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The effect of the pH at cooking on the properties of processed cheese spreads containing whey proteins

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ABSTRACT

Processed cheese spreads were made with and without whey proteins under varying cooking pH conditions. The processed cheeses were cooked at one pH value and at the end of the cooking process the pH was adjusted to the final product pH of 5.7. The rheological properties and whey protein denaturation levels of the processed cheese spreads were measured. The rheological properties and texture of the processed cheeses containing whey proteins could be markedly modified by varying the cooking pH during processing, whereas those without whey proteins were unaffected. These textural modifications could not be explained solely by the changes in whey protein denaturation during cooking. It is proposed that the interactions of the whey proteins during cooking affect the processed cheese texture, and that these interactions are affected by the pH of the processed cheese during processing.

1. Introduction

Traditionally processed cheese is made by mixing and heating natural cheese, salts, calcium chelating salts (also commonly known as melting or emulsifying salts), water and fat. The conversion of natural cheese into processed cheese was originally developed in the late nineteenth to early twentieth centuries, and was used as a preservation measure, extending the shelf life of the cheese. Nowadays, processed cheeses are made for reasons other than preservation, such as for versatility, convenience and cost reduction. With the advances in dairy technologies, new ingredients such as milk powders, whey powders, milk protein concentrates, caseins and caseinates are now available as ingredients for incorporation into processed cheese (Berger, Klostermeyer, Merkenich, & Uhlmann, 1998; Guinee, Caric, & Kaláb, 2004; Lee, Anema, & Klostermeyer, 2004). The composition and properties of some of these ingredients can be used to manipulate the textural and functional properties of the resultant processed cheese.

There are limited reports on the study of the effects of whey proteins on processed cheese properties. Ernstrom, Sutherland, and Jameson (1980) reported that the effect of cheese base (which contains whey proteins) in processed cheese resulted in excessively firm product, but the effect on processed cheese food was satisfactory. Gupta and Reuter (1993) investigated the effect of whey protein concentrate (WPC) on the properties of processed cheese and processed cheese foods. They reported a slight increase in hardness at 10% protein replacement level, but a more significant increase at 20% protein replacement level. The melt properties of the processed cheese containing WPC were markedly reduced. This reduction of the melt properties of processed cheese by the addition of whey proteins has been reported on a number of occasions (Gupta & Reuter, 1993; Schulz, 1976; Strandholm, Prochnow, Miller, Woodford, & Neunaber, 1989).

Savello, Ernstrom, and Kalab (1989) reported on the addition of native and denatured whey proteins in processed cheese made from rennet casein or acid casein. Though a general trend of melt reduction was observed, high levels of whey protein inclusion (4.5%) in the rennet casein system showed an increase in meltability. On addition of native whey proteins to the rennet casein system, oiling off was observed when the whey protein level was increased above about 1.5%. Abd El-Salam, Khader, Hamed, Al-Khamy, and El-Garawany (1997) indicated that the use of WPC in processed cheese may be complex and possibly confounded by the use of different types and levels of calcium chelating salts. Mounsey, O'Kennedy, and Kelly (2007) showed that the whey protein isolate (WPI) significantly decreased the meltability of processed cheese, but the hardness increased and then decreased above 2.6% w/w WPI. At low levels of WPI addition, there was no significant change in hardness or meltability compared with the control (no WPI).

Despite the different results reported, the general trend is that whey protein addition to processed cheese tends to decrease the meltability of the cheese (Collinge & Ernstrom, 1988; Gigante, Antunes, Petenate, & Roig, 2001; Gupta & Reuter, 1993; Mounsey et al., 2007; Savello et al., 1989; Schulz, 1976; Sood & Kosikowski, 1979), with variable effects on the firmness (Abd El-Salam et al., 1997; Ernstrom et al., 1980; Gupta & Reuter, 1993; Mounsey et al., 2007).

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Recent studies on heating milk have shown that the manipulation of the pH at the time of heating can markedly influence the interactions of the denatured whey proteins with the caseins (Anema & Li, 2003; Donato & Dalgleish, 2006; Rodriguez del Angel & Dalgleish, 2006), and that these interactions have an effect on the rheological properties of acid gels prepared from the heated milks (Anema, Lee, Lowe, & Klostermeyer, 2004; Rodriguez del Angel & Dalgleish, 2006). On heating milk (90 °C/30 min) at a relatively low pH (pH 6.5) most of the denatured whey proteins interacted with the casein micelles, and on acidification, these heated milks produced acid gels with a relatively low firmness. As the pH at heating was increased from pH 6.5 to pH 7.1, less denatured whey protein interacted with the casein micelles, the whey proteins remaining in the serum as complexes with κ-casein. The firmness of acid gel prepared from the heated milks increased as the pH of the heated milks was increased, so that acid gels prepared from milk heated at a heating pH of ~pH 7.1 had a firmness that was more than double that of acid gels prepared from milk heated at pH 6.5 (Anema et al., 2004; Rodriguez del Angel & Dalgleish, 2006). These changes in the rheological properties of the acid gels were related to the denaturation and interaction of the whey proteins, and these interactions were markedly affected by small changes in the pH at the time of heating the milk (Anema et al., 2004; Rodriguez del Angel & Dalgleish, 2006).

Though the ingredients, processing methods and equipment used in processed cheese manufacture are versatile (Berger et al., 1998; Guinee et al., 2004), processed cheese is usually prepared (cooked) at the pH of the finished product. Due to the solubility, interaction, and as well as the hydrolysis of the calcium chelator salts, the final pH of processed cheese products usually ranges from pH 5.4 to pH 5.9, depending on the processed cheese type, but is usually around pH 5.7 (Guinee et al., 2004; Lu, Shirashoji, & Lucey, 2007). It is possible that, in processed cheese samples with added whey proteins, the rheological properties and textural properties of the processed cheese may be able to be manipulated by changing the pH of the system during cooking. This work therefore reports on the effect of processing conditions, especially the pH at cooking, on the textural properties of model processed cheese spreads containing whey proteins; these are compared with processed cheese samples that did not have whey proteins added. Therefore, this work examined the effect of altering the pH at cooking on the visual and rheological properties of processed cheese spreads containing whey proteins (PC_{whey}), in comparison to those that contained no whey proteins ($PC_{control}$).

2. Materials and methods

2.1. Materials

The whey protein concentrate (WPC) used was ALACEN 392, and the rennet casein used was ALAREN 799 (90 mesh). Both were supplied by Fonterra Co-operative Group, New Zealand. Sodium chloride (NaCl), trisodium citrate (TSC) and citric acid (CA) were supplied by BDH Laboratory Supplies, Poole, England. Sunflower oil (AMCO brand) was obtained from Goodman Fielder, East Tamaki, New Zealand.

2.2. Preparation of model processed cheese samples

2.2.1. Preparation of processed cheese samples with added whey proteins

WPC (15.36 g) was added to water (67.64 g) and stirred for ~30 min to form a well dispersed solution. Rennet casein (55.08 g) and NaCl (6 g) were added to a calcium chelator solution containing water (170 g), TSC and CA to achieve different pH's of the processed cheese during cooking (see Table 1 for levels of TSC and CA added and the cook pH achieved). The mixture was rapidly stirred for a few minutes at room temperature where the slurry set into a gel-like material. The WPC and gelled rennet casein solutions were allowed to hydrate for 12 h in a refrigerator set to ~5 °C.

Model processed cheese spreads were prepared using a 2 l capacity Vorwerk Thermomix TM 21 blender cooker (Vorwerk Australia Pty. Ltd., Granville, N.S.W., Australia) as described by Lee et al. (2004). The Vorwerk cooker has temperature and speed settings which were used to heat and stir the mixtures. The sunflower oil (192 g) was heated for 1 min at a temperature setting of 100 °C and a speed setting of 1 (100 rpm). This brings the oil temperature to ~60 °C. The hydrated rennet casein, WPC and additional water (48.7 g, which includes ~11 g that evaporates during cooking) were added to the warm oil and the mixture was cooked at a temperature setting of 90 °C for 2 min at speed 4 (~2000 rpm), after which the temperature setting was lowered to 80 °C and held at this setting for 5 min. The remaining CA or TSC (as shown in Table 1) was dissolved in water (20 g) and added to the mixture. The mixture was then cooked for a further 2 min at 80 °C at speed 4 (~2000 rpm). This late addition of CA or TSC shifts the processed cheese from its cook pH (see Table 1) to a final pH of 5.7. The final temperature of the molten processed cheese was ~85 °C. The molten processed cheese was poured into plastic screw-cap containers (volume 50 ml), inverted and then stored at 5 °C. The target composition of the processed cheese spreads was 52.1% moisture, 10.1% protein (80% casein from rennet casein, 20% whey protein from WPC), 33.2% fat, 2.6% calcium chelating salts (ratio TSC:CA = 3.20:1) and 2.0% minerals. This composition is typical of processed cheese spread products (Berger et al., 1998; Guinee et al., 2004; Lee et al., 2004).

2.2.2. Preparation of processed cheese samples with no added whey proteins

Samples that contained no whey proteins were also prepared. Rennet casein (69.5 g) was hydrated with TSC, CA (see Table 2

Table 1
Levels of trisodium citrate (TSC) and citric acid (CA) added to processed cheese to attain cook pH and final pH for processed cheese samples containing whey proteins.

Required cook pH	TSC added to attain cook pH (g)	CA added to attain cook pH (g)	TSC added to attain final pH of 5.7 (g)	CA added to attain final pH of 5.7 (g)
5.62	8.64	3.57	2.79	0
5.71	11.43	3.57	0	0
5.87	11.43	3.20	0	0.37
6.07	11.43	2.80	0	0.77
6.27	11.43	2.42	0	1.15
6.55	11.43	1.85	0	1.72
6.66	11.43	1.66	0	1.91
6.80	11.43	1.47	0	2.10
6.97	11.43	1.09	0	2.48
7.60	11.43	0	0	3.57

for levels of TSC and CA added during cooking and the cook pH achieved), NaCl (6 g) and water (200 g). Sunflower oil (192 g), the hydrated rennet casein, and water (104.5 g, which includes ~11 g which evaporates during cooking) was cooked as described in Section 2.2.1. After cooking, the remaining CA (see Table 2 for levels) was dissolved in water (20 g) and was added to the mixture. The mass was cooked for a further 2 min at a temperature setting of 80 °C. The target composition of these processed cheese spreads was 52. 0% moisture, 10.1% protein (all casein from rennet casein), 33.3% fat, 2.6% calcium chelating salts (ratio TSC:CA = 2.92:1) and 2.0% minerals. Again, this composition is typical of processed cheese spread products (Berger et al., 1998; Guinee et al., 2004; Lee et al., 2004).

2.3. Measurement of moisture content and pH of processed cheese samples

The moisture content was measured using the rapid aluminium foil method, while the pH was measured using a Mettler Toledo In-Lab® 422 combination glass electrode (Mettler-Toledo GmbH, Urdorf, Switzerland), as described by Lee et al. (2004).

2.4. Measurement of the rheological properties of the processed cheese samples

The mechanical spectra of the samples were obtained using a TA AR2000 rheometer (TA Instruments Ltd., Crawley, UK) at 20 °C with a 2 cm diameter steel plate. The height of the sample was set at 2 mm. The edge of the sample was coated with a light paraffin oil, to prevent the sample from drying out. The samples were swept from 10 Hz to 0.01 Hz at a strain 0.005. Immediately after this, the sample underwent a pre-programmed temperature sweep. The temperature was increased from 5 °C to 70 °C at a rate of 1 °C/min and the mechanical spectra were recorded at a frequency of 0.1 Hz and at a strain of 0.005. The ratio of G'' to G' is expressed as phase angle in degrees.

The flow curves of the samples were obtained using the controlled shear rate mode of the rheometer at 70 °C with a 4 cm diameter, 4° stainless steel cone equipped with a moisture trap. Light paraffin oil was applied to the rim of the samples to minimise sample drying. Each sample was swept from 0 to 100 s^{-1} and then from 100 to 0 s^{-1}. The total run time was 4 min.

2.5. Measurement of the level of whey protein denaturation in processed cheese samples

Whey protein denaturation was determined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE), as described in Anema et al. (2004). Processed cheese (0.1 g) was weighed into a 2 ml Eppendorf tube. Water (0.5 ml) was added. Three glass beads were added and the sample was dispersed by agitating on a high speed mixer (Chiltern MT19 Auto Vortex Mixer, Chiltern Scientific, London, UK). The sample mixture was then clipped onto a rotation platform and rotated gently for an hour until the sample dispersed in the water. Sodium acetate buffer at pH 4.31 (0.5 ml) was added, which shifts the pH of the processed cheese dispersion to pH 4.6 and precipitates the casein and denatured whey proteins. The precipitated proteins were separated from the supernatants by centrifugation (14,000 rpm for 15 min) and the supernatants containing the remaining native whey proteins were analysed by SDS–PAGE. The level of whey protein denaturation was determined by comparing the bands for the whey proteins in the processed cheese samples with those in a sample of similar composition that was processed under conditions that involved minimal heat treatment and therefore limited whey protein denaturation. This sample was made using the same formulation as sample 2 (Table 1), but cooked at 60 °C for 10 min. The whey proteins are not denatured to any significant extent at a cooking temperature of 60 °C (Anema & McKenna, 1996; Dannenberg & Kessler, 1988).

3. Results

3.1. Two-step pH adjustment during cooking

In this work, processed cheese spreads were prepared using a two-step pH adjustment process, where the processed cheese is cooked at a pH other than its final product pH (defined as "cook pH"), and, just before the cooking process was terminated, its pH was then brought back to the product's desired pH (defined as "final pH": in this work the final pH was 5.7). The pH variation during cooking could be achieved by supplying the appropriate amount of the basic calcium chelating salt (TSC) and its conjugate acid (CA), to give the desired cook pH during processing (Table 1). The product's final pH of 5.7 was achieved by adding the remaining calcium chelating salts (TSC or CA) at the end of the cooking process (Table 1).

To confirm that the variation in the properties of the processed cheese was due to the cook pH and not the variable addition of the calcium chelating salts, similar experiments were conducted where all the calcium chelating salts were added at the start of the experiment, and the cook pH and final pH were adjusted using strong acid and base (hydrochloric acid/sodium hydroxide). The properties and general observations for the processed cheese samples were similar to those where a combination of acid/base calcium chelating salts were used to alter pH (results not shown), indicating that the observed effects were due to the pH at cooking.

3.2. Composition and visual appearance of processed cheese samples

Samples either contained whey proteins at 20% of the total protein (PC_{whey}) or contained no whey proteins ($PC_{control}$). For all processed cheese samples prepared in this work, white and homogeneous emulsions were easily formed, regardless of the cook pH values or the presence or absence of whey protein. The experimentally determined moisture contents of all processed cheese samples were within ±0.2% of the target moisture and all the final pH values were 5.7 ± 0.05.

Fig. 1 shows selected photographs of typical PC_{whey} samples cooked at different pH values. The final pH of all these samples was pH 5.7. The texture of the samples varied greatly, depending on the cook pH. Samples obtained at a cook pH of ~5.7 were very soft and runny, could easily be poured from the cooker, and, after

Table 2
Levels of trisodium citrate (TSC) and citric acid (CA) added to processed cheese to attain cook pH and final pH of processed cheese samples that contained no whey proteins.

Required cook pH	TSC added to attain cook pH (g)	CA added to attain cook pH (g)	TSC added to attain final pH of 5.7 (g)	CA added to attain final pH of 5.7 (g)
5.70	11.172	3.828	0	0
6.41	11.172	1.964	0	1.864
6.70	11.172	1.165	0	2.663
7.11	11.172	0	0	3.828

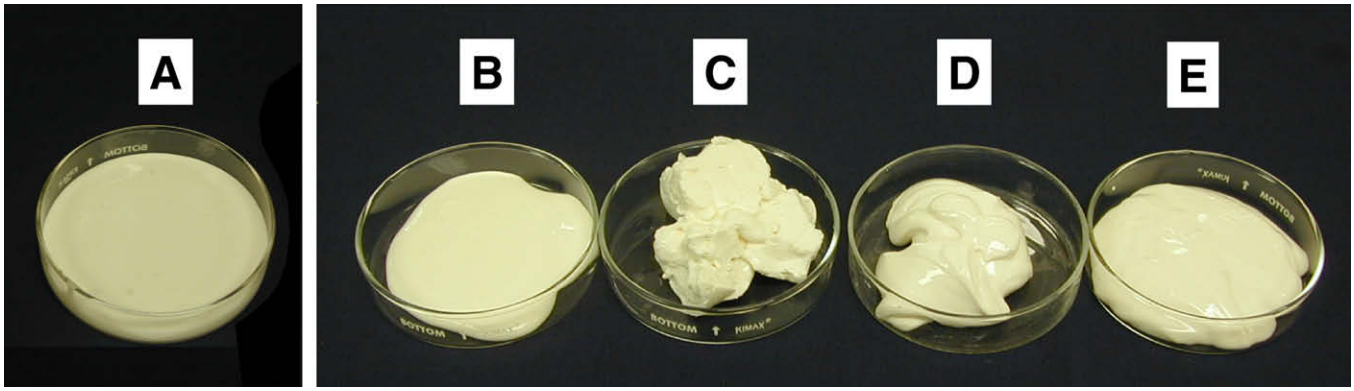

Fig. 1. Photographs of selected processed cheese samples. Sample without whey proteins cooked at pH 5.7 (A). Samples with whey proteins cooked at pH 5.7 (B), pH 6.7 (C), pH 7.0 (D) or pH 7.6 (E). The final pH of all processed cheese samples was pH 5.7.

cooling would flow when unsupported (Fig. 1B). In contrast, samples obtained at a mid-range cook pH of ~6.7 were very firm and had to be scooped from the cooker as they would not flow. After cooling, these samples did not flow when unsupported (Fig. 1C). The samples prepared at a high cook pH (e.g., ~pH 7.0 and pH 7.6) had a soft texture again, although not as soft as those cooked at pH 5.7 (Fig. 1D and E). The sample cooked at pH 7.6 could easily be poured from the cooker, and, after cooling, would flow when unsupported (Fig. 1C).

Also included in Fig. 1 is a typical $PC_{control}$ sample (Fig. 1A). For the $PC_{control}$ samples, no effect of cook pH was observed, and all samples had a soft texture similar to that of the PC_{whey} sample cooked at pH 5.7, as shown in Fig. 1B. These results indicate that the visual properties of the processed cheese samples could be markedly altered by varying the cook pH during processing, and that these textural differences were due to the presence of native whey proteins in the samples during processing.

3.3. Small strain rheological properties of processed cheese samples

To examine the textural properties more quantitatively, the rheological properties of the processed cheese samples at 20 °C were monitored. The firmness or elastic modulus (G') of the processed cheese samples plotted against the cook pH for both the $PC_{control}$ and PC_{whey} samples is shown in Fig. 2. For the $PC_{control}$ samples, the G' was low (~100 Pa) and was similar at all cook pH values. However, for the PC_{whey} samples, a complex dependence on cook pH was observed. At a cook pH of 5.7 the G' was low at about 200 Pa, although this was still higher than the $PC_{control}$ samples. As the cook pH increased, the G' increased to a maximum at a cook pH of ~6.7, where the G' was ~2500 Pa, more than a 10-fold increase over the sample cooked at pH 5.7. As the cook pH was increased above pH 6.7, the G' decreased again and was about 400 Pa at pH 7.6.

The texture of the processed cheese spread products can also be described by their different mechanical spectra, where the elastic modulus (G') and viscous modulus (G'') are measured at a range of oscillation frequencies (selected samples shown in Fig. 3). When a viscoelastic material (such as processed cheese) is subjected to a shear deformation, the behaviour of the material to the deformation is dependent on the ratio of the relaxation time to the reciprocal of the rate of deformation (i.e., the frequency of the deformation). If the relaxation time is short relative to the reciprocal of the rate of deformation (low frequencies), the system has sufficient time to return to its equilibrium state, so that a fluid-like behaviour is observed and the viscous component (G'') is larger than the elastic component (G'). However, if the relaxation time

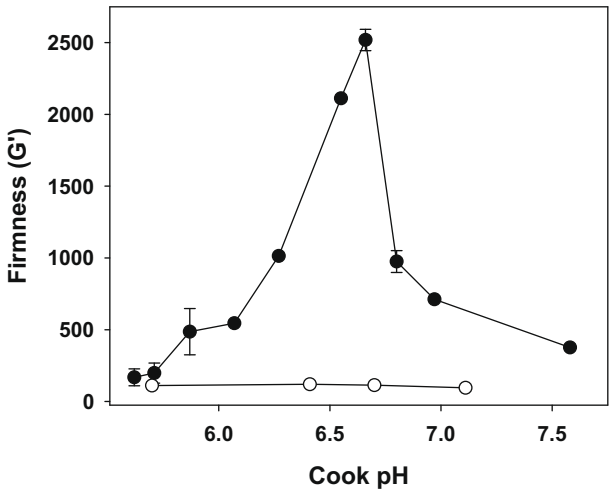

Fig. 2. Elastic modulus (G') for processed cheese spread samples at 20 °C plotted against cook pH. Samples containing whey proteins (●) and samples without whey proteins (○). All processed cheese samples had a final pH of 5.7. Error bars on selected points represent the standard deviations of repeated measurements.

is much longer than the reciprocal of the rate of deformation (high frequencies), the system will show a solid-like character and the elastic component (G') is larger than the viscous component (G''). Over a defined frequency range, a sample may have only fluid-like behaviour ($G'' > G'$), have only solid-like behaviour ($G' > G''$), or show a transition from fluid-like to solid-like behaviour ($G'' > G'$ at low frequencies and $G' > G''$ at high frequencies, with a $G' - G''$ crossover at intermediate frequencies; Harrison, Franks, Tirtaatmadja, & Boger, 1999; Muller, 1973; Ross-Murphy, 1988).

The mechanical spectra of the PC_{whey} samples cooked at low pH (~pH 5.6–5.85) displayed a $G' - G''$ crossover at around 1 Hz with G' lower than G'' at low frequencies (<1 Hz) and G' higher than G'' at higher frequencies. Samples with this type of mechanical spectra have been referred to as concentrated liquids (Lee & Klostermeyer, 2001; Ross-Murphy, 1988). At a cook pH between pH 5.85 and pH 6.97, no $G' - G''$ crossover was observed, and G' was higher than G'' at all frequencies. Samples with this type of mechanical spectra have been referred to as weak gels (Lee & Klostermeyer, 2001; Ross-Murphy, 1988). However, at a cook pH of 7.6, the processed cheese again assumed a softer structure, with mechanical spectra of a concentrated liquid as a $G' - G''$ crossover was observed with G' lower than G'' at frequencies <0.05 Hz and G' higher than G'' at higher frequencies (Fig. 3A). For the $PC_{control}$ samples, the mechan-

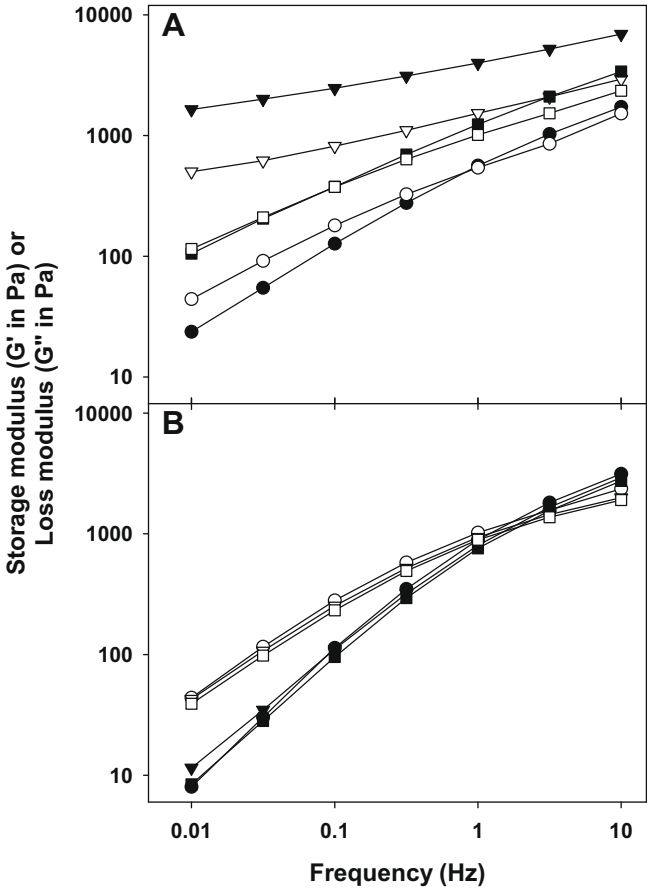

Fig. 3. Frequency sweep of selected processed cheese spread samples at 20 °C. (A) Samples with whey proteins cooked at pH 5.7 (●, ○), pH 6.7 (▼, ▽) and pH 7.6 (■, □). (B) Samples without whey proteins cooked at pH 5.7 (●, ○), pH 6.7 (▼, ▽), and pH 7.1 (■, □). Filled symbols: elastic modulus (G'); open symbols: viscous modulus (G''). All processed cheese samples had a final pH of 5.7.

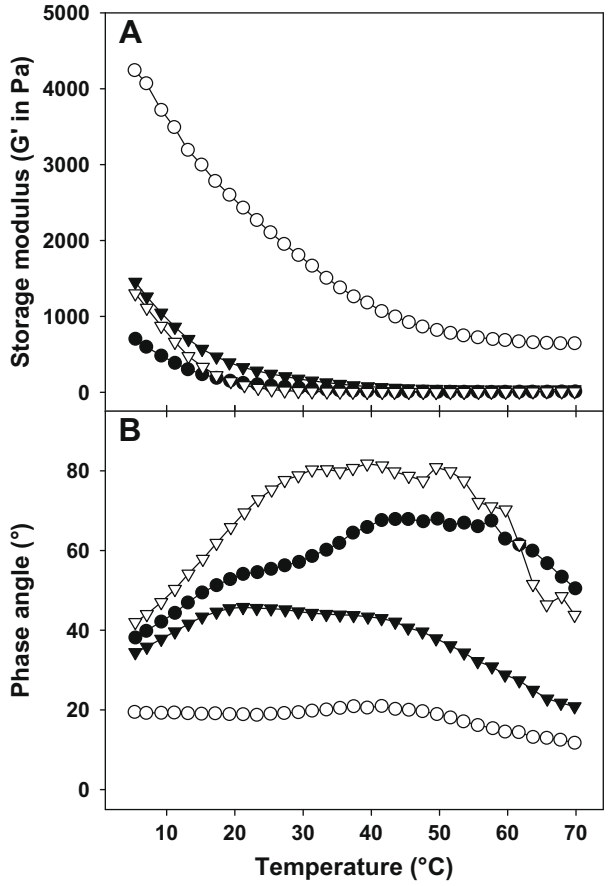

Fig. 4. Rheological properties of processed cheese samples during a temperature sweep from 5 °C to 70 °C. (A) Elastic modulus (G') plotted against temperature and (B) phase angle plotted against temperature. Samples with whey proteins cooked at pH 5.7 (●), pH 6.7 (○) and pH 7.6 (▼), and a sample without whey proteins cooked at pH 5.7 (▽).

ical spectra were similar to those for concentrated liquids at all cook pH values. The $G' - G''$ crossover was at ~1.8 Hz (Fig. 3B).

To examine the effect of temperature on the properties of the processed cheese spread samples, the rheological properties of the cheeses were monitored during a temperature sweep from 5 °C to 70 °C (selected samples are shown in Fig. 4). The G' for all samples decreased with increasing temperatures, as would be expected with the increase in thermal motion of molecules, the decrease in ordering of water molecules (increased entropy) and the decrease in hydrogen bonding with increasing temperature (Dickinson & McClements, 1995; McClements, 2005a, 2005b).

The melting point of samples is often determined by their G''/G' ratio, expressed as phase angle in degrees. A sample is often said to "soften" or "melt" when the G'' becomes greater than G', which is equivalent to the point where the phase angle is greater than 45°. For the PC_{whey} samples, the sample at a cook pH of 6.7 had the highest G' and lowest phase angle throughout the temperature range. The phase angle for this sample did not attain 45° even at the highest temperature, indicating that the sample did not melt. Samples with a cook pH higher or lower than pH 6.7 samples were softer (lower G') throughout the temperature range, and had higher phase angles, although many of the samples did not have a phase angle above 45 °C (e.g., the sample at a cook pH of 7.6 in Fig. 4). The $PC_{control}$ samples attained the highest phase angles during the temperature sweeps, indicating that this sample became the softest during heating.

The phase angle of all PC_{whey} and $PC_{control}$ samples increased with temperature up to a maximum and then decreased again at higher temperatures (Fig. 4B). This is a rather unexpected observation, as it may be expected that the samples will continue to soften with increasing temperature throughout the temperature range, i.e., an increasing phase angle with increasing temperature. It is unknown why the phase angle increases to a maximum and then decreases again with increasing temperature. As this effect is observed in both the PC_{whey} and $PC_{control}$ samples, it cannot be attributed to the whey proteins and their denaturation at elevated temperatures.

It has been reported that inter-protein interactions become increasingly important with increasing temperature in both non-fat processed cheese model systems (Lee, Buwalda, Euston, Foegeding, & McKenna, 2003) or fat-containing processed cheese systems (Heertje & Lewis, 1993). Lee et al. (2003) reported that these protein interactions may be related to the "creaming reaction" that is reported to occur in processed cheese during processed cheese manufacturing (Berger et al., 1998; Heertje & Lewis, 1993; Lee et al., 2003). Increases in inter-protein interactions in the system would be expected to result in an increase in the network structure, thus decreasing the phase angle. Hence, the decrease in phase angle with an increase in temperature (Fig. 4B) may be the result of increased inter-protein interactions on further heating. Other factors such as increased hydrophobic interactions may also become more important at elevated temper-

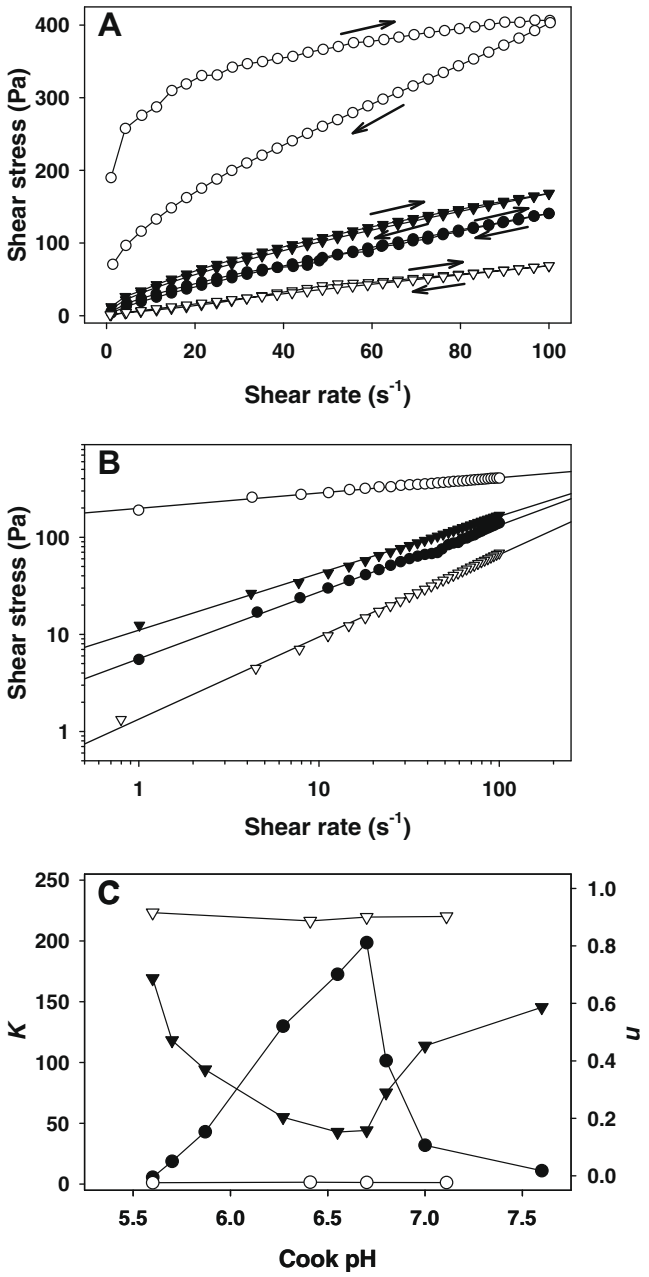

Fig. 5. (A) Flow curves of selected processed cheese spreads at 70 °C. Samples with whey proteins cooked at pH 5.7 (●), pH 6.7 (○) and pH 7.6 (▼), and a sample without whey proteins cooked at pH 5.7 (▽). (B) Log-log plot of the up sweep of flow curves in (A). (C) Influence of cook pH on the consistency index, K (●, ○), and the flow behaviour index, n (▼, ▽) for processed cheese sample with whey proteins (●, ▼) and without (○, ▽) whey proteins added.

atures and contribute to the decreased phase angles at these elevated temperatures.

3.4. Flow curves of processed cheese samples at 70 °C

The flow curves of selected processed cheese samples, as measured at 70 °C, are shown in Fig. 5A. These plots show the shear stress rising with increasing shear rate on the up sweep, and decreasing with decreasing shear rate on the down sweep. There was hysteresis between the up and down curves with the down curves having lower shear stress at any given shear rate than the up curves. These curves are typical of thixotropic pseudoplastic materials. As expected, the PC_{whey} sample prepared at a cook pH of 6.7 had higher shear stress and more hysteresis at any given shear rate than those samples prepared at a cook pH of 5.7 or 7.6. The $PC_{control}$ sample had the lowest shear stress at any given shear rate.

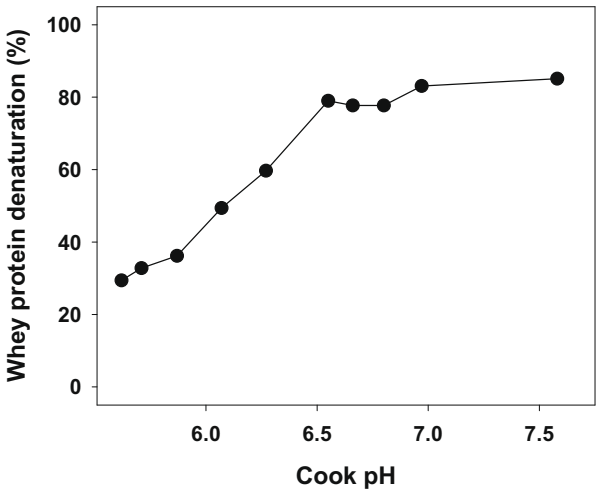

Fig. 6. Level of whey protein denaturation in processed cheese samples containing whey proteins cooked at different pH values.

For the up sweep, plots of the log (shear rate) against log (shear stress) produced linear lines (Fig. 5B) indicating that all the processed cheese samples were typically pseudoplastic. These plots could be fitted using the power law relationship ($r^2 > 0.984$); hence, they could be described by the K and n constants defined by the mathematical equation:

$$\tau = KD^n$$

where τ is the shear stress and D is the shear rate. K is known as the "consistency index" and n is the "flow behaviour index" (Lee et al., 2004; Muller, 1973). The latter is a measure of the departure from Newtonian behaviour. If $n = 1$, the sample is Newtonian. If $n < 1$, the sample shear thins during the test, and if $n > 1$, the sample shear thickens.

The n and K values plotted against cook pH for the PC_{whey} and $PC_{control}$ processed cheese samples are shown in Fig. 5C. For the PC_{whey} samples, the n varied from 0.15 to 0.68, indicating the samples had shear-thinning characteristics. The value of n was the lowest whereas K was the highest at cook pH of around 6.7, which indicates that the samples were thickest at this cook pH (Fig. 5C). At cook pH below pH 6.7, the n increased and K decreased as the pH decreased, and at cook pH above pH 6.7, the n increased and K decreased as the pH increased. The $PC_{control}$ samples had relatively constant n and K values of around 0.9 and 1.2, respectively (Fig. 5C), indicating that these samples had lower shear-thinning characteristics (i.e., of lower "consistency") than the PC_{whey} samples. Cook pH had little effect on the n and K values of the $PC_{control}$ samples (Fig. 5C). For all processed cheese samples, the results for the flow curves (Fig. 5A) and for the consistency and flow behaviour indices (Fig. 5C) were consistent with those for the firmness (Figs. 1 and 2). These rheological results indicate that the cook pH may affect the network structure within the processed cheese, with a high degree of network structure (more connectivity) resulting in a higher firmness and greater shear-thinning characteristics.

3.5. Whey protein denaturation in PC_{whey} processed cheese samples

Fig. 6 shows the whey protein denaturation level for the PC_{whey} samples, as estimated by SDS–PAGE analysis. About 30% of the whey proteins were denatured in the PC_{whey} samples cooked at

pH 5.7, and this level of denaturation increased with increasing cook pH, up to a cook pH of 6.7, where about 85% of the whey proteins were denatured. The levels of whey protein denaturation at cook pH above pH 6.7 were similar, with between 85% and 90% of the total whey protein being denatured during processing. These results on whey protein denaturation are consistent with literature reports, as a strong pH dependence of the denaturation has been observed for whey proteins in milk, albeit over a narrower pH range (Law & Leaver, 2000), as well as in synthetic milk serum (Paulsson, Hegg, & Castberg, 1985).

4. Discussion

A few previous studies have investigated the use of whey proteins in processed cheese (Collinge & Ernstrom, 1988; Gigante et al., 2001; Gupta & Reuter, 1993; Mounsey et al., 2007; Savello et al., 1989; Schulz, 1976; Sood & Kosikowski, 1979). Almost all these studies indicate that the incorporation of whey proteins into the cheese decreases the processed cheese product meltability, which is consistent with our observations, although the effect on melt is markedly dependent on the cook pH (Fig. 4). There have been few reports on the effect of whey proteins on firmness of the processed cheese, and the results that are available are often contradictory with some reports, suggesting marked changes to firmness, while others report smaller changes (Abd El-Salam et al., 1997; Ernstrom et al., 1980; Gupta & Reuter, 1993; Mounsey et al., 2007; Savello et al., 1989). The work presented here has shown that the textural and the melt properties of processed cheese spread samples are very dependent on the presence of whey proteins and that these properties can be manipulated by the cook pH.

The results shown in Figs. 1–3 show that, in the PC_{whey} samples, the cook pH can have a marked effect on the textural properties of the processed cheese. A 10-fold (or more) increase in firmness could be achieved by altering the pH during cooking (Figs. 1 and 2). This indicates that the careful manipulation of the cook pH would allow the texture of the processed cheese to be modified, from a system that flows on standing (concentrated liquid), to a system that retains structure even when unsupported (weak gel). The observation that the $PC_{control}$ did not show a dependency on cook pH clearly indicates that the whey proteins are involved in this effect (Figs. 1–3).

As native whey proteins are soluble at all pH values and will not modify the texture of dairy products, the denaturation of the whey proteins during processing to produce processed cheese is required for modifying the textural properties of the processed cheese spreads. However, a comparison between the G' value (Fig. 2) and the level of whey protein denaturation in the PC_{whey} samples (Fig. 6) indicates that the effect of cook pH on the texture of PC_{whey} processed cheese samples cannot be explained simply by differences in the level of whey protein denaturation. This is especially evident when the cook pH is $\geqslant$6.5, where, the G' of the processed cheese spreads markedly decreased even though the level of whey protein denaturation was similar in these samples.

It is proposed that the whey proteins are denatured during the cooking of the processed cheese and that these denatured whey proteins interact with other (denatured) whey proteins and casein proteins from the rennet casein. These interactions may involve thiol-disulphide exchange reactions between the denatured whey proteins and between the denatured whey proteins and *para*-κ-casein/α_{s2}-casein; however, as these thiol-disulphide exchange reactions are pH-dependent (Torchinsky, 1981), they may be more important at the higher cook pH than at lower pH. The pH-dependency of thiol-disulphide exchange reactions may also account for some of the pH-dependency of the textural properties of the PC_{whey} processed cheese samples, as different types of interactions (disulphide bonds, hydrophobic interactions, ion-pairing or salt bridges, etc.) may be occurring to different extents as the cook pH is altered. The pH dependence of the interactions involved in the processed cheese, which form network structures, result in processed cheese samples with modified network connectivity and therefore a difference in G' and in visible firmness, compared to equivalent samples with no added whey proteins (Figs. 1 and 2).

For the PC_{whey} samples, the observation that the textural properties increase with cook pH to a maximum at ~pH 6.7, and then decrease again as the cook pH is increased further (Figs. 1–5) may be a consequence of different types/extents of interactions at the different cook pH values, e.g., a predominance of thiol-disulphide exchange reactions at higher cook pH when compared with lower cook pH. However, it is also possible that there is a balance between the rate of denaturation and the rate of interactions of the denatured whey proteins. At lower cook pH, the denaturation rate of the whey proteins is slower and this allows for an efficient incorporation of the denatured whey proteins into the processed cheese gel structure and a strong gel network is formed. The pH dependence of the texture of the processed cheese at cook pH below pH 6.7 may simply be a consequence of the pH dependence of the whey protein that is denatured and incorporated into the processed cheese structure, with a stronger network as more whey protein is denatured and incorporated (Figs. 2 and 6). This is consistent with the change of mechanical spectrum from that of concentrated liquid at a cook pH of 5.7 to that of a weak gel at a cook pH of 6.7 (Fig. 3).

As the cook pH is increased the rate of denaturation of the whey proteins increases (Fig. 6). It is possible that, at a cook pH above 6.7, the rate of denaturation is so rapid that the denatured whey proteins are aggregating at a rate that does not allow efficient incorporation of the proteins into the processed cheese structure. It is also possible that at a high cook pH, the increased charge on the proteins during processing may limit the extent of intermolecular interactions and therefore reduce the interconnectivity of the network. In both these situations, a weaker gel network with lower interconnectivity may result, when compared with structures that are formed at cook pH 6.7. As the structure is weakened, the mechanical spectra of the samples are again reverted to those resembling concentrated liquids (Fig. 3).

This effect of cook pH on the denaturation and subsequent aggregation of the proteins may be related to a change in reaction mechanism between diffusion-limited and reaction-limited aggregation processes (Horne, 1989; Ikeda, Foegeding, & Hagiwara, 1999; Vetier, Desobry-Banon, Eleya, & Hardy, 1997). Under conditions where the rate of denaturation of the whey proteins is slow (i.e., at low cook pH), the aggregation process may be diffusion-limited, where the aggregation rate is determined by the time taken for the aggregating particles to encounter each other. This diffusion-limited aggregation may dominate up to a cook pH of 6.7, where the maximum in firmness is obtained. At higher pH (i.e., above pH 6.7) the rate of denaturation of the whey proteins is rapid and under these conditions, the aggregation process may become reaction-limited, where the rate of aggregation is determined by the time for interacting particles to overcome the repulsive barriers between the particles.

The fractal dimension is a measure of the aggregate packing density and has been shown to be dependent on the mechanism of aggregation (Ikeda et al., 1999; Mellema, Walstra, van Opheusden, & van Vliet, 2002; Vetier et al., 1997). In diffusion-limited aggregation, a lower aggregate density is observed and a fractal dimension of 1.7 to 1.8 is observed. In contrast, in reaction-limited aggregation, a higher aggregate density is encountered and fractal dimensions of 2.0 to 2.2 are observed. Studies on heat-induced whey protein gelation have shown that the gelation mechanism

can be changed from reaction-limited to diffusion-limited aggregation processes by increasing the ionic strength of the system (Ikeda et al., 1999). This effect of ionic strength was attributed to the shielding of charges on the interacting proteins, thereby increasing the aggregation probability and the dependence on diffusion of aggregating particles. Lowering the pH of the system may have a similar effect to increasing the ionic strength.

As reaction-limited and diffusion-limited processes give different aggregate structures, future work examining the microstructure and fractal dimensionality of the aggregates may give useful insights into structure–functionality relationships, and how this may be linked to aggregation mechanisms. Although these aggregation models and fractal dimensions are useful in defining aggregate structures and possible mechanisms, there are some limitations in their use, as they assume that the aggregation is irreversible, rigid and that no rearrangements can occur. This may not always be the case in biological systems, such as milk protein aggregates, where significant rearrangements may occur (Mellema et al., 2002; Vetier et al., 1997). These biological systems often display high experimental fractal dimensions, which may be due to extensive cluster rearrangements during aggregation or storage (Meakin & Jullien, 1988; Vetier et al., 1997).

5. Conclusions

This work shows that different textural properties could be obtained in processed cheese spreads containing whey proteins by varying the cook pH. Varying the cook pH in a processed cheese system containing whey proteins affected the whey protein denaturation and interactions to form different structures, which ultimately control the rheological and textural properties of the final product. In the samples where no whey proteins were present ($PC_{control}$), varying the cook pH did not affect the textural properties of these processed cheese spreads. It is therefore highly likely that the observed effect of cook pH on the rheological properties of the processed cheese spreads containing whey proteins (PC_{whey}) was due to the interaction of whey proteins with caseins. Under the right environment, whey proteins, particularly β-lactoglobulin, interact with the caseins to form a strong network structure, resulting in a firm and self-supporting processed cheese with relatively low protein concentrations and high moisture levels.

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