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Removal of milk fat globules from whey protein concentrate 34% to prepare clear and heat-stable protein dispersions

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ABSTRACT

Whey protein concentrates (WPC) are low-cost protein ingredients, but their application in transparent ready-to-drink beverages is limited due to turbidity caused by fat globules and heat instability. In this work, fat globules were removed from WPC 34% (WPC-34) to prepare heat-stable ingredients via the Maillard reaction. The removal of fat globules by acid precipitation and centrifugation was observed to be the most complete at pH 4.0, and the loss of protein was caused by micrometer-sized fat globules and protein aggregates. Spray-dried powder prepared from the transparent supernatant was glycated at 130°C for 20 and 30 min or 60°C for 24 and 48 h. The 2 groups of samples had comparable heat stability and degree of glycation, evaluated by free amino content and analytical ultracentrifugation, but high-temperature, short-time treatment reduced the color formation during glycation. Therefore, WPC-34 can be processed for application in transparent beverages.
Key words: whey protein concentrate 34%, milk fat globule, acid precipitation, glycation for heat stability

INTRODUCTION

Whey protein ingredients have high contents of EAA and versatile functionalities enabling various applications in the food industry (Vardhanabhuti et al., 2009). Whey protein concentrate 34% (**WPC-34**) is a cost-effective ingredient with about 34% mass being proteins. When compared with WPC-80 and whey protein isolate (**WPI**), fewer purification steps are used in the production of WPC-34 and, therefore, WPC-34 contains big particulates such as milk fat globules (**MFG**) that cause turbidity and precipitation (Hwang and Damodaran, 1995). Conversely, recent studies have found that the MFG membrane (**MFGM**) contains abundant bioactive lipids and proteins that have the potential of inhibiting the growth of cancer cells, lowering cholesterol, inhibiting pathogens, and assisting in the cure of Alzheimer's disease (Kanno, 1990; Spitsberg, 2005). The MFGM has excellent surface activities and can be applied as the carrier material to deliver bioactive ingredients in functional foods (Thompson and Singh, 2006; Thompson et al., 2006). Therefore, processes separating WPC to fractions with and without MFGM may add value to the dairy industry.

Hwang and Damodaran (1995), Damodaran (2010), and Damodaran (2011) selectively precipitated MFGM using various approaches. In an early study (Hwang and Damodaran, 1995), chitosan was used to form complexes with MFGM in Cheddar cheese whey at pH 4.5, and the complexes were removed by centrifugation to form transparent supernatants, resulting in 92% removal of lipids and 6.8% reduction of protein. In a later study, divalent cations at 0 to 50 m*M* were studied to precipitate MFGM at 30°C and pH 5.2 (Damodaran, 2010). Calcium and magnesium ions were ineffective, whereas complete precipitation was observed for zinc ions above 20 m*M*. In the latest study, diafiltration of Cheddar cheese whey at pH 4.2 and 35°C for 30 min was also observed to be effective in precipitating MFGM that was speculated to be caused by removal of calcium ions that are critical to the structure of MFGM (Damodaran, 2011). A loss of 14% protein was reported after diafiltration. These studies are important to produce whey protein ingredients in applications requiring visual clarity.

Transparent beverages are possible products containing whey protein ingredients. To produce ready-to-drink beverages, thermal processing is needed to ensure microbiological safety and quality. For acidic beverages with pH <4.6, thermal processing conditions are not regulated, and the beverage industry uses hot-fill processes equivalent to heating at about 88°C for 2 min (Etzel, 2004). For products with pH >4.6, UHT processing can be used (e.g., at 138°C for at least 8 s for dairy products; McGarrahan, 1982). Conversely, whey proteins are known to aggregate during heating, especially at acidity near the isoelectric point of whey proteins (~pH 5.0) when the net charge of proteins is low (Bryant and McClements, 1998; Baier and McClements, 2005). Glycation of whey proteins with reducing

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saccharides is an effective method to improve the stability of proteins against aggregation during heating, and we recently showed that WPI glycated with lactose at 80°C for 2 h enabled transparent dispersions at pH 3.0 to 7.0 with 0 to 150 m*M* NaCl or $CaCl_2$ after heating at 88°C for 2 min and 138°C for 1 min (Liu and Zhong, 2013). Because cheese whey and WPC contain lactose, whey protein-lactose conjugates can be produced by integration with the whey protein-manufacturing processes.

The first objective of the present study was to exploit a simple process to prepare transparent samples by acid precipitation of MFG from WPC-34 suspension. The second objective was to evaluate the heat stability and structure of clarified WPC (**cWPC**) and conjugates prepared from cWPC (**gWPC**).

MATERIALS AND METHODS

Materials

The WPC-34 was kindly donated by Grande Cheese Co. (Grande, WI). Precast 15% gradient polyacrylamide gels and protein markers (catalog no. 161-0375) were purchased from Bio-Rad Laboratories Inc. (Hercules, CA). Nile Blue A dye was obtained from Sigma-Aldrich Corp. (St. Louis, MO). Bovine serum albumin used in the protein assay was purchased from BioWorld LLC (Atlanta, GA). Other chemicals used in the present study were from either Thermo Fisher Scientific Inc. (Pittsburgh, PA) or Sigma-Aldrich Corp.

Precipitation of MFG from WPC-34 Suspension

The WPC-34 was hydrated at a powder concentration of 2% (wt/vol) in deionized water for about 2 h under mixing on a stir plate until no visible powder was observed. The suspension was adjusted to pH 2.5 to 6.5 with 1.0 *N* NaOH or 1.0 *N* HCl. After standing at ambient conditions (21°C) for 4 h, the samples were centrifuged for 15 min at 8,000 × *g* (model Sorvall RC 5B Plus; DuPont, Wilmington, DE). Both the supernatant and precipitates were collected for further analysis. To improve the removal of MFGM, the supernatant was further incubated at ambient conditions for another 24 h and centrifuged again at 4,000 × *g* for 15 min at 10°C. Then, the turbidity of the supernatant was compared for visual appearance by photographing and determining the absorbance at 600 nm (Evolution 201 UV-visible spectrophotometer; Thermo Scientific, Waltham, MA). The protein concentration in the supernatant was measured by the bicinchoninic acid method using an assay kit from Thermo Fisher Scientific Inc. (Rockford, IL), with BSA as a protein standard.

Preparation of cWPC powder

The WPC-34 powder was suspended in deionized water and processed as above for the treatment adjusting to pH 4.0. After centrifugation, the transparent supernatant was collected, adjusted to pH 7.0 with 1.0 *N* NaOH, and spray-dried (model B-290; Büchi Labortechnik AG, Flawil, Switzerland). The spray dryer was operated at conditions as follows: an inlet air temperature set at 160°C, a compressed air pressure of 600 kPa, an outlet temperature controlled at about 65°C, an air flow rate of 32 m^3/h, and a feed flow rate of ca. 4 mL/min. The powder collected from the spray dryer was designated as cWPC and used in further experiments.

SDS-PAGE

The SDS-PAGE was carried out under the reducing conditions with a 15% precast polyacrylamide gel obtained from Bio-Rad Laboratories Inc. The WPC-34 was prepared at 2% (wt/vol) concentration and then subjected to different treatments described in previous sections. Fifty microliters of each collected sample was mixed with 200 μL of the SDS-PAGE loading buffer and then heated at 95°C for 5 min in a water bath. Five microliters of each sample was loaded into each well of gel. Electrophoresis in a Mini Protean Tetra Cell (Bio-Rad Laboratories Inc.) was conducted at a constant voltage of 200 V and a current of 400 mA until the indicator dye reached the gel bottom. After staining with Coomassie Blue for 10 min, the gel was destained until bands became visible.

Characterization of Protein Particles and MFG by Confocal Laser Scanning Microscopy

The microstructural characteristics of MFGM and protein were studied using a Leica SP2 confocal laser scanning microscope (Leica Microsystems GmbH, Mannheim, Germany). The instrument was equipped with an Argon ion laser excited at a wavelength of 633 nm for protein observation and a HeNe laser excited at 543 nm for fat observation. One milliliter of WPC sample [2% (wt/vol) powder suspended in distilled water at pH 7.0] was mixed with 10 μL of a 1.0% (wt/vol) Nile Blue A solution to stain both proteins and lipids before imaging.

Fat, Protein, and Lactose Contents in WPC and cWPC

The total lipid content was determined using the method of AOAC International (2012; method 989.05).