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Effect of whey protein aggregates of various sizes on the formation and properties of rennet-induced milk gels

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ABSTRACT

Whey protein (WP) aggregates that ranged in diameter from 41 to 299 nm but had the same composition were produced by varying the protein concentration at heating at neutral pH and low ionic strength, followed by dilution. The effect of WP aggregates on the formation and properties of rennet-induced milk gels were evaluated. Whey protein aggregates had no effect on the kinetics of casein-omacropeptide release during renneting but significantly affected the clotting of casein micelles and the properties of milk gels. The maximum clotting rate and the strength of rennet-induced milk gels decreased as the dose of WP aggregates added to milk increased. However, WP aggregates with larger diameter and higher voluminosity had a less detrimental effect on milk clotting than smaller aggregates did. The higher surface hydrophobicity and faster diffusion of smaller WP aggregates could have been responsible for adsorption onto renneted casein micelles, which reduced milk clotting rate and gel strength. The permeability of rennet-induced milk gels decreased as the dose of WP aggregates added to milk increased, but the protein concentration at heating had no effect. Whey protein aggregates reduced the contraction capacity of the gels during cooking (42 °C). Drained curd mass fraction and curd moisture increased as the dose of added WP aggregates increased and were affected less by larger WP aggregates. The results of this study suggest that small WP aggregates (<100 nm) interact with casein micelles and interfere with rennet gel formation and contraction.

1. Introduction

In the traditional cheese-making process, the curd matrix is formed by caseins, which constitute about 80% of the milk proteins whereas the remaining 20%, the whey proteins (WPs), are lost to a large extent in the whey. In recent decades, many efforts have been made to increase WP levels in cheese (Hinrichs, 2001; Lelièvre, 1995). The main reason for those efforts is economic, since the retention of WPs in cheese increases cheese yield and thereby cheese-making profits (Jensen & Stapelfeldt, 1993). There is also a nutritional interest, because WPs are high in essential amino acids (Ismail, Ammar, & El-Metwally, 2011). Different approaches have been proposed to increase WP recovery in cheese, such as the heat treatment of milk to denature and complex the WPs with casein micelles (Lawrence, 1993; Lelièvre, 1995). However, this practice can interfere with gel formation and cause texture defects (Banks, Law, Leaver, & Horne, 1994; Guyomarc'h, 2006; Singh & Waungana, 2001). The ultrafiltration of unheated or heated milk has also been used to concentrate the total protein fraction and increase the retention of WPs in cheese (Banks et al., 1994; Jensen & Stapelfeldt, 1993). This technology requires the adaptation of the processing conditions and is preferably used for soft cheeses (Hinrichs, 2001). The heat denaturation of WPs before they are added to cheese milk has been proposed as another way to incorporate WPs into the cheese matrix. The heat denaturation of WPs under controlled conditions induces the formation of aggregates through hydrophobic interactions and intermolecular disulfide bonds (Alting et al., 2004; Guyomarc'h, 2006). During the cheese-making process, WP aggregates are trapped in the pores of the casein network. It is common knowledge that WP aggregates must be smaller than the matrix pore size (<10 μm) in order for the continuity of the casein network to be preserved (Hinrichs, 2001; Lelièvre, 1990, 1995). Several studies have focused on WP denaturation and aggregation (Nicolai, Britten, & Schmitt, 2011). It has been shown that the size of WP aggregates depends on many factors such as the protein concentration at heating (Alting, Hamer, de Kruif, Paques, & Visschers, 2003; Ju & Kilara, 1998), the pH and calcium concentration at heating (Britten & Giroux, 2001), the

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heating time and temperature (Ju & Kilara, 1998; Spiegel, 1999), and the lactose content (Spiegel, 1999). Aggregate size can be further controlled by homogenization, whether applied during or after heat treatment (Spiegel & Huss, 2002; Zoon, 1994).

The influence of denatured WPs on the renneting properties of milk (Steffl, Hafenmair, Hechler, & Hinrichs, 1999; Steffl, Schreiber, Hafenmair, & Kessler, 1999a, 1999b) and the characteristics of cheese (Lebeuf, Lacroix, & Paquin, 1998; Mead & Roupas, 2001) has been studied. The addition of heat-denatured WPs to milk decreased the strength and contraction capacity of rennet-induced gels, resulting in cheese with increased moisture content (Hinrichs, 2001). These changes were attributed to the strong water-binding capacity of denatured WP aggregates and the steric hindrance of the paracasein network. However, little attention has been paid to the possible interaction between WP aggregates and casein micelles during milk coagulation. Soluble protein aggregates isolated from heated milk were shown to interact with casein micelles during milk renneting (Kethireddipalli, Hill, & Dalgleish, 2011). A similar interaction between denatured WP aggregates and casein micelles may interfere with rennet gel formation and contraction.

The objective of this study was to determine the influence of WP aggregates of different sizes and characteristics on the formation and properties of rennet-induced milk gels. Whey protein aggregates were produced at neutral pH and low ionic strength by varying only the protein concentration at heating, in order to avoid any change in chemical composition.

2. Materials and methods

2.1. Materials and reagents

Hilmar 9400 WP isolate containing 88.7% (w/w) protein in dry matter was purchased from Hilmar Ingredients (Hilmar, CA, USA). Low-heat skim milk powder containing 35% (w/w) protein in dry matter was provided by Agropur (Granby, QC, Canada). IdaPro milk protein isolate containing 81.8% protein (w/w) in dry matter was obtained from Idaho Milk Products (Jerome, ID, USA). Milk permeate was prepared from fresh skim milk (50 °C, pH 6.6) by ultrafiltration (2×) using a pilot-scale system with a Romicon membrane that had a 10-kDa cut-off (Koch Membrane Systems, Wilmington, MA, USA). Chy-Max Extra rennet was purchased from Chr. Hansen, Inc. (Milwaukee, WI, USA). Mazu DF 204 antifoam was obtained from BASF Corporation (Mount Olive, NJ, USA). Sifto table salt was purchased from a local market. All other reagents were of analytical grade.

2.2. Preparation of whey protein aggregates

Whey protein isolate was dispersed in deionized water to a protein concentration of 5%, 10%, 15%, or 20% (w/v) and stirred for at least 3 h. Then, the WP isolate dispersions were adjusted to pH 7.0 with 1 and 0.1 N NaOH. After overnight storage at 4 °C, the dispersions were brought to 80 °C in a water bath at an average heating rate of 8 °C/min and then held at this temperature for 15 min. After heat treatment, the samples were rapidly cooled to room temperature in an ice bath. All samples (5%–20% protein) were diluted in water to the same final protein concentration of 4.5% (w/w) and mixed with a blender for 90 s. This treatment was required to facilitate dispersion, since gelation occurred at the high protein concentrations (15% and 20%). Mazu antifoam (0.05% [w/v]) was added to the samples before blending. The pH was then adjusted to 6.6, and the dispersions were homogenized in a single-stage Emulsiflex-C5 homogenizer (Avestin, Ottawa, ON, Canada) at 20 MPa for two passes. Sodium azide was added at 0.02% (w/v) to prevent microbial growth, and the samples were stored at 4 °C until use.

2.3. Characterization of whey protein aggregates

2.3.1. Degree of denaturation

The proportion (%) of denatured WPs was determined by measuring the solubility at pH 4.6. The WP aggregate dispersions were diluted in a 0.1 M citrate-phosphate buffer solution (pH 4.6) to a final protein concentration of 1% (v/v) and then centrifuged at 15,000× *g* for 20 min. The protein concentration in the supernatants was determined by measuring the optical density at 280 nm. The denatured WP content was obtained from the difference with the total WP concentration in the dispersion and was reported as a percentage.

2.3.2. Aggregate size

The Z-average hydrodynamic diameter was measured in duplicate at 25 ± 0.1 °C by photon correlation spectroscopy using a Zetasizer Nano ZS (Malvern Instruments Inc., Southborough, MA, USA). Before measurement, the WP aggregate dispersions were diluted with filtered (0.22 μm pore size) deionized water to obtain a count rate in the appropriate range.

2.3.3. Aggregate voluminosity

The viscosity of the WP aggregate dispersions (η) relative to the solvent (water) viscosity (η_0) was determined in triplicate at 25 ± 0.1 °C using Ubbelohde 1C capillary viscometers (International Research Glassware, Kenilworth, NJ, USA). Before measurement, the samples were diluted to a 0.8% (v/v) protein concentration with deionized water. The effective volume fraction of the dispersed phase was calculated according to the method of Boulet, Britten, and Lamarche (1998) using Lee's equation (Eq. (1)), which relates the volume fraction (Φ_v) to the relative viscosity (η/η_0):

$$\eta/\eta_0 = 1 + 2.5\Phi_v + 7.031\Phi_v^2 + 37.371\Phi_v^3 \quad (1)$$

The voluminosity was obtained by dividing the volume fraction of the dispersed phase by the protein concentration and was reported in millilitres per gram.

2.3.4. Accessible thiol groups

The accessible thiol groups were measured spectrophotometrically using Ellman's reagent as described by Shimada and Cheftel (1989). The WP aggregate dispersions were diluted to a 0.2% (v/v) protein concentration in 0.09 M Tris, 4 mM EDTA, and 0.09 M glycine buffer (pH 8.0). Before absorbance reading, the samples were deproteinized by the addition of powdered ammonium sulphate (1 g) followed by filtration (0.45 μm pore size). Measurements were taken in duplicate, and the concentration of accessible thiol groups was reported in micromoles per gram of protein.

2.3.5. Surface hydrophobicity

The surface hydrophobicity of the WP aggregates was determined by fluorescence spectroscopy using the fluorescent probe 1-anilinonaphthalene-8-sulfonic acid (ANS) according to the method of Alizadeh-Pasdar and Li-Chan (2000). The initial slope (S_0) of the fluorescence versus protein concentration (percent) plot was calculated by linear regression and used as an index of the protein surface hydrophobicity.

2.4. Milk reconstitution with whey protein aggregates

The WP aggregate dispersions were diluted in water at concentrations ranging from 0% to 0.8% (w/w). Low-heat skim milk