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Reducing the level of added disodium phosphate alters the chemical and physical properties of processed cheese

Timothy P. Guinee · Brendan T. O’Kennedy

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Abstract The intake of sodium in modern western diet is excessive, with potentially harmful effects on health. Consequently, there is a drive to reduce the sodium content of foods. A major source of sodium in processed cheeses (PCs) is the added emulsifying salts (sodium phosphates or citrates) which play a key role in product formation and stabilisation. The present study investigated the impact of reducing the level of disodium orthophosphate (DSP) on the properties of PC. PCs were formulated in triplicate with 0.35, 0.75, 1.29, 2.0, 2.7 or 3.4 g.100 g⁻¹ DSP, and standardised with respect to intact casein content, moisture, protein-to-fat ratio, calcium/casein ratio, and pH. DSP at ≥ 0.75 g.100 g⁻¹ was necessary to form a homogeneous, stable PC. Increasing DSP significantly increased the storage modulus (G'), firmness and fracture stress of the unheated PC, and reduced the flowability and fluidity (loss tangent max) of the heated PC. These changes coincided with a significant increase in the level of water-soluble protein (WSP) and reduction in the ratio of calcium to WSP in the water-soluble extract prepared using a PC/water at a ratio of 1:2. Our results indicate that there is scope to reduce the sodium level of PCs by reducing the level of added DSP to a minimum level (e.g., ≥ 0.75 g.100 g⁻¹ in current study), but that reduction from levels (grams per 100 grams) of 2.0–3.4 to 0.75–1.29 markedly alters the textural and cooking properties of the resultant PCs.

磷酸钠对再制干酪物化特性的影响

摘要 现代西方国家饮食中过量钠的摄入对人体健康造成了潜在的危害。因此很有必要减少食品中钠的含量。再制干酪中钠的主要来源是添加的乳化盐(磷酸钠或柠檬酸钠),这些钠盐对再制干酪的成型和稳定起着关键的作用。本文研究了降低磷酸氢二钠添加量对再制干酪特性的影响。再制干酪中磷酸氢二钠的添加量分别为0.35、0.75、1.29、2.0、2.7和3.4 g.100 g⁻¹,分别进行了三个重复,以酪蛋白含量、水分、蛋白与脂肪的比值、钙与酪蛋白的比值以及pH为评价标准。当磷酸氢二钠添加量 ≥ 0.75 g.100 g⁻¹时才能形成均一和稳定的

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再制干酪。增加磷酸氢二钠的添加量能够显著地增加未加热再制干酪的储能模量(G')、硬度和破裂应力,但是降低了加热再制干酪的流动性。在制干酪和水比例为1:2时提取的干酪液相中,这些性质的变化与水溶性蛋白含量的显著增加和钙与水溶性蛋白的比值下降相一致。实验结果表明通过减少磷酸氢二钠添加量来达到降低再制干酪中钠的含量是有限范围的(如本研究的范围是 $\geq 0.75 \text{ g}\cdot 100 \text{ g}^{-1}$)当磷酸氢二钠含量从 $2.0\sim 3.4 \text{ g}\cdot 100 \text{ g}^{-1}$ 降低到 $0.75\sim 1.29 \text{ g}\cdot 100 \text{ g}^{-1}$ 时,显著地改变了再制干酪的质地和烹饪特性。

Keywords Processed cheese · Disodium orthophosphate · Water-soluble extract · Rheology · Meltability

关键词 再制干酪 · 磷酸氢二钠 · 水溶性提取物 · 流变学 · 熔融性

1 Introduction

Cheesemaking procedures, especially for hard cheese varieties, ensure a dehydration of the casein from $\sim 2.7\text{--}3.0\%$ in the aqueous phase of milk to a concentration level which is in the intermediate moisture zone (39–40% casein in the aqueous phase). At this casein concentration (Bouchoux et al. 2009), the aqueous system is “jammed”, a term used to denote that the originally dispersed casein has now become a continuum where no bulk free (non-bound and non-entrapped) solvent is available to confer liquidity to the cheese. The casein concentration, whereby significant jamming or solid-like behaviour is observed rheologically, is dependent on the volume of solvent that the paracasein matrix can control. However, the voluminosity of the paracasein matrix is temperature dependant, decreasing with increase in temperature, and this principle is exploited during cheesemaking to change the properties of the final cheese (Horne 1998).

This decrease in the voluminosity of the paracasein matrix, on heating cheese (e.g., to $60\text{--}90\text{ }^\circ\text{C}$), can be observed as a reversal of the jamming phenomenon, whereby fluidity/liquidity is conferred on the cheese sample through the expulsion of solvent from the casein matrix (Dave et al. 2001; Pastorini et al. 2002) which increases the mobility of the cheese mass. This heat-induced reversal, which may be considered as the basis of the melting and/or stretching of cheese on heating, occurs to a degree dependent on various factors such as the protein-in-moisture concentration, the protein-to-fat ratio, and the strength of the links between the paracasein polymers as affected by pH and calcium level. Whilst cheese may melt at high temperatures (e.g., $80\text{--}100\text{ }^\circ\text{C}$) under quiescent conditions, its inability to emulsify the oil that is expressed during the heating step, even under shear, resulted in the development of emulsifying salts (ES) for the manufacture of heat-stable PC. ES play a key role in manufacture of PCs, by affecting the hydration of the protein (e.g., calcium phosphate para- κ -casein, or casein) from cheese or milk protein ingredients. The hydrated protein then emulsifies the fat released during the heating/shearing steps of the PC manufacturing process. The principal ES used in cheese processing are sodium phosphates, especially sodium orthophosphates such as disodium hydrogen phosphate (DSP, Na_2HPO_4), and sodium citrates, especially trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$).

The increase in global consumption of cheese products has been paralleled by a growth in demand for more consistency in relation to composition, quality, and

concentrations of certain components (e.g., sodium, Na), which when consumed in excessive quantities may adversely affect health. In particular, high levels of dietary sodium have been linked to hypertension and cardiovascular disease (He and McGregor 2007). The levels of Na in PCs (e.g., 1 to 1.5 g.100 g⁻¹ in retail PCs available on Irish market) is generally much higher than that of natural cheeses (e.g., ~0.7 and 0.26 g.100 g⁻¹ in Cheddar and Swiss cheeses, respectively). Whilst, the proportions of Na in PCs depend on the types and levels of added ingredients, ES are generally a major contributor. For a typical PC containing 1.1% (w/w) sodium and formulated from cheddar cheese (80%, w/w), butter (0.5%), ES (0.5% trisodium citrate dihydrate, 0.5% disodium hydrogen orthophosphate and 0.5% trisodium orthophosphate) and water (19.8%), the respective contributions of cheese and ES to the total Na are ~53% and 47%. Owing to their relatively high Na content, various approaches are used to reduce Na content of PCs, including reduction in ES content and the use of potassium phosphates/citrates; however, the latter approach is generally not favoured because of the risk of predisposing consumers to hyperkalemia (Evans 2005).

The current study examines the effect of altering the level of DSP (0.35% to 3.4%) on the physicochemical and rheological properties of PC; a minimum level of 0.35% was chosen, as the maximum level of added sodium phosphates permitted in generic cheese, designated as unripened cheese, is 0.35% (expressed as P₂O₅) (FAO/WHO 2007).

2 Materials and methods

2.1 Raw materials

Commercial cheddar cheeses, ranging in age from 4 to 6 weeks, were obtained from a local manufacturer. The cheese had mean pH, pH 4.6-soluble N, Ca and P values of ~5.34, 9.3 g.100 g⁻¹ of total N, 746 mg.100 g⁻¹, and 457 mg.100 g⁻¹, respectively. The following ingredients were used: disodium phosphate anhydrous (Albright and Wilson Lt., Cheshire, England), sodium chloride (Irish International Trading Corp. PLC, Cork, Ireland), deionized water, food grade lactic acid (80%, w/w; Biocel Ltd., Rockgrove, Little Island, Co. Cork, Ireland), hydrochloric acid (4 M, Biocel Ltd), and sodium hydroxide(30%, w/v; Micro-Bio, Ireland, Ltd., Industrial Estate, Fermoy, Co. Cork) .

2.2 Manufacture of PC

The formulations used for the different PC treatments are given in Table 1. Cheddar cheese was cut into cubes (~64 cm²), shredded (Hallde RG-350 machine, AB Hallde Maskiner, Kista, Sweden), and added (~2 kg) to the processing kettle (Stephan UMM/SK5; A. Stephan u. Söhne GmbH & Co., Hameln, Germany). Butter, the dry ingredients (ES and potassium sorbate) and water were then added on top of the cheese. Under standard processing conditions, the blend was agitated for 1 min, heated using indirect steam injection to 80 °C whilst continuously shearing at 2,400 rpm (usually ~1–1.5 min) and held for 1 min. Either lactic acid (80 g.100 g⁻¹), or NaOH (35 g.100 g⁻¹), was then added in sufficient quantities to regulate the pH to a target value of ~5.8–5.9. The levels required were determined in

Table 1 Formulation of processed cheeses with different levels of added DSP

Ingredients added (g.100 g ⁻¹)	Treatment of processed cheeses					
	0.35 DSP	0.75 DSP	1.29 DSP	2.0 DSP	2.7 DSP	3.4 DSP
Cheddar cheese	82.1	81.8	81.1	79.7	78.3	76.2
DSP	0.35	0.7	1.3	2.0	2.7	3.4
NaCl	0.2	0.2	0.2	0.2	0.2	0.2
Lactic acid (80 g.100 g ⁻¹)	0.0	0.0	0.07	0.37	0.77	1.17
NaOH (35 g.100 g ⁻¹)	0.4	0.1	0.0	0.0	0.0	0.0
Potassium sorbate	0.09	0.09	0.09	0.09	0.09	0.09
Water	16.9	17.0	17.3	17.7	18.2	19.0

0.75 DSP, 1.29 DSP, 2.0 DSP, 2.7 DSP, 3.4 DSP denote processed cheese formulations with 0.75, 1.29, 2.9, 2.7 and 3.4 g.100 g⁻¹ added disodium orthophosphate as emulsifying salt. Three replicate trials were undertaken, and the mean intact casein content of cheddar cheese was ~90.8; calcium level, ~815 mg.100 g⁻¹

preliminary trials, in which the quantity of lactic acid/NaOH was increased incrementally and subsamples (~40 g) were withdrawn after each increment, cooled and tested for pH after ~24 h. Following processing and pH adjustment, the hot molten PCs were hot-filled into 2 kg plastic containers and placed at 4 °C. Three separate trials, each with five different PCs corresponding to different levels of added DSP, were undertaken over a 2-week period. PCs made with 0.35, 0.75, 1.29, 2.7 and 3.4 g.100 g⁻¹ DSP were denoted as 0.35 DSP, 0.75 DSP, 1.29 DSP, 2.7 DSP and 3.4 DSP, respectively.

2.3 Analyses

2.3.1 Compositional analyses of cheddar cheeses

Cheddar cheese used for processing was analysed for protein, fat, moisture and Ca using standard IDF methods (Guinee et al. 2007), and for pH and pH 4.6-soluble N level, as previously described (Guinee and O'Kennedy 2009). The pH was measured on a slurry prepared from 20 g of grated PC and 12 g distilled water. The intact casein (IC) content was calculated by the following equation: IC=100-pH 4.6SN%.

2.3.2 Compositional analyses of PCs

PCs were analysed in triplicate for protein, fat, moisture, Ca, P, and pH, as described above. The water-soluble protein (WSP) level, which was used as an index of casein hydration/solubility, was measured on a homogenate prepared by blending grated PC (60 g) with warm water (120 g) at 55 °C using a stomacher (Stomacher, Lab-Blender 400; Seward Medical, London) for 5 min (Kuchroo and Fox 1982). The resultant homogenate was held at 40 °C for 1 h, centrifuged at 3,000 x g for 20 min at 4 °C (Mistral 3000i centrifuge; MSE Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG, England) to sediment the undissolved residue, and the filtrate was passed through glass wool to remove fat. The filtrate was denoted WSE and its N

content was determined by macro-Kjeldahl (Foss Tecator 2020 Digestor and Auto-sampler Analyser; Foss, Calmount Road, Ballymount, Dublin 12, Ireland). The WSP was expressed as grams per 100 grams filtrate, or as percent of total N in the cheese (WSP%), calculated according Eq. 1:

$$\text{WSP (\% total Protein)} = \frac{\frac{N_f}{100} \times [W_w + (\frac{C_m}{100}) + W_c] \times \frac{100}{W_c}}{P_c}$$

where %N_f is percent% nitrogen of the WSE (grams per 100 grams), W_w is weight of added water (g), C_m is moisture content of the PC (grams per 100 grams), W_c is weight of PC (g), and P_c is protein content of PC (grams per 100 grams). The pH 4.6 SN% gave a measure of the extent of proteolysis in the PCs.

The calcium and phosphorous contents of the WSE were measured by analysis of a filtrate prepared by mixing the WSE and trichloroacetic acid (15 g.100 g⁻¹) at a weight ratio of 1 to 4, and filtering the mixture through a Whatman No 42 filter paper. The phosphorous content was determined using the spectrometric method (IDF 1982) and calcium by atomic absorption spectrometric method (IDF 2007). Ca and P, as percent of total Ca and P, were calculated using the equation above, where Ca_f (calcium in WSE) and Ca_c (total calcium in PC) replaced N_f and P_c, respectively.

2.3.3 Rheological and functionality of PCs

Cube (25 mm) samples of PCs were compressed to 30% of original height at a rate of 60 mm.min⁻¹ on a TA-HDi Texture Profile analyzer (Stable Micro Systems, Vienna Court, Lammas Road, Godalming, Surrey GU7 1YL, England) located at room temperature (Guinee and O’Kennedy 2009). Six samples were taken from each PC, placed in an airtight plastic bag, and held at 4 °C overnight prior to compression. Fracture stress was defined as the force per unit area to induce fracture, fracture strain as the fractional displacement at fracture, and firmness as the force required to compress the cheese to 30% of its original height.

Heat-induced flowability was measured as the percentage increase in diameter on heating PC discs (45 mm diameter; 6 mm thick) at 280 °C for 4 min (Guinee and O’Kennedy 2009), according to the principle of the Schreiber test (Park et al. 1984), and as the percentage increase in length of a cylinder of PC (~15 g; diameter, 22 mm; length, 37.5 mm) on heating in an enclosed tube at 180 °C for 7.5 min (Guinee and O’Kennedy 2009), according to the principle of the Olson/Price test (Olson and Price 1958).

2.3.4 Heat-induced changes in viscoelasticity

The changes in viscoelasticity on heating cheese discs (40 mm diameter, 2 mm thick) from 25 to 80 °C were measured by low-amplitude strain oscillation in a controlled stress rheometer (CSL₅₀₀² Carri-Med; TA Instruments, Inc., New Castle, DE, USA), as described previously (Guinee and O’Kennedy 2009). The cheese discs were placed between two parallel serrated plates (40 mm diameter) of the rheometer cell, tempered at 25 °C for 15 min and subjected to an harmonic low-amplitude shear strain (γ) of 0.005 at an angular frequency of 1 Hz. The temperature was then increased

to 80 °C over 15 min, during which time the storage modulus (G'), loss modulus (G'') and phase angle (δ) were computed continuously. The loss tangent was calculated as the tangent of δ .

2.4 Statistical analysis

The data were analysed using a randomised complete block design which incorporated five treatment PCs (different DSP levels, 0.75–3.5 g.100 g⁻¹) and three blocks (replicate trials for treatments). As 0.35 g.100 g⁻¹ DSP was not sufficient to form a stable PC, no data for 0.35 DSP PC were included in the statistical analyses. Analysis of variance was carried out using a SAS procedure (SAS 2003), where the effect of treatment and replicates were estimated for all response variables. Duncan’s multiple comparison test was used as a guide for pair comparisons of the treatment means. The level of significance was determined at $P < 0.05$. Descriptive analysis was performed on one batch of samples and the data are included as observations only.

The data for some response variables were also analysed by linear regression to establish possible correlations between the response variables (e.g., salt-in-moisture and pH). The significance of correlations were determined by applying Students t test to r^2 with $n-2$ df where n is the actual number of data points, and df is the degrees of freedom.

3 Results and discussion

3.1 Observations during manufacture of PCs

The addition of DSP at 0.35% was insufficient to enable the formation of a stable PC, as reflected by the presence of large quantities of free water and oil in the formulation after the normal cooking period and which persisted even on extending the processing time to 8 min at 80 °C. It was likely that at this concentration, the added DSP did not raise the pH of the PC blend sufficiently to ensure adequate dissociation of the added DSP per se and chelation of calcium from the para- κ -casein of the cheese; hence, the casein was insufficiently hydrated to give emulsification of free fat released on heating/shearing. In contrast to the above, previous studies (Shirashoji et al. 2006, 2010) reported no problems in the manufacture of PC (pH ~ 5.55, moisture ~ 37.5 or 39 g.100 g⁻¹) when using trisodium citrate or sodium hexametaphosphate as ES at a level of 0.25 g.100 g⁻¹. The inter-study differences in the level of ES required for the satisfactory manufacture of PC may reflect differences in the type of cheese used (e.g., levels of calcium and intact casein), functionality of ES (e.g., degree of phosphorous polymerisation, ion exchange capacity) and/or processing conditions (e.g., time). Pre-clustering of phosphate groups in the form of hexametaphosphate or polyphosphates are analogous to a localised concentration of the active group, in this case phosphate. This localised increase in phosphate should have a synergistic effect on the affinity of phosphate for calcium bound to the casein, thereby exhibiting functional effects at lower overall phosphate levels.

Stable PC was formed at DSP levels $\geq 0.75\%$ (w/w); however, the 0.75 DSP PC was notably more liquid on completion of processing (80 °C, prior to packing/

cooling) than PCs formed with higher DSP levels (1.29–3.4 g.100 g⁻¹). The higher liquidity may reflect a lower degree of calcium chelation and, hence less water uptake and swelling of the protein particles which would be expected to favour a less constrained, more discontinuous matrix structure, and in turn a greater system mobility and liquidity.

3.2 Composition of PCs

The compositions of the PCs are shown in Table 2. The moisture content of all PCs was similar as formulations were designed to ensure fixed moisture content (Table 1). Consequently, the levels of fat and protein decreased significantly, by ~1.8 and 1.0 g.100 g⁻¹, respectively, as the level of added DSP was increased from 0.75 to 3.4 g.100 g⁻¹. Similarly, the increase in added DSP significantly reduced the level total calcium, by ~45 mg.100 g⁻¹, but the calcium-to-protein ratio remained unchanged since cheese was the only source of Ca in the PCs. In contrast, the level of P increased linearly at a rate of ~0.15 g.100 g⁻¹ for every 1 g.100 g⁻¹ of added DSP, which is slightly lower than expected based on the fact that P accounts for ~17.2% of the molecular mass of Na₂HPO₄·2H₂O. The pH of the PCs did not significantly differ, as lactic acid was added in increasing quantities to standardise the pH to ~5.85 as the level of added DSP was increased.

3.3 Composition of WSE

Compositional analysis of the serum, or juice, of natural cheese is frequently used to explain the effects of various parameters (e.g., maturation, calcium level, pH) on the microstructure and physical properties of cheese, e.g., mozzarella (Kindstedt and Guo 1998). However, studies at our institute and elsewhere (Shirashhoji et al. 2006) have

Table 2 Composition of experimental PCs made with different levels of DSP

Cheese composition	0.75 DSP	1.29 DSP	2.0 DSP	2.7 DSP	3.4 DSP
Moisture (g.100 g ⁻¹)	48.2 ^a	48.3 ^a	48.3 ^a	48.0 ^a	48.4 ^a
Fat (g.100 g ⁻¹)	26.2 ^a	25.8 ^{ab}	25.4 ^{ab}	25.1 ^c	24.4 ^d
Protein (g.100 g ⁻¹)	19.6 ^a	19.6 ^a	19.2 ^{ab}	18.8 ^b	18.6 ^b
Protein/fat	0.75 ^a	0.76 ^a	0.76 ^a	0.75 ^a	0.76 ^a
Ca (mg.100 g ⁻¹)	671 ^a	666 ^a	642 ^{ab}	635 ^b	626 ^b
Ca (mg.g ⁻¹ protein)	34.2 ^a	34.0 ^a	33.4 ^a	33.7 ^a	33.7 ^a
P (mg.100 g ⁻¹)	582 ^a	635 ^b	782 ^c	882 ^d	968 ^e
P (mg.g ⁻¹ protein)	29.7 ^a	32.4 ^b	40.7 ^c	46.8 ^d	52.2 ^e
pH	5.81 ^a	5.85 ^a	5.85 ^a	5.84 ^a	5.87 ^a
pH 4.6SN (g.100 g ⁻¹ total N)	9.8 ^a	8.9 ^a	9.0 ^a	9.5 ^a	9.1 ^a

0.75 DSP, 1.29 DSP, 2.0 DSP, 2.7 DSP, 3.4 DSP denote processed cheeses made with 0.75, 1.29, 2.9, 2.7 and 3.4 g.100 g⁻¹ added disodium orthophosphate as emulsifying salt. Values presented are the means of three replicate samples for each processed cheese; values within a row not sharing a common letter (a, b, c) differ significantly ($P < 0.05$). Ca, calcium; P, phosphorous; pH 4.6SN, pH 4.6-soluble nitrogen as a percentage of total nitrogen

found that it is generally not possible to express serum from PCs using methods normally applied to natural cheese, such as centrifugation at 12,500 \times g (Guo and Kindstedt 1995). Consequently, we extracted the PCs with water at a PC/water ratio of 1:2, and analysed the resultant WSEs to gain an insight on the effect of DSP level on the physical properties of the PCs per se. A PC/water ratio of 1:2 was chosen as Kuchroo and Fox (1982) reported that the level of water-soluble N extracted from cheddar cheese remained relatively constant as the cheese/water ratio was varied from 1:1 to 1:5, and used an extraction ratio of 1:2 for further studies. The relative constancy of WSP with cheddar cheese/water extraction ratio may be expected on consideration of the cheese as a constrained structure comprised of insoluble fused paracasein micelles that are cemented largely via calcium-, and calcium phosphate-induced crosslinks; this is especially the case in young cheese where little of the protein is hydrolysed. The WSP test involves extracting (or washing) this structure with excess water under defined conditions of temperature and shear prior to separating the sediment through centrifugation.

The compositions of the WSEs of the different PCs are shown in Table 3. The increase in soluble protein on processing, from ~11% in the cheddar cheese to ~76–79% in PCs with 2.0–3.4% DSP confirms previous findings (Guinee and O'Kennedy 2009). Protein solubilisation may be attributed to the perturbation of the cross-

Table 3 Composition of water-soluble and water-insoluble extracts of experimental PCs made with different levels of disodium orthophosphate (DSP)

Composition	0.75 DSP	1.29 DSP	2.0 DSP	2.7 DSP	3.4 DSP
Water-soluble cheese extract (WSE)					
Protein (g.100 g ⁻¹)	2.8 ^c	5.1 ^b	6.1 ^a	5.9 ^{ab}	6.1 ^{ab}
Protein (% of total protein)	34.7 ^b	69.0 ^a	78.7 ^a	77.2 ^a	76.7 ^a
Ca (mg.100 g ⁻¹)	47.0 ^a	45.3 ^a	31.3 ^{ab}	27.4 ^b	32.4 ^{ab}
Ca (% of total Ca)	17.7 ^a	17.5 ^a	12.2 ^a	11.9 ^a	12.9 ^a
Ca/protein ratio (mg.g ⁻¹ protein)	18.2 ^a	8.9 ^b	5.2 ^b	4.7 ^b	5.2 ^b
P (mg.100 g ⁻¹)	27.9 ^d	51.2 ^{cd}	81.3 ^c	123.7 ^b	170.5 ^a
P (% of total P)	12.1 ^d	20.0 ^{cd}	25.8 ^{bc}	35.0 ^{ab}	43.9 ^a
P (mg.g ⁻¹ protein)	10.8 ^c	10.2 ^c	13.4 ^{bc}	21.1 ^{ab}	28.4 ^a
Ca/P (M)	1.31 ^a	0.69 ^b	0.29 ^c	0.17 ^c	0.15 ^c
Water-insoluble cheese extract (WISE)					
Protein (g.100 g ⁻¹)	24.6 ^a	13.3 ^b	7.9 ^c	8.3 ^c	6.8 ^c
Ca/protein ratio (mg.g ⁻¹ protein)	43.9 ^c	83.2 ^{bc}	141.7 ^{ab}	133.7 ^{ab}	172.2 ^a
Ca (mg.100 g ⁻¹)	1,107 ^a	1,069 ^a	1,089 ^a	1,083 ^a	1,109 ^a
P (mg.100 g ⁻¹)	992 ^a	981 ^a	1,123 ^a	1,107 ^a	1,054 ^a
Ca/P (M)	0.86 ^a	0.84 ^a	0.75 ^a	0.76 ^a	0.80 ^a

0.75 DSP, 1.29 DSP, 2.0 DSP, 2.7 DSP, 3.4 DSP denote processed cheeses made with 0.75, 1.29, 2.9, 2.7 and 3.4 g.100 g⁻¹ added disodium orthophosphate as emulsifying salt. The water-soluble extracts were prepared from homogenates obtained by blending processed cheese and water at a weight ratio of 1 to 2. Values presented are the means of three replicate samples for each processed cheese; values within a row not sharing a common letter (a, b, c, d) differ significantly ($P < 0.05$). Ca, calcium; P, phosphorous; Ca/P (M), molar ratio of Ca to P

linkages between the paracasein micelles, as confirmed by the removal of most of the calcium and inorganic phosphate from the paracasein (Table 3). This would be expected to give a less constrained structure in which most of the protein is potentially water-soluble when the level of DSP is sufficiently high (e.g., $\geq 1.29 \text{ g} \cdot 100 \text{ g}^{-1}$).

Based on the protein content of the PCs ($\sim 18.6\text{--}19.6 \text{ g} \cdot 100 \text{ g}^{-1}$) and its expression as concentration in the moisture phase ($\sim 29 \text{ g} \cdot 100 \text{ g}^{-1}$; Table 2), the dilution step during the preparation of the WSE achieves a potential maximum of $\sim 7.3\%$ protein in the aqueous phase which may be in a “soluble” or non-sedimentable form. However, the actual protein content (grams per 100 grams) in the extracted WSEs was lower than the calculated maximum but increased significantly with added DSP from ~ 2.8 (35% of total protein) in 0.75 DSP PC to ~ 6.0 ($\sim 75\%$ of total protein) in 2.0 DSP PC, and remained relatively constant with further increases in DSP (Table 3, Fig. 1). This result raises a number of questions: why at all levels of DSP in the range 0.75 to $3.4 \text{ g} \cdot 100 \text{ g}^{-1}$ is some protein water-soluble and some not? and why did the level of WSP not increase as the level of DSP was raised from 2.0 to $3.4 \text{ g} \cdot 100 \text{ g}^{-1}$, but did as DSP is increased from 0.75 to $2.0 \text{ g} \cdot 100 \text{ g}^{-1}$? An insight may be obtained by consideration of the method of PC manufacture (Table 1). The protocol suggests that two different aqueous solvent systems are involved, especially at the early stages of processing; these include the ‘internal’ solvent (cheese serum) within the natural cheese and the ‘external’ solvent comprised of water and dissolved solutes (e.g., emulsifying salt, disodium phosphate) added to the cheese during processing. The internal cheese solvent is constrained within the paracasein micelles, which have become significantly deswelled and dehydrated during the cheesemaking process. The interactions between the paracasein micelles (calcium crosslinks, ionic and hydrophobic forces) make re-swelling in the presence of added water irreversible under the normal conditions. Similarly, rennet casein, which may be considered as a dehydrated skim milk cheese, is insoluble in water owing to the relative high

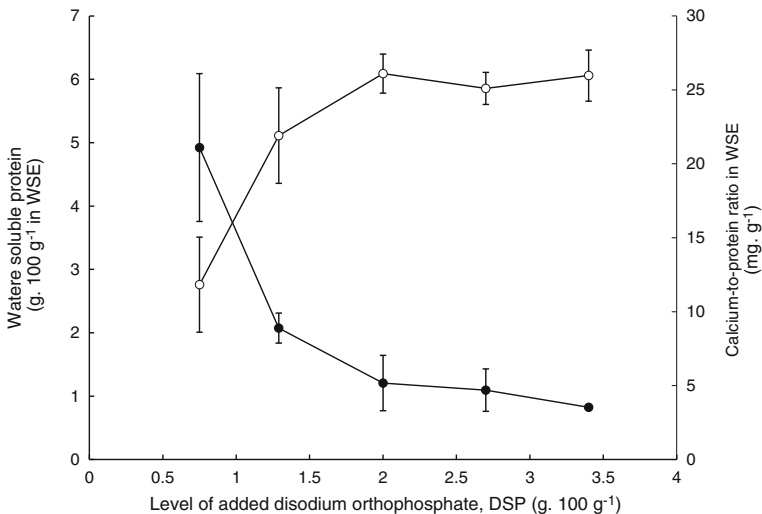


Fig. 1 Effect of disodium orthophosphate level on the concentration of water-soluble protein (*open circle*) and ratio of calcium to protein (*filled circle*) in the water-soluble cheese extracts prepared with a processed cheese/water ratio of 1:2

hydrophobicity of the para- κ -casein (Swaisgood 2003). This is significant from a cheese dispersion viewpoint where both aqueous solvent systems (internal solvent of natural cheese and added external solvent) have to equilibrate over a period of ~ 3 min during processing. When shredded cheese, added water and disodium phosphate are added to the processing kettle, a number of possible scenarios could coexist. The disodium phosphate could fully dissolve in the added water giving, in the case of 2.0 DSP, an initial phosphate concentration of $\sim 720 \text{ mmol.L}^{-1}$, or it could partially dissolve and remain as powder in various states of hydration on the surfaces of the shredded cheese. In either setting, the concentration of phosphate in the external solvent at the beginning of processing will be orders of magnitude higher than that in the internal solvent, and higher than that present when equilibration throughout the solvent system approaches the calculated concentration of $\sim 280 \text{ mmol.L}^{-1}$. It is likely that a critical molarity of DSP in the moisture phase is the driving force for protein solubilisation. Hence, variation in DSP concentration at a micro-level within the forming PC mass prior to equilibration is likely to be a factor for having both water-soluble and water-insoluble protein at all DSP levels.

In agreement with previous studies (Guinee and O’Kennedy 2009; Shirashoji et al. 2010), most ($>80\%$) of the Ca in the PCs was water-insoluble and this level increased significantly as the level of DSP increased. Coinciding with the increase in protein content of the WSE, the calcium-to-protein ratio decreased significantly with DSP, especially as the level was increased from 0.75 to $\geq 1.29 \text{ g.}100 \text{ g}^{-1}$ (Fig. 1). The difference in calcium-to-protein ratio suggests that whilst extracted soluble protein may be considered loosely as “soluble”, it is likely to consist of structural entities (para- κ -casein aggregates) varying in calcium phosphate content and size, all of which remain stable to centrifugal sedimentation at the low g forces ($3,000\times g$) applied during the preparation of the WSE. Variation in Ca/casein ratio of the soluble protein is likely to alter the voluminosity of the aggregates in the native serum phase of the PC and, hence, their contribution to rheology and cooking properties of the PC. In contrast to the trend for Ca, the concentration of water-soluble P and the ratio of P to protein in the WSE increased linearly as DSP was increased (Table 3). Hence, the molar ratio of Ca to P in the WSE decreased with increasing DSP (Fig. 2).

As a corollary to the trend noted for the WSE, increasing the level of DSP significantly reduced the level of protein and increased the calcium-to-protein ratio in the water-insoluble cheese extract (WISE; Table 3). However, the concentrations of total insoluble Ca and P, and the molar ratio of Ca to P (0.84–0.75) in the WISE did not change significantly with DSP level (Fig. 2). This suggests that the stoichiometric ratio of calcium to phosphorous in the insoluble calcium phosphate complex formed is unaffected by DSP level under the current processing conditions and that excess P remains in the WSE; hence, linear regression indicated a strong positive correlation between DSP level and concentration of P in the WSE ($r=0.99$).

3.4 Effect of ratio of PC-to-water ratio on composition of WSE

On extraction with a PC/water ratio of 33.3:66.7, $\sim 80\%$ of the protein in the PC was water-soluble. Nevertheless, protein that is extracted in the WSE may not be ‘soluble’ in the native, undiluted PC as the concentrations of the paracasein, Ca and P in the aqueous

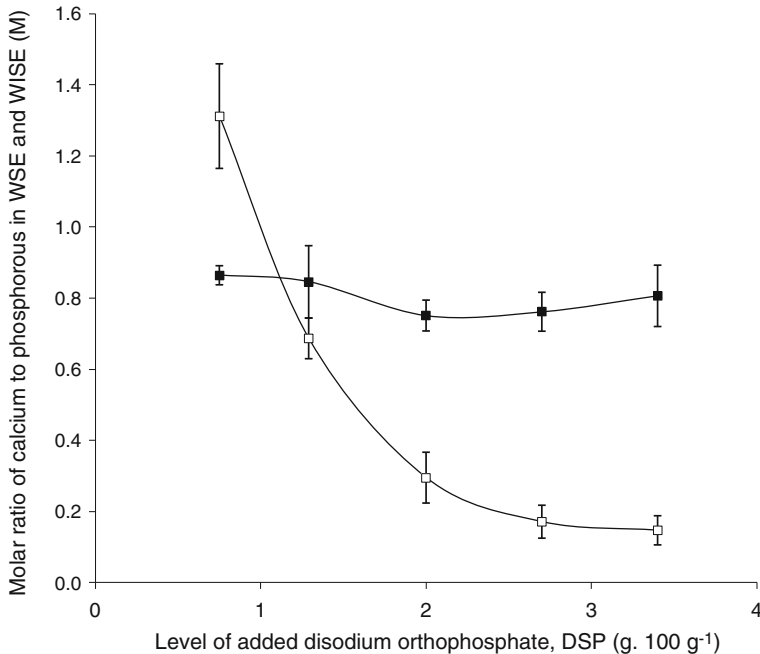


Fig. 2 Effect of disodium orthophosphate level on the molar ratio of calcium to phosphorous in the water-soluble extracts (WSE) prepared with a processed cheese/water ratio of 1:2 (open square) and the resultant water-insoluble extracts (WISE; filled square)

phase of the PC are higher and may contribute to structures (e.g. aggregates and matrix). Such structures could affect the rheology of the heated and unheated PC. Therefore, interpretation based on dilute WSEs may obscure the real effects of different variables, such as DSP level, on the physical properties of the PC. PCs made with relatively low ($1.29 \text{ g} \cdot 100 \text{ g}^{-1}$; LDSP) and high ($3.4 \text{ g} \cdot 100 \text{ g}^{-1}$ HDSP) levels of DSP were, therefore, extracted with PC/water ratios $>33.3:66.7$ to simulate the serum phase of the PC more closely and at ratios $<33.3:66.7$ to determine the potential extractability of protein, Ca and P.

Increasing PC/water ratio markedly reduced the actual volume of WSE, especially in the case of the HDSP PC, which yielded no WSE when the PC/water ratio was increased to 64.7:35.3 (Table 4). The latter result clearly indicates that the PC/water macerate of the 3.4 DSP PC was too viscous to separate on centrifugation, possibly because of the high level of protein structuring the water phase. Similarly, Shirashoji et al. (2006) obtained no WSE on using a 50:50 PC/water extraction ratio for PCs made with trisodium citrate at concentrations $\geq 1.5 \text{ g} \cdot 100 \text{ g}^{-1}$. The inter-study difference in the PC/water ratio at which no WSE was obtained is probably attributable to differences in level of 'solubilised' protein in the cheese serum as affected by the type of ES and the characteristics of the natural cheese (e.g., levels of calcium and intact casein). The concentration of protein in the WSE increased linearly as the PC/water ratio increased, reaching $\sim 12.2 \text{ g} \cdot 100 \text{ g}^{-1}$ at a ratio of 57:43 (Table 4). The consistently lower protein level in the HDSP PC may reflect a higher viscosity and a higher degree of fat emulsification. This would lead to more of the 'solubilised' protein being on the

Table 4 Effect of varying the extraction ratio of cheese to water on the composition of water-soluble extracts of processed cheeses made with relatively low (LDSP) or high (HDSP) levels of disodium orthophosphate

Extraction ratio (Cheese/water)	DSP level	9.1:89.9	16.7:83.3	33.3: 66.7	50:50	57.1:42.9	64.7: 35.3
Actual volume of WSE (mL.100 g ⁻¹ cheese + water)	LDSP	96.8	94.6	90.2	81.6	69.4	75.8
	HDSP	94.2	87.6	67.8	43.0	30.6	0
Predicted volume of WSE (mL.100 g ⁻¹ cheese + water)	LDSP	95.4	91.4	82.8	74.2	70.4	64.6
	HDSP	95.2	91.4	82.6	74.0	70.2	64.4
Protein (g.100 g ⁻¹)	LDSP	1.8	3.3	6.9	11.4	12.5	13.1
	HDSP	1.4	2.5	5.9	9.4	12.2	–
Ca (mg.100 g ⁻¹)	LDSP	17	25	28	40	90	353
	HDSP	9	13	19	19	221	–
Ca (mg.g ⁻¹ protein)	LDSP	9.5	7.6	4.0	3.5	7.2	–
	HDSP	6.6	5.2	3.2	2.0	18.2	26.9
P (mg.100 g ⁻¹)	LDSP	16	24	29	40	41	94
	HDSP	48	87	173	252	219	–

The water-soluble extracts were prepared from homogenates obtained by blending processed cheeses and water at different ratios. Values presented are the means of three replicate samples for each processed cheese; values within a row not sharing a common letter (a, b, c) differ significantly ($P < 0.05$)

LDSP and HDSP 1.27 and 3.4 g.100 g⁻¹ added disodium orthophosphate, Ca calcium, P phosphorous

surface of smaller fat globules, which are expected to be largely separated from the aqueous phase during the preparation of the WSE. The lower volume (which is indicative of a higher viscosity) and lower protein level of the HDSP WSE at PC/water ratios $>50:50$ suggest that the protein contributes to a higher degree of structuring and immobilisation of the aqueous phase of the HDSP PC than in the LDSP PC.

The Ca content of the WSEs of both PCs remained relatively constant as the PC/water ratio was increased to 50:50 and thereafter rapidly increased. The trend suggests that the WSEs were sufficiently liquid (non viscous) to allow sedimentation of the insoluble calcium phosphate complexes (inclusions) at PC/water ratios of $\leq 50:50$, but too viscous at ratios $>50:50$. A different trend was noted for P content, which essentially increased progressively in the WSEs of both PCs as the PC/water ratio was increased, but at a higher rate in the HDSP PC. The pattern is consistent with the concomitant increase in the content of water-soluble P (not involved in the formation of calcium phosphate complexes) as the quantity of cheese increases.

3.5 Functional properties of PCs

The functional properties of the PCs are shown in Table 5 and Fig. 3.

Table 5 Rheological and cooking properties of PCs made with different levels of disodium orthophosphate (DSP)

	0.75 DSP	1.29 DSP	2.0 DSP	2.7 DSP	3.4 DSP
Rheological properties on unheated cheese					
Fracture strain (–)	0.86 ^a	0.84 ^a	0.75 ^a	0.76 ^a	0.80 ^a
Fracture Stress (kPa)	52.0 ^b	45.1 ^b	65.3 ^{ab}	100.8 ^a	94.8 ^a
Firmness (N)	76.8 ^b	92.9 ^a	94.2 ^a	98.7 ^a	101.1 ^a
Cooking properties of heated cheese					
Flow Schreiber Method (%)	75.3 ^a	75.4 ^a	38.0 ^b	8.2 ^c	4.5 ^c
Flow Olson/Price Method (%)	404 ^a	388 ^a	189 ^b	15 ^c	10 ^c
Viscoelastic properties in heating from 25 to 78 °C					
$G'_{25\text{ °C}}$ (kPa)	19.5 ^b	14.1 ^b	13.8 ^b	21.5 ^{ab}	29.6 ^a
LT_{max} (–)	2.12 ^a	1.79 ^a	0.96 ^b	0.69 ^b	0.49 ^b
$G'_{78\text{ °C}}$ (Pa)	65 ^c	61 ^c	410 ^{bc}	1121 ^{ab}	1595 ^a

0.75 DSP, 1.29 DSP, 2.0 DSP, 2.7 DSP, 3.4 DSP denote processed cheeses made with 0.75, 1.29, 2.9, 2.7 and 3.4 g.100 g⁻¹ added disodium orthophosphate as emulsifying salt. Values presented are the means of three replicate samples for each processed cheese; values within a row not sharing a common letter (a, b, c) differ significantly ($P < 0.05$). $G'_{25\text{ °C}}$ and $G'_{78\text{ °C}}$, storage modulus at 25 and 78 °C, respectively; LT_{max} , highest value of loss tangent (tan of phase angle between stress and strain)

3.5.1 Rheology of unheated PCs

Increasing DSP level significantly increased the fracture stress, fracture strain and firmness of the unheated PC (8 °C). This effect may reflect an increased degree of fat emulsification (Rayan et al. 1980; Savello et al. 1989), as a consequence of the higher level of WSP, reduction in the calcium-to-casein ratio, and lower degree of casein aggregation. The emulsifying capacity of casein is generally inversely related to its content of calcium or calcium phosphate, which determine the degree of casein structuring and dimensions of resultant particles/aggregates and its efficiency in stabilising oil-in-water emulsions (Mulder and Walstra 1974). The change in rheological properties with increasing DSP may also reflect a higher degree of structuring and immobilisation of the aqueous phase by the protein; results from WSEs (Tables 3, 4) showing lower WSE volumes and lower calcium/protein ratios at the higher DSP levels would support this suggestion.

The values for fracture stress and firmness for all PCs were in the same order of magnitude as those previously reported for PCs and determined under similar conditions (Guinee and O'Kennedy 2009). Compared to natural cheeses such as cheddar and low-moisture part-skim mozzarella that are frequently used in similar ingredient applications as PCs (e.g., slices for sandwiches, shredded cheese for salads, toppings in dishes such as pizza and quiche), the range of fracture stress and firmness values are notably lower, e.g., low-moisture part-skim mozzarella cheese (moisture content of ~48 g.100 g⁻¹) and cheddar with typical respective firmness values of ~200–500 N and 200–400 N, and fracture stress values of 300–150 kPa (cheddar; Fenelon and Guinee 2000; Guinee et al. 2001). The generally lower values of the latter

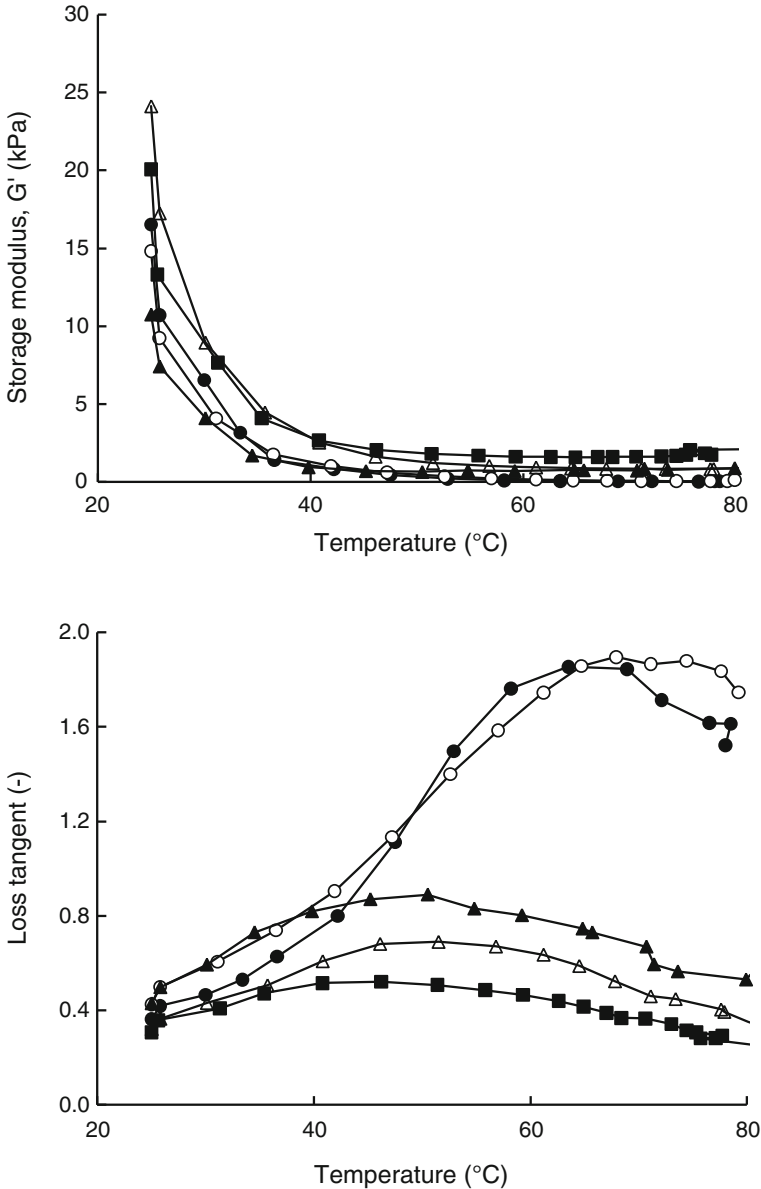


Fig. 3 Changes in the viscoelastic characteristics of processed cheese heated from 25 to 80 °C, as influenced by level ($\text{g}\cdot 100\text{ g}^{-1}$) of added disodium orthophosphate: 0.75 (filled circle), 1.3 (open circle), 2.0 (filled upright triangle), 2.7 (open upright triangle) and 3.4 (filled square)

parameters in PCs may be attributed *inter alia* to their lower protein content, their lower content of calcium attached to the para- κ -casein, higher level of WSP, the more uniform distribution of fat, and finer protein matrix (Auty et al. 2001; Kaláb et al. 1987; Marchesseau et al. 1997).

3.5.2 Flowability and viscoelastic changes on heating

The flowability of the 0.75 DSP and 1.29 DSP PCs were similar and significantly higher than that of the 2.0 DSP PC, which in turn was higher than that of the 2.7 DSP and 3.4 DSP PCs (Table 5). These trends are consistent with those of Brickley et al. (2008) who found that flow at 60 °C generally decreased with increasing DSP level in the range 0.5 to 4 g.100 g⁻¹, to a degree dependent of type of cheese base used in formulation and pH of final PC. The reduction in meltability as DSP increased could reflect an increase in degree of fat emulsification (Rayan et al. 1980; Savello et al. 1989) and a greater degree of immobilisation and structuring of the aqueous phase. A higher degree of fat emulsification would give greater fat surface area and a higher number of casein-covered, emulsified fat particles, which increase the effective protein concentration of, and enhance the structural integrity of, the PC matrix (Marchesseau et al. 1997). It was noteworthy, that surfaces of the 2.7 DSP and 3.4 DSP PCs tended to be very dry and scorched (with little evidence of free fat) following heating, in contrast to the 0.75 DSP and 1.29 DSP PCs which were moist and had a desired surface sheen that was indicative of sufficient oil release during cooking.

A typical profile of the changes in viscoelasticity of the different PCs on heating from 25 to 80 °C is shown in Fig. 3. Heating of all PCs resulted in a continual decrease in storage modulus, G' . This effect was most pronounced in the temperature range 25 to 40–45 °C, where liquefaction of fat is occurring, and then much more slowly with further heating to 80 °C. In contrast, the loss tangent (LT), $\tan \delta$, which may be considered as an index of the fluidity of the melted cheese mass, increased to a maximum value (LT_{\max}) at 45–65 °C and thereafter tended to decrease. The temperature-induced increase in LT_{\max} may be attributed to the combined effects of fat liquefaction and protein interaction, e.g., by more extensive hydrophobic bonding (Bryant and McClements 1998). The resultant shrinkage of the protein matrix and the release of water from the proteins would favour greater mobilisation of the protein matrix in the melting PC mass. Pastorino et al. (2002) reported that heating of non-fat mozzarella cheese from 10 to 50 °C resulted in an increase in protein aggregate size and suggested that this was likely to be associated with an increase in the hydrophobic-induced re-association of β -casein and calcium in the cheese serum with other proteins in the matrix.

The subsequent decrease in LT, which has been frequently reported for natural cheeses and PCs (Guinee and O'Kennedy 2009; Shirashoji et al. 2010), may reflect moisture loss from the sample on prolonged heating, and or heat-induced structural changes in the sample such as a possible formation of a calcium phosphate like structure throughout the heated cheese mass (Relyveld 1977) or increased protein aggregation mediated by calcium phosphate complexes and/or through alteration in the proportions of different interactions (hydrophobic or electrostatic) (Bryant and McClements 1998; Jeurnink and de Kruif 1995; Marchesseau and Cuq 1995). Analogously, prolonged holding of processed PC formulations at high temperature during manufacture results in the resultant PCs having a higher degree of protein aggregation (as reflected by the presence of thicker, more electron-dense protein strands, Kaláb et al. 1987), higher firmness, and lower flowability (melting; Glenn et al. 2003; Rayan et al. 1980; Shirashoji et al. 2006). Commercially, the physical

changes observed on prolonged holding are referred to as ‘creaming’, a term that was used originally to signify a thicker, creamier texture in high-moisture PC spreads.

Increasing DSP significantly reduced LT_{\max} and increased the value of G' at 80 °C (G'_{80}) (Table 5), indicating a decrease in the fluidity of the melted cheese; linear regression analysis showed significant relationships between DSP level and the latter parameters, with LT_{\max} decreasing by ~ 0.65 and G'_{80} increasing by 0.65 Pa, respectively per 1 g.100 g⁻¹ increase in added DSP. Similar effects were previously reported on increasing the level of ES (sodium hexametaphosphate or trisodium citrate) in PCs from 0.25 to 2.75 g.100 g⁻¹ (Shirashoji et al. 2006, 2010).

4 Conclusion

The effect of varying the level of added DSP on the properties of PC was studied. A critical minimum concentration of ~ 0.75 g.100 g⁻¹ DSP was required to ensure the manufacture of a stable product; at a lower DSP level (0.35 g.100 g⁻¹), the heated/sheared cheese exuded free oil and water at the end of processing. The level of water-soluble protein in the PC, as determined from a water-soluble extract prepared using a cheese/water ratio of 1:2, increased from 35% to 75% of total protein as added DSP was increased from 0.75 to 2.0 g.100 g⁻¹ and remained relatively constant thereafter. Increasing DSP significantly increased the fracture stress and firmness of the unheated cheese, and reduced the degree of flow and fluidity of the melted PCs, with the effects being most pronounced as the DSP was increased to ≥ 2.0 g.100 g⁻¹. These changes, as added DSP was increased, are likely to be associated with the concomitant increase in the water solubility of the protein, which would favour increases in degree of fat emulsification and viscosity of the aqueous phase. In contrast, a reduced degree of swelling and solubilisation of the protein at the lower DSP levels (0.75 and 1.27 g.100 g⁻¹), would support a more liquid aqueous phase and greater mobility of the melted cheese. Hence, it is noteworthy that a number of studies have shown that added phosphates can result in gelation of micellar casein suspensions (Fox et al. 1965; Mizuno and Lucey 2007; Panouillé et al. 2004). However, these studies were on dilute casein concentrations (~ 5 – 10 g.100 g⁻¹), which were not rennet-treated, making extrapolation to concentrated, PC structures difficult.

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