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Structural changes induced by high-pressure processing in micellar casein and milk protein concentrates

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ABSTRACT

Reconstituted micellar casein concentrates and milk protein concentrates of 2.5 and 10% (wt/vol) protein concentration were subjected to high-pressure processing at pressures from 150 to 450 MPa, for 15 min, at ambient temperature. The structural changes induced in milk proteins by high-pressure processing were investigated using a range of physical, physicochemical, and chemical methods, including dynamic light scattering, rheology, mid-infrared spectroscopy, scanning electron microscopy, proteomics, and soluble mineral analyses. The experimental data clearly indicate pressure-induced changes of casein micelles, as well as denaturation of serum proteins. Calcium-binding α_{S1}- and α_{S2}-casein levels increased in the soluble phase after all pressure treatments. Pressurization up to 350 MPa also increased levels of soluble calcium and phosphorus, in all samples and concentrations, whereas treatment at 450 MPa reduced the levels of soluble Ca and P. Experimental data suggest dissociation of calcium phosphate and subsequent casein micelle destabilization as a result of pressure treatment. Treatment of 10% micellar casein concentrate and 10% milk protein concentrate samples at 450 MPa resulted in weak, physical gels, which featured aggregates of uniformly distributed, casein substructures of 15 to 20 nm in diameter. Serum proteins were significantly denatured by pressures above 250 MPa. These results provide information on pressure-induced changes in high-concentration protein systems, and may inform the development on new milk protein-based foods with novel textures and potentially high nutritional quality, of particular interest being the soft gel structures formed at high pressure levels.

Key words: high-pressure processing, micellar casein concentrate, milk protein concentrate, pressure-induced milk protein gel

INTRODUCTION

Milk proteins represent the structural basis for dairy foods such as cheese and yogurt, which largely owe their textural and sensory attributes to the formation of protein networks by heat, pH, enzymatic modification, or a combination of these (Farrell, 1999). High-pressure processing (**HPP**) has emerged in recent years as a nonthermal processing technique able to inactivate microorganisms in foods, and also able to alter the structure and functionality of proteins, including milk proteins. High-pressure-processing-induced physicochemical changes in dairy systems include reduced turbidity, color, and particle size in milk (Gaucheron et al., 1997; Anema et al., 2005b; Orlien et al., 2006), increased viscosity in concentrated milks (Merel-Rausch et al., 2006), and altered rennet and acid coagulation kinetics and coagulum properties (Anema et al., 2005a; Zobrist et al., 2005). Most of the research on pressure-induced changes in milk proteins has been conducted at naturally occurring, low protein concentrations. Such changes could, however, open interesting opportunities in high-concentration protein systems, where HPP treatment may facilitate the creation of unique structures and functionalities. In this context, it becomes very interesting to explore the effect of HPP treatment on commercially available milk protein concentrates, such as milk protein concentrate (**MPC**) and micellar casein concentrate (**MCC**). Due to the strong interest in food products with a high protein content, both MPC and MCC have become increasingly available and used in recent years. Although these dairy ingredients offer nutritional and functional benefits compared with other protein sources, their utilization is still largely limited to traditional uses, such as protein fortification of cheese or yogurt.

The hypothesis of this work was that high-pressure treatment of high-protein-concentration systems will enable the formation of unique structures, without the use of heat or chemical additives. The outcome of the treatment may be different for MCC compared with MPC, due to the different level of serum proteins in the

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Table 1. Casein and calcium concentrations in the micellar casein concentrate (MCC) and milk protein concentrate (MPC) preparations

Sample	Protein level/sample code (%)	Actual casein concentration (g/100 g)	Total calcium concentration (mg/g)
MCC, untreated	2.50	2.29	0.61
	10	8.38	2.22
MPC, untreated	2.50	1.93	0.54
	10	7.08	1.99

2 types of protein ingredients. Therefore, the objective of this study was to investigate the effect of HPP treatments at pressures between 150 and 450 MPa on MCC and MPC, at both low and high protein concentration. The results of this research further the understanding of pressure-induced changes in concentrated protein systems and also provide a basis for the development of milk-protein-based products with novel structures and textures.

MATERIALS AND METHODS

Protein Concentrates

Commercial MCC (83.53 g/100 g of total protein, 71.00 g/100 g of casein, 2.00 g/100 g of fat, 0.50 g/100 g of lactose, 9.00 g/100 g of ash, 1.88 g/100 g of total calcium, and 5 g/100 g of moisture) and MPC (81.00 g/100 g of total protein, 63.76 g/100 g of casein, 2.00 g/100 g of fat, 6.00 g/100 g of lactose, 4.50 g/100 g of ash, 1.79 g/100 g of calcium, and 5 g/100 g of moisture) powders were obtained from the American Casein Company (AMCO, Burlington, NJ).

The casein to serum protein ratio, calculated based on the total protein and casein concentrations, was 85:15 in MCC and 79:21 in MPC (same as in skim milk). The protein powders were reconstituted with deionized (**DI**) water to produce suspensions with target protein concentrations of 2.5 and 10% (weight basis), respectively. The actual casein and calcium concentrations of the suspensions are shown in Table 1.

Protein Sample Preparation

The suspensions were stirred at 500 rpm and 25°C for 30 min. After that, 100 mL of suspension was high-shear-mixed using an Ultraturrax T25 batch disperser equipped with the S25N-18G fixture (IKA Works Inc., Wilmington, NC) for 7.5 min, at 21,500 rpm. The resulting suspensions were then stirred at 400 rpm and 25°C for another 90 min to enable full hydration and settling of foam. Finally, 50-mL aliquots were filled in vacuum storage bags (Seal-a-Meal, Sunbeam Products Inc., Boca Raton, FL) and double-sealed using a vacuum sealer (AGW Multivac, Sepp Haggenmüller KG, Wolfertsschwenden, Germany). The packaged MCC and MPC samples were stored overnight at 4°C before HPP treatment.

HPP Treatment

The protein samples were processed using a 10-L HPP unit (Elmhurst Research Inc., Albany, NY) located at the Department of Food Science at Rutgers University (New Brunswick, NJ). Samples were subjected to pressure treatments at 150, 250, 350, and 450 MPa for a holding time of 15 min. Initial and maximum temperatures of the pressurizing medium (filtered water), as well as pressurization and depressurization rates are shown in Table 2.

Composition Analyses

The chemical composition of the samples was determined at Dairy One Laboratory (Ithaca, NY). Lactose content was determined by infrared spectroscopy (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands), the fat content by ether extraction (AOAC 989.05; AOAC International, 2010a), and the total nitrogen by the Kjeldahl method (AOAC 991.20; AOAC International, 2010b). The NPN was determined by Kjeldahl method (AOAC 991.21; AOAC International, 2010b), and the noncasein nitrogen also by the Kjeldahl method (AOAC 998.05; AOAC International, 2010c).

Table 2. Temperature and pressure data for the pressure treatments

Treatment pressure level (MPa)	Initial temperature (°C)	Maximum temperature (°C)	Maximum pressure (MPa)	Pressurization rate (MPa/s)	Depressurization rate (MPa/s)
150	14.6 ± 4.5	18.3 ± 2.6	158.1 ± 2.9	2.4 ± 0.2	35.1 ± 4.6
250	14.8 ± 3.4	20.2 ± 2.6	261.6 ± 2.0	2.8 ± 0.1	51.4 ± 0.6
350	14.7 ± 4.9	21.4 ± 1.8	360.6 ± 0.7	3.1 ± 0.1	71.2 ± 0.7
450	13.7 ± 5.5	22.3 ± 2.0	463.6 ± 2.4	3.1 ± 0.1	84.4 ± 7.8