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Dynamic ultra-high pressure homogenisation of milk casein concentrates: Influence of casein content

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ABSTRACT

Milk casein concentrates with different casein-to-protein ratios (0.82 and 0.93), pH (5.8, 6.2 and 6.7) and level of applied ultra-high pressure homogenisation (UHPH) (0, 150 and 300 MPa) were studied. Samples with a casein-to-protein ratio of 0.93 at pH 5.8 and subjected to UHPH treatment at 300 MPa had the highest apparent viscosity and particle size followed by similar samples with a casein-to-protein ratio of 0.82. Water mobility was reduced at higher casein-to-protein ratio and with higher pH, while increased water mobility was observed in samples with a casein-to-protein ratio of 0.82 at pH 5.8 subjected to UHPH treatment at 300 MPa. The ^{1}H and ^{31}P NMR spectra revealed minor structural effects of adding casino-phospho-peptides and changing pH, but no effect of UHPH treatment, which indicate that UHPH treatment did not induce measureable structural changes in the individual proteins but rather induced changes in the tertiary structure or degree of polymerisation.
Industrial relevance: Milk depleted from whey protein is advantageous to use in cheese production since it can be further processed without detrimental denaturation of whey protein which will result in reduced maturation of cheese. In combination with pH control and ultra-high pressure homogenisation of the milk casein concentrate structural changes in the protein can be obtained and new interesting milk based products can be developed.

1. Introduction

Processing of milk can induce structural changes of the milk proteins at both micro- and macro molecular levels (Kulozik, 2008). In this context ultra-high pressure homogenisation (UHPH) represents a purely physical and mechanical method for this purpose (Roach & Harte, 2008). Previously the effects of UHPH treatment on isolated systems of whey protein, caseins or on systems based on casein to whey protein ratio of 80:20 as found in bovine milk have been assessed (Fox & McSweeney, 1998). UHPH treatment induces denaturation and aggregation of whey proteins as well as changes in the casein micellar structure depending on e.g. chemical composition of the solution, velocity gradients, inlet and outlet temperature, holding time and especially the mechanical forces introduced by homogenisation (Dumay et al., 2013).

Only few reports describe the effect of UHPH treatment applied to pure whey protein (Bouaouina et al., 2006; Grácia-Juliá et al., 2008) or pure casein systems (Roach & Harte, 2008). Grácia-Juliá et al. (2008) studied the effect of UHPH treatment on whey protein isolate and observed protein aggregation at pressures exceeding 225 MPa (holding time < 1 s). They furthermore observed that the mechanical forces were more important than heating in relation to denaturation. Roach and Harte (2008) analysed a native casein suspension and reported reduced solubilisation of casein at 250 MPa and above. In addition constant concentrations of α-lactalbumin and β-lactoglobulin were observed. Other researchers studied the effects of UHPH treatment on protein denaturation in skim milk and observed less or no denaturation of whey proteins at pressures up to 200 MPa or at holding times less than 0.7 s (Hayes & Kelly, 2003; Pereda et al., 2009; Sandra & Dalgleish, 2005). However, denaturation of β-lg has been reported to occur at longer holding times (Datta et al., 2005; Hayes et al., 2005). Escobar et al. (2011) compared to cheeses prepared from raw milk and UHPH treated (300 MPa, 2 s) raw milk and found no evidence of denaturation of β-lg due to UHPH treatment. On the other hand, Zamora et al. (2007) reported reduced amounts of β-lg and α-la in cheese whey due to UHPH treatment, because of incorporation into the curd, and later Zamora et al. (2012) showed that UHPH treatment increased incorporation of both whey protein and caseins in the curd. The UHPH treatment procedures

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in both of these studies were based on piston-gap homogenisers with an inlet temperature of 30 °C, maximum homogenisation temperature of 90 °C and a holding time 0.7 s.

Since denaturation and solubilisation of proteins depend on storage conditions and temperature before and after UHPH treatment it is very difficult to make a direct comparison of the studies mentioned above as they adopt different experimental set-ups.

Milk depleted from whey protein is advantageous to use in cheese production since it can be further processed without detrimental denaturation of whey protein which will result in reduced maturation of cheese due to interactions between β-lactoglobulin and κ-casein (Hyslop, 2003). For that reason milk casein concentrates with two different casein-to-protein ratios processed by dynamic UHPH treatment have been analysed with respect to apparent viscosity, particle size, soluble whey protein and using low as well as high-field NMR to assess the impact on micro- and macrostructure of the samples.

2. Materials and methods

2.1. Preparation of samples

Raw milk (Arla Foods, Brabrand, Denmark) was skimmed (55 °C) and skim milk (3000 L) was heated (63 °C, 15 s) and cooled to 5 °C (Pasilac Therm A/S, Silkeborg, Denmark). The following day skim milk was pre-heated to 55 °C in 300 s (Pasilac Therm, Silkeborg, Denmark). The MF process was then carried out in batch mode at 50 °C using a pilot unit (500 L h^{-1}) constructed by Arla Foods (Arla Foods, Videbæk, Denmark), equipped with 24 pcs FR3B-6338 SW-membranes (Synder, Vacaville, CA, USA) with a nominal molecular mass cut-off of 800 kDa and a UF unit (Arla Foods, Videbæk, Denmark) equipped with 16 pcs HFK328-6338 SW-membranes (Koch, Wilmington, DE USA) with a nominal molecular mass cut-off of 5 kDa. The skim milk was concentrated to 5% (w/w) casein and diafiltration was carried out using five volumes of UF permeate from the MF permeate (casein-to-protein 0.93, C:P93), while the other milk casein concentrate solution (casein-to-protein 0.82, C:P82) was standardised from C:P93 by adding UF concentrate and UF permeate. The two milk casein concentrates (MCC) were pasteurised (72 °C, 15 s) and cooled to 8 °C (Pasilac Therm A/S, Silkeborg, Denmark).

From each fraction C:P93 and C:P82 respectively, six samples were prepared in an experimental design that was created using Modde 9.1 (Umetrics, Umeå, Sweden). Within each fraction two samples were acidified to pH 5.8, one sample to pH 6.2 and three samples were not acidified (pH 6.7). Acidification was performed with 20% (w/w) citric acid (Jungbunzlauer, Basel, Schweiz). The same amounts of liquid (water, citric acid or water and citric acid) were added to all samples to obtain the same dilution factor. One sample at pH = 5.8 and one at pH = 6.7 was UHPH treated at 300 MPa and one at pH = 6.2 was UHPH treated at 150 MPa (DEE International Inc., Debee 2000, Boston, MA, USA). The samples were cooled and stored at 5 °C. All samples were analysed after 2–10 days. The experimental design is summarised in Table 1. Two groups of samples were prepared: one with a casein-to-protein ratio of 0.93 (C:P93) and one with 0.82 (C:P82) and for each MCC the pH was adjusted to 5.8, 6.2 or 6.7. Subsequently, samples at pH 5.8 and pH 6.7 were UHPH treated at 0 and 300 MPa, while samples at pH 6.2 were subjected to a pressure of 150 MPa. There is a difference in pH between the experimental design and the actual measured values of pH, because of equilibrium setting after acidification. All trials were performed in duplicate.

Table 1
Experimental design[(1)]. The sample code, given by the abbreviations C:Px_y_z, where x is the casein-to-protein ratio, y is the pH and z is the pressure in MPa during ultra-high pressure homogenisation (UHPH).

Sample code	Casein-to-protein ratio	pH	UHPH treatment (MPa)
C:P93_5.8_0	0.93	5.8	0
C:P93_5.8_300	0.93	5.8	300
C:P93_6.2_150	0.93	6.2	150
C:P93_6.7_300	0.93	6.7	300
C:P93_6.7_0_1	0.93	6.7	0
C:P93_6.7_0_2	0.93	6.7	0
C:P82_5.8_0	0.82	5.8	0
C:P82_5.8_300	0.82	5.8	300
C:P82_6.2_150	0.82	6.2	150
C:P82_6.7_300	0.82	6.7	300
C:P82_6.7_0_1	0.82	6.7	0
C:P82_6.7_0_2	0.82	6.7	0

(1) All treatments were performed on milk casein concentrate based on skim milk. All experiments were performed in duplicate.

2.2. Chemical analyses

The pH was measured using a PHM-240 pH-metre (Hach Lange, Brønshøj, Denmark). Total solid content, fat and nitrogen were determined according to IDF standard methods (IDF, 1987, 2004a, 2008). Protein was obtained by multiplying the nitrogen content by a Kjeldahl factor of 6.38 for milk protein, while the casein content was determined by multiplying the nitrogen content by 6.36 (Van Boekel & Ribadeau-Dumas, 1987). Non-casein nitrogen was determined according to the IDF standard method (IDF, 2004b) and ash content was determined according to NMKL standard method (NMKL, 2005). Determination of lactose was done using a lactose/day-galactose enzymatic Boehringer Mannheim test-kit (Roche, Basel, Schweiz). Total content of magnesium, sodium, chloride, potassium and phosphate was determined using inductively coupled plasma (ICP) spectroscopy (Perkin-Elmer Optima 4300 DV, Boston, MA, USA).

2.3. Total calcium and serum calcium

The total calcium was determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) (Perkin-Elmer Opima 4300 DV, Boston, MA, USA). Samples of 0.5 g were weighed to a quartz tube and 5 mL of concentrated 69% (w/w) HNO_3 (SCP Science, Quebec, Canada) was added, and the sample was digested in a microwave (Anton Paar Multiwave 3000, Graz, Austria) and further diluted with ultrapure water.

The serum calcium content was released from the MCC with centrifugation and filtration and the calcium in the serum was determined by ICP-OES: 0–30 mL of sample was centrifuged at 17,090 ×*g*, at 4 °C for 2 h (Beckman Coulter Inc., Brea, CA, USA). The sample was filtered through a pleated filer (Grade 580, retention: >20 μm, Frisenette ApS, Knebel, Denmark) and the filtrate, was centrifuged at 126,000 ×*g*, at 4 °C for 1 h (Bechman coulter optima™ L-80 XP ultracentrifuge, Birkerød, Denmark). Subsequently samples were filtered through a 3 kDa cut-off filter (Sartorius Stedim biotech, Göettingen, Germany) and centrifuged at 5170 ×*g* at 4 °C for 16 h.

2.4. Total whey protein and soluble whey protein

To determine the total whey protein content 200 μL of sample was transferred into an Eppendorf tube. One mL of reduction buffer (100 mM sodium citrate; 6 M Urea) (Merck KGaA, Darmstadt, Germany) and 20 μL of 1 M 1.4-dithioerythritol (DTE) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were added to the sample and mixed. The sample was incubated for 60 min at 37 °C, and centrifuged at 9300 ×*g*, at 5 °C in 10 min, 200 μL of the supernatant was transferred to a vial and analysed with a high-performance liquid chromatography (HPLC) system (Agilent Technologies 1290, Santa Clara, CA, USA) equipped with a BioSuite™ C18 PA-B column (C_{18}, 3.5 μm; 2.1 × 250 mm, Waters, Milford, MA, USA) and a UV-detector to detect whey protein at a wavelength of 214 nm (Agilent Technology, Santa Clara, CA, USA). Mobile phase A was 0.1% trifluoroacetic acid (TFA) (Fluka, St. Louis, MO, USA) in water and mobile phase B was 0.1%