a final concentration of approximately 3.5% protein. The preparation of liquid and powder samples for scanning electron microscopy were conducted according to the method described by Mimouni et al. (2010a). Both pretreated milk and MPC samples were coated with platinum particles using a JFC-1600 auto fine coater (Jeol) and were then sprayed onto double-sided carbon tape and mounted onto electron microscopy stubs.

Changes in the free sulfhydryl (**SH**) content of milk protein during manufacturing were determined according to the method of Ou et al. (2004), based on Ellman's reaction using 5,5′-dithio-2-nitrobenzoate (DTNB; Sigma-Aldrich Co., St. Louis, MO) at 412 nm by using a JH756 UV-Visible spectrophotometer (Jinghua Instrument Co., Shanghai, China). Free SH content was obtained by dividing the absorbance value by the SH molar extinction coefficient (13,600). The results were expressed in micromoles of SH per gram of protein.

The surface hydrophobicity index ($\mathbf{H_0}$) of milk protein was measured at wavelengths of 390 nm (excitation) and 470 nm (emission) with a Cary Eclipse Luminescence Spectrophotometer (Varian, Palo Alto, CA) using 1-anilinonaphthalene-8-sulfonic acid (**ANS**; Sigma-Aldrich Co.) as probe, as described by Sava et al. (2005).

Each parameter was analyzed in triplicate and results are shown as means ± standard deviations. The results were subjected to one-way ANOVA using SPSS 17.0 software (SPSS Inc., Chicago, IL). Duncan's multiple range test was performed to determine significant differences among samples. Differences were considered significant at $P < 0.05$.

The composition of SM was within the normal range (Table 1). The microstructure of SM (Figure 1A) showed dispersed individual casein micelle spheres. The diameters of these micelles were mainly in the range of 40 to 150 nm. The edge of each micelle was very clear, and individual spheres could be recognized easily. Skim milk had the highest H_0 but relatively low free SH content (Table 2). The SM sample was collected after pasteurization (85°C for 15s). When milk is heated at temperatures above 70°C during commercial processing, several physicochemical alterations occur in the milk constituents, including denaturation of whey proteins and the formation of hydrophobic or disulfide-bonded aggregates with the κ-casein of the casein micelles (Patel et al., 2006). Pasteurization could promote the formation of free SH groups, which can react with casein to form disulfide bonds (Westergaard, 2004). This process is consistent with the observation that SM had the lowest free SH content among samples. Heat treatment during pasteurization also likely exposed more ANS-binding hydrophobic groups.

Although the percentage of each component increased with UF ($P < 0.05$; Table 1), multivalent calcium,