Influence of pH and heat treatment of whey on the functional properties of whey protein concentrates in yoghurt

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Abstract

Our aim was to investigate how two conditions of whey processing, pH and heat treatment, affect the physical properties of stirred yoghurts fortified to 45 g protein kg^{-1} with whey protein concentrates (WPC). Cheddar whey was heated at pH 6.4 or pH 5.8 at 72 °C for 15 s, eventually heated further at 82 or 88 °C for 78 s, ultrafiltered, and spray dried. Resulting WPC contained 38% protein; the denaturation level of the whey protein was 10–53%. There were significant ($P<0.05$) differences in physical properties of WPC fortified yoghurts: water-holding capacity ranged from 33% to 46% and elastic modulus ranged from 63 to 145 Pa depending on whey processing. WPC with low denaturation level produced yoghurts with high elastic modulus and water-holding capacity. Minimizing the heat treatment during whey processing maximized the functional properties of WPC to be used in yoghurt.

1. Introduction

The manufacture of yoghurt involves milk fortification with dairy ingredients to increase protein content from 3.5% to 4–5%. Traditionally, skim milk powder is used for milk fortification. However, availability and the low cost of whey protein concentrates (WPCs) make them attractive and they are now commonly used to replace skim milk powder in yoghurt formulation. WPCs are produced by ultrafiltration and drying of whey, and contain 34–88% protein. Significant variation in the functionality of WPC in yoghurt has been reported. Yoghurt fortified with various samples of commercial WPC had water holding capacity ranging from 45% to 63% Brookfield viscosity from 10 to 30 Pa s, apparent viscosity from 0.16 to 1.1 Pa s (Guinee, Mullins, Reville, & Cotter, 1995), complex viscosity from 13 to 24 Pa s (Sodini, Montella, & Tong, 2005), and firmness from 50 to 79 g (Modler, Larmond, Lin, Froelich, & Emmons, 1983). Previous studies have reported that various functional properties of WPC such as solubility, heat-gelation, foaming and emulsion capacity can be influenced by the whey processing conditions (De Wit, Klarenbeek, & Adamse, 1986; Mangino, Liao, Harper, Morr, & Zadow, 1987; Morr & Foegeding, 1990). Among the different process parameters, heating plays a determinant role in whey protein functionality (Mangino et al., 1987; Morr, 1987). The source of whey has been shown also to affect the properties of the WPC (De la Fuente, Hemar, Tamehana, Munro, & Singh, 2002; Ji & Haque, 2003).

No information is available about the effect of whey processing on WPC functionality in yoghurt. The objective of the present work was to determine how WPC manufacture influences the performance of the whey protein concentrates in yoghurt, by focusing on two processing variables, (i) whey pH, to simulate differences in whey source, and (ii) whey heat treatment.

2. Material and methods

2.1. Processing of the WPC

2.1.1. Whey processing

Four batches of fresh whey were prepared. Fresh milk (265 kg per batch) was obtained from the Cal Poly Dairy between May and June 2003 and processed for Cheddar manufacture. Milk was skimmed and pasteurized (74 °C—15 s) using a universal pilot plant plate pasteurizer (Processing Machine and Supply Co, Philadelphia, PA) processed with a flow rate of $0.053\,L\,s^{-1}$. Pasteurized skim milk was filled into a cheese vat (Kusel, Watertown, WI), heated at 32 °C, $0.26\,mL\,kg^{-1}$ calcium chloride was added, and the milk was inoculated with 0.02% (v/w) commercial mesophilic culture R604 (Chr. Hansen, Milwaukee, WI, USA). After 45 min of acidification, 0.02% (v/w) chymosin (Single strength CHY-MAX, 630 international milk clotting units per mL, Chr. Hansen) was added. Coagulation was allowed for 25 min. Then, the curd was cut into small cubes, healed for 5 min, and cooked at 39 °C until pH 6.4. When the pH reached 6.4, whey was collected for the first set of WPC (2 batches). For a second set of WPC (2 other batches), when the pH reached 6.4, food grade lactic acid 88% (Archer Daniels Midland Company, Decatur, IL, USA) was added to the blend of curd and whey at a level of about $0.6\,mL\,kg^{-1}$, in order to lower the pH to 5.8. After 30 min to allow for equilibrium to become established, the whey was collected. For each batch, about 220 kg of whey were collected. Skim milk was used instead of whole milk to eliminate any fat variation in the whey generated.

2.1.2. WPC manufacture

Whey was clarified by filtration with cloth, and heat treated with a universal pilot plant plate pasteurizer (Processing Machine and Supply Co) at 72 °C for 15 s (flow rate $0.053\,L\,s^{-1}$) to reduce the likelihood of any further microbial growth. Each batch was divided into three lots. One was directly concentrated by ultrafiltration to a volumetric concentration factor of 6× using a 10,000 molecular weight cut off membrane Durasan™ PW3838C* (Niro, Hudson, WI, USA) installed in a DDS ultrafiltration system (De Danske Sukkerfabrikker, Nakskov, Denmark). The two others were submitted to another heating, 82 or 88 °C for 78 s, using the pilot plant plate pasteurizer operating at a flow rate of $0.033\,L\,s^{-1}$. Then, each lot was ultrafiltered. About 10 kg of retentate per lot were collected. The retentate was spray dried in a Niro Filterlab Spray Dryer. About 1 kg of spray dried WPC was collected for each treatment.

2.1.3. Experimental design

The different combinations of pH and heat treatment tested have been replicated, thus 12 different samples of WPC have been produced. Each sample was analyzed to determine dry matter, fat, ash, total nitrogen (TN), non-protein nitrogen (NPN), nitrogen soluble (SN) at pH 4.6, and used as ingredient for milk fortification in yoghurt-making to determine functionality in yoghurt.

2.2. Yoghurt processing

2.2.1. Mix preparation

Milk was standardized to a low fat level ($10\,g\,kg^{-1}$) by blending pasteurized non-fat milk and pasteurized homogenized whole milk. The milk which had an average protein content of $34 \pm 1\,g\,kg^{-1}$, was then fortified with whey protein concentrate to give a final total protein content of $45\,g\,kg^{-1}$. The standardized and fortified milk was packaged into 1-L flasks, heated in a water-bath without agitation 30 min at 85 °C, then cooled for 1 h in an ice bath, and stored overnight at 4 °C.

2.2.2. Yoghurt manufacture

The day after, the milk was pre-heated to 42 °C, inoculated with 0.02% (w/v) commercial yoghurt culture Yog-Fast 17 (Chr. Hansen), then incubated at the same temperature until pH 4.50 was reached. Fermentation time was 6.5 ± 0.5 h. The fermented milk was stirred, and cooled to 25 °C before packaging to slow down the acidification, as usually done in industrial yoghurt manufacture, by placing the bottles in a ice bath for 15 min and manually stirring the yoghurt with a stainless steel bored disk by 60 up and down movements after 1, 7, and 14 min. The cooled stirred fermented milk was then poured into 100-mL cups at a flow rate of approximately $6\,L\,h^{-1}$ by means of a 50-mL syringe (orifice 1 mm diameter) and was stored overnight at 4 °C. The shear created by pumping the yoghurt with a syringe was to simulate the texturization of stirred yoghurt, which occurs commercially by pumping the set yoghurt through perforated screens and filling heads for packaging.

2.3. Chemical analyses

2.3.1. Determination of pH, nitrogen and protein

The pH of 10% (w/w) reconstituted whey protein concentrate was determined in duplicate by use of a pH-meter Φ34 (Beckman, Fullerton, CA, USA). The levels of TN, SN at pH 4.6, and NPN were determined in the whey protein concentrates via the Kjeldahl method. All measurements were carried out in duplicate.

The true protein was calculated as $(TN-NPN) \times 6.38$. The NPN, expressed in protein equivalent, was calculated as $NPN \times 6.38$. The ratio of insoluble protein at pH 4.6 on total protein was calculated as $100 \times (TN-SN)/TN$ and was used to characterize the amount of protein denaturation in WPC as proposed by De Wit, Klarenbeek, and Hontelez-Backx (1983).

2.3.2. Determination of moisture, fat, and ash in WPC

Moisture was determined by drying each sample for 5 h in a vacuum oven at 100 °C (American Dairy Products Institute, 1990). Fat content was determined by the Babcock method (Association of Official Analytical Chemists, 1995). Ash content was determined by ignition at 550 °C in an electric muffle furnace (Association of Official Analytical Chemists, 1995). All the measurements were carried out in triplicate.

2.4. Functional properties of WPC in yoghurts

After keeping one day at 4 °C, yoghurts were analyzed to determine their water-holding capacity and viscoelastic properties. The pH was 4.40 ± 0.04.

2.4.1. Water-holding capacity

The water-holding capacity of yoghurt was determined using a procedure adapted from Guzman-Gonzalez, Morais, Ramos, and Amigo (1999). A sample of about 20 g of yoghurt (Y) was centrifuged for 30 min at $1250 \times g$ and 20 °C (height = 4.8 cm). The whey expelled (WE) was removed and weighed. The water-holding capacity (WHC) was defined as

$$\mathrm{WHC}\,(\%) = 100 \times (\mathrm{Y} - \mathrm{WE})/\mathrm{Y}.$$

The measurement was carried out in triplicate.

2.4.2. Viscoelasticity

A control-stress rheometer (model SR5000; Rheometric Scientific Inc., Piscataway, NJ, USA), equipped with a concentric cylinder device consisting of a cup (32-mm diameter) and a bob (29.5-mm diameter, 44.25-mm length), maintained at 10 °C, was used to study the viscoelastic behavior of the stirred yoghurt. About 17 mL of yoghurt sample were transferred into the cup of the rheometer and the bob was lowered until the whole bob surface was covered. About 5 min were allotted to allow the sample temperature to equilibrate to 10 °C. Measurements were carried out using a stress sweep (1–100 Pa, frequency 1 Hz, 20 cycles per decade of stress). The elastic modulus, in Pa, and the loss tangent, were calculated at 1 Pa. Three replicates were performed and a fresh sample was used for each replicate. The coefficient of variation between the three replicates was between 1% and 10%.

2.5. Statistical analyses

Results were evaluated statistically using Minitab™ 13.1 Software (Minitab Inc., State College, PA, USA). A two-factor analysis of variance with interaction was performed to determine the effects of both whey pH and whey heat treatment on denaturation of whey proteins in WPC and physical properties of the resulting yoghurts. Multiple comparison of means was performed using Tukey's pairwise comparison at a α-level of 5%.

3. Results and discussion

3.1. Characterization of whey protein concentrates

Composition, pH, and degree of denaturation of the whey protein concentrates are given in Table 1. The pH on a 10% dilution (w/w) was 6.5 and 6.0 for WPC involving whey at pH 6.4 and 5.8, respectively. The slight increase of pH noticed in WPC compared with whey was probably due to increasing buffering capacity for 3.8% protein solution as compared with whey containing only 0.6–0.7% protein. The composition of the WPC is comparable with industrial WPC preparations as reported in previous studies (De Wit et al., 1983; Guzman-Gonzalez et al., 1999; Mangino, 1992; Sodini et al., 2005). A significant decrease of ash content ($P < 0.001$) is noticed with increasing heat treatment, from 59–59.5 to 54.6–57.5 g kg^{-1}. This has been also reported by Mangino et al. (1987) and Morr (1985). Heating causes the conversion of soluble Ca and Mg salts to insoluble complexes in whey, resulting in partial precipitation of Ca and Mg salt during heat treatment, and decreasing concentration in heated whey (Morr, 1985). The degree of denaturation of the whey protein ranged from 10% to 53%. In other studies, commercial WPC have been reported to be in the range 9–65% (De Wit et al., 1986), 5–23% (Guzman-Gonzalez et al., 1999), and 19–26% (Sodini et al., 2005). The effect of whey pH and heat treatment and the interaction were significant ($P < 0.001$).

3.2. Functional properties in yoghurts

WPC-fortified yoghurt exhibited different water-holding capacity and viscoelastic properties as showed in Fig. 1. In

Table 1
Composition on a dry matter basis, pH value, and degree of denaturation of the whey protein concentrates

Sample code (°C)	True protein[a]	NPN[a]	Fat[a]	Ash[a]	PH[b]	Denaturation[c] (%)
6.4/72	344 (±9)	43.7 (±2.2)	39.3 (±4.2)	59.5 (±0.3)	6.54 (±0.03)	13.3 (± 1.4)
6.4/82	343 (±5)	41.7 (±1.0)	39.6 (±3.7)	58.2 (±0.4)	6.49 (±0.04)	29.9 (± 5.3)
6.4/88	352 (±4)	41.4 (±0.9)	38.8 (±3.1)	57.5 (±0.2)	6.48 (±0.03)	40.8 (± 3.7)
5.8/72	328 (±7)	41.4 (±0.6)	21.2 (±2.9)	59.0 (±1.0)	6.02 (±0.01)	9.9 (±0.1)
5.8/82	345 (±3)	41.8 (±2.0)	26.6 (±4.0)	56.3 (±0.7)	6.01 (±0.01)	39.8 (± 0.6)
5.8/88	356 (±4)	44.8 (±1.8)	31.5 (±2.8)	54.6 (±0.6)	6.01 (±0.02)	52.9 (± 1.5)

[a]Results expressed in g kg−1 are the average (± standard deviation) of two independent trials of WPC manufacture and two or three analysis replicates (n = 4 or n = 6).
[b]Measured in a 10% (w/w) reconstitution.
[c]Determined by pH 4.6 non-soluble nitrogen.

all cases, the effects of whey pH and heat treatment and the interaction were significant ($P<0.05$).

3.2.1. Water-holding capacity

Water-holding capacity (Fig. 1a) ranged from 33% to 46%. Both heat treatment and acidity of the whey had a negative effect on the water-holding capacity of the yoghurt. The highest water holding capacities were obtained when yoghurt was fortified using WPC from whey with low heat treatment. These results agree with those of Schorsch, Wilkins, Jones, and Norton (2001), who found a lower syneresis in acid gel issued from casein–whey mixtures, when whey proteins were not pre-heated before to be mixed with casein, heated and acidified. Water-holding capacity was significantly ($P<0.05$) higher for yoghurts enriched with WPC issued form whey at pH 6.4 (average water-holding capacity 44%), than for the ones enriched with WPC issued form whey at pH 5.8 (average water-holding capacity 39%). The initial pH was significantly ($P<0.001$) different between the yoghurt mixes (6.48 and 6.35, respectively) before fermentation. According to the study of Vasbinder and de Kruif (2003), a more acidic pH of the mix before heat-treatment contributes to a more inhomogeneous coverage of the casein micelles by the denatured whey protein during the heating. It can be suggested that a more inhomogeneous coverage of the casein micelles would allow a more open structure of the gel network, making the removal of the water during centrifugation easier.

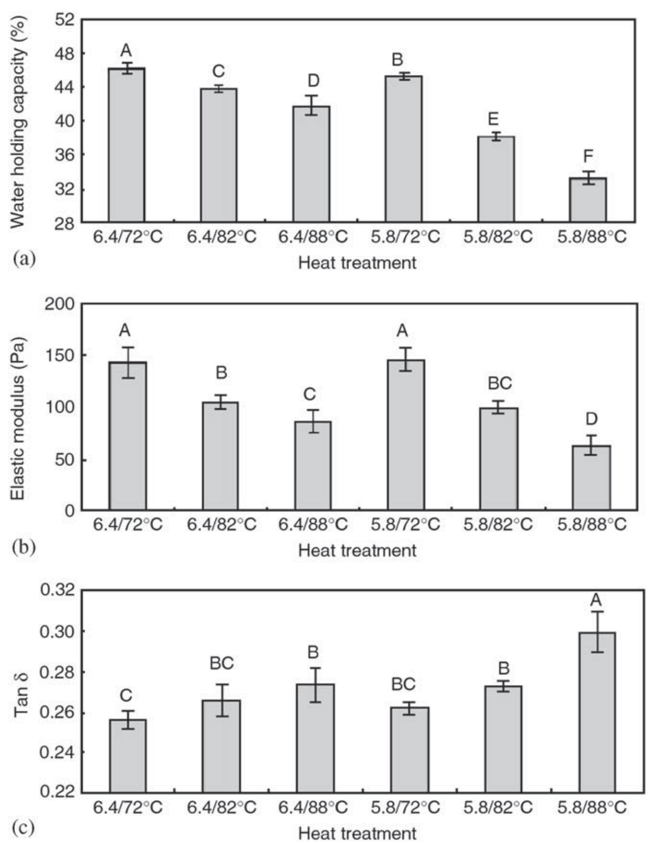

Fig. 1. Physical properties of yogurt fortified with whey protein concentrates: (a) water-holding capacity, (b) elastic modulus, (c) loss tangent ($\tan\delta$). Data are the results of two independent trials of WPC manufacture. The error bars represent the standard deviations. WPC with no common letters differ according to the Tukey's pairwise comparison ($\alpha=0.05$).

3.2.2. Viscoelasticity

Elastic modulus (Fig. 1b) decreased with increasing heat treatment and decreasing pH of whey, ranging from 145 (72 °C for 15 s, pH 6.4) to 63 Pa (88 °C for 78 s, pH 5.8). These results are different than the results obtained by Cho, Lucey, and Singh (1999) with lactic acid gel involving recombined milk fortified with heated WPC or unheated WPC. In their study, a higher elastic modulus was observed for lactic gel with heated WPC than with unheated WPC. However, in case of recombined milk system, fortification is followed by homogenization, which generates a new layer of milk protein around the fat globules. This layer, which was thicker when WPC was pre-heated (as demonstrated by a higher protein load), was involved during the gel build-up (Cho et al., 1999). In our study, WPCs were added after milk homogenization and the mechanism of whey protein involvement in the gel network is likely to be different.

Finally, loss tangent δ (Fig. 1c) increased with increasing heat treatment of whey and decreasing whey pH, ranging from 0.256 to 0.299. These values agree with the ones generally observed for stirred yoghurt (Rohm & Kovac, 1994) and show its viscoelastic character. The results demonstrate a more elastic behavior of stirred yoghurt enriched with WPC made from non-acidic and mildly heated whey.

3.2.3. Relation between protein denaturation in WPC and functional properties in yoghurts

Fig. 2 is a plot of the elastic modulus, the loss tangent δ, and the water-holding capacity of the yoghurts as a function of protein denaturation in WPC. Significant ($P<0.01$) linear relationships can be seen between denaturation and water-holding capacity ($R>0.89$), elastic modulus ($R>0.93$), and loss tangent δ ($R>0.80$). The lower the denaturation of protein in the WPC, the higher the elastic modulus and the water-holding capacity of the WPC-fortified yoghurt. Mechanism of gelation in a milk system with pre-heated whey protein as proposed by Schorsch et al. (2001), based on a rheological study and microstructure observations, could explain these results. These authors demonstrated that the gel structure after acidification is more particulate and heterogeneous when the whey protein are pre-heated before to be mixed with the casein micelles. A difference of structure according to the thermal history of the whey protein could explain the difference in rheological and physical

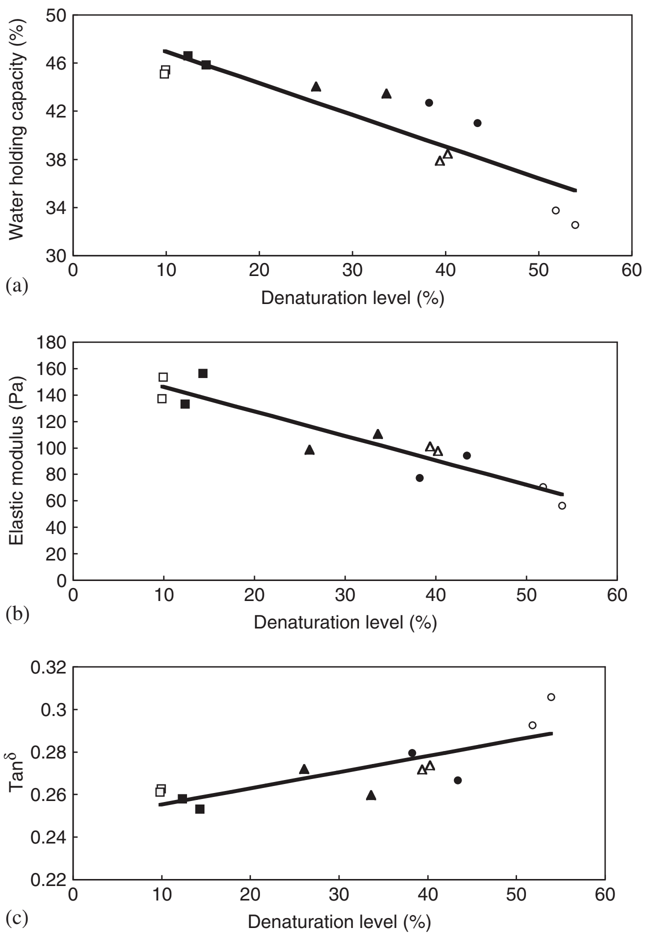

Fig. 2. Relationship between denaturation of whey protein concentrates used for milk fortification and physical properties of yogurt: (a) water-holding capacity, (b) elastic modulus, (c) loss tangent (tan δ). Data are the results of two independent trials of WPC manufacture. Closed and open symbol are used for WPC produced with a whey at pH 6.4 and 5.8, respectively. The whey was heated at 72 °C for 15 s (■ □), eventually heated further at 82 °C for 78 s (▲ △), or at 88 °C for 78 s (● ○).

properties of WPC-fortified yoghurt observed in this work.

4. Conclusion

Whey pH and the heat treatment in manufacture of whey protein concentrates had a significant effect on physical properties of resulting fortified yoghurt. A high pH and a mild heat treatment during whey processing seemed to be more favorable to produce functional whey protein concentrates for yoghurt manufacture. A linear relationship was observed between water-holding capacity and viscoelasticity of fortified yoghurt, and the level of denaturation of whey proteins in whey protein concentrates. A high level of denaturation of the whey protein in WPC was detrimental to their functionality in yoghurt.

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