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Changes in functionality of whey protein and micellar casein after high pressure – low temperature treatments

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ABSTRACT

Changes in functional properties of micellar caseins (MC) and whey proteins (WPI) due to high pressure – low temperature (HPLT) treatments were investigated and compared to changes induced via high pressure treatments at room temperature (HP). Single whey protein solutions, micellar casein dispersions and two mixtures (micellar caseins:whey proteins weight mixing ratios 80:20 and 20:80) were treated at a concentration of 2% (w/w) and at two different pH values (7.0 and 5.8). Oscillating pendant drop and shear experiments were performed to identify changes in the rheological behavior at the air/water interface and in bulk, respectively. Foaming and emulsification experiments were conducted to investigate further impacts on the functional behavior. Both, HPLT and HP treatments led to a decreased emulsion stability for emulsions from WPI solutions independent from the treatment pH, while the foam stability was increased for these samples. In comparison, the changes for MC dispersions exhibited the same tendency but less pronounced. HPLT treatments of MC rich samples always led to the formation of a few very large flocs which had a major influence on the functional behavior. The rheological behavior of these samples changed from a Newtonian to a shear-thickening behavior. The elastic part of the surface dilatational modulus was increased for a pure WPI solution after HPLT and HP treatments while the viscous part remained unaffected. However, changes in functional properties highly depended on the sample composition and results for mixtures differ from those for pure dispersions.

1. Introduction

Proteins play a major role as functional ingredients in food as they offer the potential to create and stabilize disperse systems like foams, emulsions and gels. In general functionality can be regarded as 'any property of a food or food ingredient except its nutritional ones that influences its utilization' (Pour-El, 1981). Dispersed systems are thermodynamically instable as their free energy is higher than for the two single phases. In consequence, additives are needed to stabilize the dispersed phase within the continuous phase. Proteins are suitable for this challenge due to their amphiphilic character and, thus, they are widely used in food technology to stabilize foams and emulsions. However, the ability to create and stabilize dispersed food systems strongly depends on the structural properties, extrinsic factors and the process of creation. The creation and stabilization process can be divided into two steps – a diffusion of the protein to the interface and an arrangement at the phase boundary (Dalgleish, 1997). As a third step protein–protein interactions may stabilize especially foams by building a viscoelastic film (Kinsella, 1981). It is obvious that these steps require different molecular properties. Small and flexible molecules are able to be fast at the interface and, thus, help to create a disperse system (Grunden, Vadehra, & Baker, 1974). In contrast, proteins with a slow adsorption tend to cause higher long term stabilities in the case of foams (Kinsella, 1981). However, the structural reasons for the different functional properties of similar proteins are not fully understood until today. It is generally accepted that changes in the molecular structure like refolding or disulfide exchanges can induce large changes in the functional behavior of proteins. Under high pressure, reactions with a negative reaction volume are favored which provide the opportunity to modify protein structures (Belloque, Lopez-Fandino, & Smith, 2000; Gaucheron et al., 1997; Gekko & Hasegawa, 1989; Gekko & Noguchi, 1979; Heremans & Wong, 1985; Huppertz, Fox, & Kelly, 2004a; Wong & Heremans, 1988) and, thus, their functional properties. Former studies showed that high pressure – low temperature (HPLT) treatments induce different structural changes in milk proteins in comparison to high pressure treatments at room temperature (Baier, Purschke, Rawel,

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Schmitt, & Knorr, submitted for publication; Gebhardt, Toro-Sierra, & Kulozik, 2012; Kolakowski, Dumay, & Cheftel, 2001). These findings indicate the possibility of specific changes in functionality. However, HPLT effects on functionality changes are rarely investigated. Volkert, Puaud, Wille, and Knorr (2012) found changed sensorial properties of frozen dairy foams after HPLT treatments which could indicate a changed protein functionality. Reports on HP effects at room or elevated temperature identified the potential of pressure induced changes of protein functionality (Dumoulin & Hayashi, 1998; Galazka, Dickinson, & Ledward, 2000; Messens, VanCamp, & Huyghebaert, 1997). Pittia, Wilde, Husband, and Clark (1996) reported reduced emulsifying capacity and a decreased foamability of β-lactoglobulin (β-Lg) after pressure treatments from 300 to 900 MPa. However, other authors reported an increased foam stability depending on the treatment pH and the dwell time (Ibanoglu & Karatas, 2001). Galazka, Dickinson, and Ledward (1999) reported an increased droplet size and a faster creaming for a globular plant protein when treated as a pure substance but a slightly increased stability when treated in the presence of polysaccharides. Consequently, changes in functionality depend on the one hand on the exact sample composition and on the other hand on the treatment conditions. In comparison to the mentioned studies HPLT treatments offer two new options for modification beside pressure: cold denaturation (Hawley, 1971; Smeller, 2002) and effects caused by crystallization. The subzero temperature domain of the phase diagram of water enables different freezing processes. According to the nomenclature of Urrutia Benet, Schlüter, and Knorr (2004) pressure assisted freezing (PAF) denotes the cooling of a sample below the freezing line at almost constant pressure. Regarding the phase diagram of water this process enables the freezing to higher ice modifications (Bridgman, 1912) with different crystal structures and lower density in comparison to the common atmospheric ice (ICE I). Another process option is to induce the nucleation by pressure release, which is called pressure shift freezing (PSF). The sample is pressurized and undercooled in the liquid state and the crystallization is instantaneously induced when the pressure is released and the freezing line of ICE I is passed. The aim of this study is to identify the potential of HPLT treatments to modify the functionality of milk proteins.

2. Material and methods

2.1. Material

Whey protein isolate powder (WPI) was obtained from Fonterra (WPI 895, Fonterra, Auckland, New Zealand). This WPI is obtained by ion exchange and ultrafiltration of sweet whey. The protein content of the powder was 92.63% (w/w), furthermore it contained 0.18% (w/w) fat, 5.87% (w/w) moisture and 1.6% (w/w) ash. Micellar casein powder (MC) was obtained from the Hungarian Dairy Research Institute (MPI-85 MC, Hungarian Dairy Research Institute, Mosonmagyaróvár, Hungary). These micelles were manufactured by microfiltration and ultrafiltration of skimmed milk. The powder contained 85.1% (w/w) protein, 1.5% (w/w) fat, 4.9% (w/w) water and 7.5% (w/w) ash.

2.2. Methods

2.2.1. Sample preparation

All protein dispersions were treated at a concentration of 2% (w/w). The WPI solutions were prepared by diluting a specific amount of powder in deionized water and stirring it for 1 h at room temperature. The MC dispersions were prepared by giving a specific amount of powder to preheated deionized water (50 °C), stirring it for 1 h and gently homogenizing it in a high pressure homogenizer (ElmusiFlex-C5, Avestin, Inc., Ottava, Canada) at a maximum pressure of 30 MPa. Protein dispersions were prepared on a w/w ratio and pH values were either 7 (native) or set to 5.8 by the usage of HCl and NaOH (1 M, Merck KGaA, Darmstadt, Germany). The samples were double packed in polyethylene (PE) pouches to strictly avoid a penetration of the pressure transmitting medium (PTM). All samples were freshly prepared and kept at 4 °C until analyses.

2.2.2. HPLT treatments

The HPLT treatments were conducted in an experimental HPLT unit containing a high pressure vessel with 265 mL volume (Sitec Sieber AG, Zurich, Switzerland) connected to an air driven high pressure pump (DS XHW-1373, Haskel, CA, USA). The vessel was equipped with a heating-cooling jacket and tempering was realized with a cryostat (Ultra-Kryomat RUK 50-D, Lauda, Germany). An 80% (v/v) ethanol water mixture was used as tempering medium as well as pressure transmitting medium (PTM, freezing point below −59 °C). Two type K thermocouples enabled temperature measurements of the PTM at the bottom of the vessel and inside of a sample at the top of the vessel. The pressure was measured with a pressure transducer (Intersonde HP28, Watford, England). Samples were treated at 500 MPa at surrounding temperatures of −15 (pressure shift freezing – PSF), −35 (pressure assisted freezing – PAF) or 25 °C (room temperature – RT) for a constant dwell time of 20 min. The samples were thawed at room temperature before further preparations or analytics. Table 1 shows the resulting soluble protein fractions at sample pH (7.0 or 5.8) and after setting the pH to 4.6. Values were determined via RP-HPLC (Baier, Schmitt, & Knorr, submitted for publication).

2.2.3. Rheological analysis of casein based flocs

Viscosity measurements were performed to identify changes in the rheological behavior of samples which contained large flocs. A MCR 301 rotational viscometer with a CC 27 single gap cylinder (Anton Paar GmbH, Ostfildern-Scharnhausen, Germany) was used to analyze the rheological properties. The single gap cylinder had a gap of 1.13 mm and a sample volume of 19.35 mL. Shear experiments were performed at 20 °C with a linear ramp of 60 s up to 500 1/s, a dwell time of 60 s at 500 1/s and a ramp of 60 s to 0 1/s. Shear experiments were performed in duplicates. The Herschel-Bulkley model was applied to characterize the flow curves and the hysteresis area was determined. The Herschel-Bulkley model is given by the following equation:

$$\tau = \tau_0 + k \cdot \dot{\gamma}^n$$

where τ represents the shear stress, τ_0 is the yield stress, k is the consistency, $\dot{\gamma}$ is the shear rate and the exponent n represents the flow behavior ($n = 1 \rightarrow$ Newtonian fluid, $n < 1 \rightarrow$ shear thinning fluid, $n>1$ shear thickening fluid). For $\tau_0 = 0$ (no yield stress) the Herschel-Bulkley model becomes the power law model.

2.2.4. Determination of emulsification properties

Protein samples were diluted with a 10 mM phosphate buffer (pH 7.0) to a concentration of 0.4% (v/v). Commercial sunflower oil (12.5% v/v) was added to the diluted protein dispersions and pre-homogenized with an Ultra-Turrax T25 (IKA) with a S25 18 G homogenizing tool at 9.500 U/min for 5 min. Pre-homogenized samples were high pressure homogenized in a ElmusiFlex-C5 (Avestin, Inc., Ottava, Canada) at a homogenization pressure of 30 MPa.

Particle size distributions of fresh emulsions were determined by using a HORIBA LA-950 (Retsch Technology, Haan, Germany). Samples were stabilized by mixing 1:1 with a sodium phosphate