International Dairy Journal 47 (2015) 64–71

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Rheological properties and microstructure of high protein acid gels prepared from reconstituted milk protein concentrate powders of different protein contents

Gopinathan H. Meletharayil [a], Hasmukh A. Patel [a, *], Thom Huppertz [a, b]

[a] Dairy Science Department, South Dakota State University, Brookings, SD 57007, USA
[b] NIZO food research BV, P.O. Box 20, 6710 BA Ede, The Netherlands

ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form
9 February 2015
Accepted 17 February 2015
Available online 4 March 2015

ABSTRACT

Milk protein concentrate (MPC) powders were manufactured by pilot-scale ultrafiltration and diafiltration of skim milk to different protein concentration factors, followed by spray-drying. MPC powders were reconstituted at 7.5% (w/w) protein. Suspensions were subsequently standardised to 15% (w/w) total solids with lactose and heated at 90 °C for 10 min at pH 6.9. Acid gels were prepared from the heated suspensions using glucono-delta-lactone. Protein interactions in the heated dispersions and rheological and microstructural properties of the acid gels were studied. Suspensions from the higher protein MPCs showed no dissociation of κ-casein into the serum phase after heating, whereas elastic moduli at pH 4.6 and the gelation pH of the acid gels were higher and water holding capacity and gelation time was lower in acid gels prepared with high-protein MPCs. Confocal microscopy images revealed an increase in the pore size of the acid gels prepared with high-protein MPCs.

1. Introduction

In recent years, an image of health and wellness benefits has been driving the on-going popularity and sales of high protein yoghurts, such as Greek style yoghurt (GSY) in the US markets. High protein yoghurts, like GSY, Labneh and Shrikhand, were traditionally prepared by filtering yoghurt through a muslin cloth to remove the whey and achieve the desired total solids (TS) levels. In the current industrial production of high protein yoghurt, mechanical separators, like Quarg separators, are used to remove the whey to achieve the desired protein and total solid levels (Kulkarni, Belsare, & Lele, 2006). One of the drawbacks of this process is the large quantity of acid whey that is produced, which is a cause of environmental concern. One approach to address this challenge is to eliminate the de-wheying step by manufacturing GSY from milk protein concentrates (MPCs) to achieve the desired high protein content of the formulation, followed by fermentation (Bong & Moraru, 2014).

Various grades of MPC, varying in protein content, can be produced by ultrafiltration (UF) or a combination of UF combined with diafiltration (DF) of skim milk, followed by evaporation and spray-drying. The final protein concentration factor achieved with the UF/DF process determines the protein content of the powder, which generally varies from 50% (w/w; MPC50) to 90% (w/w; MPC90) in dry matter (Mulvihill & Ennis, 2003). MPC powders with different protein contents have been studied and used as ingredients in a range of food applications, including cheese (Havea, 2006), ice cream (Alvarez, Wolters, Vodovotz, & Ji, 2005), yoghurt (Guzman-Gonzalez, Morais, & Amigo, 2010) and dairy beverages (Giroux, Houde, & Britten, 2010). However, the use of MPC powders of different protein content in the production of high protein acid gels, similar to GSY, and their effect on the rheological and microstructural properties has not been reported in literature.

It is known that the UF and DF processes employed in the production of these powders lead to different mineral to protein ratios in the MPC with varying protein content, which creates different ionic environments, both in terms of ionic strength and ionic composition, when these powders are reconstituted in water. The ionic strength and composition of the serum phase may have an important role in influencing the protein interactions during the heating of these dispersions, which may influence the gelation process during the manufacture of high protein acid gels. To prove this hypothesis, the objective of this study was to determine the effects of tailored protein interactions on the rheological and

* Corresponding author. Tel.: +1 605 690 5490.
E-mail address: Hasmukh.Patel@sdstate.edu (H.A. Patel).

http://dx.doi.org/10.1016/j.idairyj.2015.02.005
0958-6946/

microstructural properties of acid gels from reconstituted MPC powders at 7.5% (w/w) protein and 15% (w/w) total solids.

2. Materials and methods

2.1. Manufacture and composition of MPC powders

MPCs were produced from pasteurised (72 °C for 15 s) skimmed bovine milk as described by Crowley et al. (2014). The six MPC samples used in the experiments were MPC50, MPC60, MPC70, MPC80, MPC85, and MPC90. Total solids, protein, lactose, moisture and ash content of the MPC powders were determined as reported by Crowley et al. (2014). All chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA, USA) unless otherwise indicated.

2.2. Preparation of MPC dispersions

MPC dispersions for the preparation of acid gels were prepared by reconstitution of the MPC powders and lactose in demineralised water to attain 7.5% (w/w) protein and 15% (w/w) total solids. The composition of standardised reconstituted MPC dispersions from MPC powders was calculated from the measured MPC powder composition and is given in Table 1. The ingredients were mixed with the calculated quantity of demineralised water, stirred for 20 min at ambient temperature and left overnight at 4 °C to allow for proper hydration and equilibration of constituents. Sodium azide (0.02%, w/w) was added to each sample as a preservative. The pH of each of the samples was adjusted to 6.9 with 1 M HCl or 1 M NaOH under stirring. Samples were then allowed to equilibrate at least for 2 h and further (minor) readjustments of pH were done, if necessary. The samples were then heated at 90 °C for 10 min in a water bath under stirring and then cooled rapidly to 4 °C in an ice bath. For the preparation of acid gels, samples were tempered to 30 °C and 1.75–1.85% (w/w) of glucono delta-lactone (GDL) was added to the samples so that a final pH of 4.6 ± 0.05 was attained at the end of a 4 h incubation period at 30 °C.

Table 1
Compositions of milk protein concentrate (MPC) dispersions with added lactose calculated from the composition MPC powders.

MPC	Protein (%, w/w)	Total solids (%, w/w)	Ash (%, w/w)	Calcium (mg g^{-1})	Inorganic phosphorous (mg g^{-1})
MPC50	7.5	15	1.18	2.62	1.91
MPC60	7.5	15	0.97	2.47	1.70
MPC70	7.5	15	0.89	2.43	1.62
MPC80	7.5	15	0.74	2.30	1.44
MPC85	7.5	15	0.68	2.30	1.38
MPC90	7.5	15	0.67	2.31	1.34

2.3. Determination of the distribution of proteins between the serum and micellar phase

To obtain supernatants for distribution of caseins and whey proteins in heated and unheated dispersions, samples from unheated and heated MPC dispersions were centrifuged at 100,000 × *g* for 1 h at 20 °C in a TLA-120.2 rotor in a Beckman Coulter Optima TLX ultracentrifuge (Beckman Instruments, Brea, CA, USA). The clear supernatants were carefully removed. The protein profile of the supernatants was determined by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing and reducing conditions as described by Anema (2008) with slight modifications. The gels were run at 200 V for 1 h, followed by staining in 0.1% (w/v) amido black 10B in 10% (v/v) acetic acid and 25% (v/v) 2-propanol for 1 h. This was followed by a destaining step using 10% (v/v) acetic acid solution until a clear background was obtained. The stained SDS-PAGE gels were scanned to TIFF images using a Microtek gel scanner, Bio-500 (Microtek International Inc., Taiwan).

2.4. Particle size analysis

Particle size measurements on heated and unheated dispersions from different treatments were performed by photon correlation spectroscopy (PCS) at 25 °C using a Malvern Zetasizer Nano-ZS (Malvern Instruments Ltd., Malvern, UK). Samples were diluted to 50 times in a calcium-imidazole buffer (5 mM $CaCl_2$, 20 mM imidazole, 30 mM NaCl, pH 7.0) prior to measurement.

2.5. Acid–base titration curves

Acid–base titration curves of MPC dispersions from different treatments were determined as described by Lucey, Hauth, Gorry, and Fox (1993). Samples were titrated from their initial pH of 6.9 to 3.0 with 0.5 M HCl, and subsequently from pH 3.0 to 7.0 with 0.5 M NaOH in the forward direction. pH was continuously measured using a Corning pH/ion meter model 450 (Corning Glass Works, Medfield, MA, USA) fitted with a Thermo Orion combination pH probe (Thermo Electron Corp., Louisville, CO, USA).

2.6. Rheological properties

The acid gel formation was monitored using a Stresstech HR high resolution controlled stress rheometer (ATS Rheosystems, Rheological instruments Inc., Borden-town, NJ, USA) whereby the elastic modulus (G′) and loss modulus (G″) of the acid gels were measured every 5 min. Of the 30 mL of MPC dispersion mixed with GDL (mixed for 5 min after addition of GDL), 14 mL was transferred into a cup and bob measuring geometry consisting of two coaxial cylinders (inner diameter 25 mm; outer diameter 27.5 mm). After loading the samples in the geometry, the surface of the sample was covered with one mL vegetable oil to prevent evaporation and drying off on the surface. The sample was oscillated at 0.1 Hz and with an applied strain of 1%. Measurements were taken every 5 min for 240 min. Gelation was defined arbitrarily as the point at which the elastic modulus of the gel was greater than 1 Pa. The pH of the remaining sample was followed by placing it in a water bath maintained at 30 °C and pH measured using a pH meter (Eutech instruments, Cyber scan pH110, Singapore) with data continuously logged into a computer.

2.7. Water holding capacity

Water holding capacity (WHC) of acid gels was measured by weighing 20 g of MPC dispersions into 50 mL centrifuge tubes and acidifying it to pH 4.6 with the appropriate quantity of GDL. The tubes were incubated at 30 °C for 4 h and transferred to a refrigerator overnight before testing. The tubes were then centrifuged at 3000 × *g* for 15 min at 4 °C as outlined by Imm, Lian, and Lee (2000). The supernatant was collected and weighed. WHC was defined as the weight of the pellet expressed as a percentage of the weight of the total sample.

2.8. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) was used to study the microstructure of the acid gels as described by Ozcan-Yilsay, Lee, Horne, and Lucey (2007), with some modifications. Fifteen grams of MPC dispersion tempered to 30 °C and 140 μL of 0.2% (w/w) acridine orange (Sigma Chemical Co., St. Louis, MO, USA) were