



# Different additives to enhance the gelation of surimi gel with reduced sodium content



Deysi Cando, Beatriz Herranz, A. Javier Borderías, Helena M. Moreno \*

Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), José Antonio Novais 10, 28040 Madrid, Spain

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## ABSTRACT

This study tested the effect of adding tetra-sodium pyrophosphate, cystine and lysine as surimi gelation enhancers (*Alaska Pollock*) in order to reduce the sodium content of gels up to 0.3%. These gels were compared with others that contained 3% NaCl content (the amount typically used for surimi processing). To induce protein gelation, gels were first heated and then set at 5 °C/24 h. Once the physicochemical and rheological properties of the gels were determined, cystine and lysine were found to be the most effective additives improving the characteristics of low NaCl surimi gels. The action of these additives is mainly based on the induction of myofibrillar protein unfolding thus facilitating the formation of the types of bonds needed to establish an appropriate network. It was found that a setting period was needed for gel processing to maximize the effect of the additives.

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## 1. Introduction

Alaska Pollock has been extensively utilized in the surimi industry, 220,000 MT being produced in 2012. Half of this production is high-grade surimi (SA, FA and A), most of which is sold to Japan to make Kamaboko and other high-quality surimi products such as different seafood analogues and others. Lower grades (KA, KB and RA) are sold to the United States and Europe for crabstick production and to Japan and Korea for fried products and for the production of other surimi-based products (Park, 2014).

In the creation of any sort of surimi based product, the gelation process is the most important step in achieving the desired texture, which is directly related to myofibrillar protein functionality (Duangmal & Taluengphol, 2010). Sodium chloride (NaCl) at a concentration of 1–3% is needed to facilitate protein solubilization resulting in gel (Kim & Park, 2008). In order to manufacture healthier products in line with the NAOS strategy (NAOS Strategy, 2005), producers face the challenge of reducing NaCl content in the product, which is no easy task considering the technological

implications of the gelation process, i.e. myosin protein gelation always requires prior myosin solubilization with salt (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014). Consequently, several authors have studied the use of different kinds of ingredients to improve protein gelation. In this study three additives, tetra-sodium pyrophosphate, cystine and lysine, were selected as gelation enhancers to solubilize proteins in the presence of low amounts of NaCl. The action of these additives is controversial as we report below: phosphates are commonly used in surimi gelation to dissociate protein complexes, enhancing gel-forming ability (Matsukawa, Hirata, Kimura, & Arai, 1995). Although phosphate compounds have proven promising as a processing agent, they most likely have a detrimental effect on gel properties as they may chelate the  $\text{Ca}^{2+}$  ion. This could impede the setting of surimi induced by endogenous transglutaminase (Julavittayanukul, Benjakul, & Visessanguan, 2006). Cystine is a weak oxidant that maximizes the formation of cross-linkages (Chen, Chow, & Ochiai, 1999) and, as such, can be considered a candidate to improve surimi properties. Lysine is a polyaminoacid contributing to the formation of covalent crosslinks between the  $\epsilon$ -amino group and the  $\gamma$ -carboxamide group of glutamyl residues of adjacent proteins when acting as a substrate of transglutaminase

\* Corresponding author.

E-mail address: [hmoreno@ictan.csic.es](mailto:hmoreno@ictan.csic.es) (H.M. Moreno).

(Dickinson, 1997). Moreover, lysine at neutral pH is positively charged, as is arginine, but other aminoacids such as glutamic and aspartic acid are negatively charged. An ionic attraction, called a salt bridge, may occur between these groups, resulting in the formation of intra- and intermolecular bonds. Moreover, Liu, Kanoh, and Niwa (1995) reported that L-lysine in Alaska Pollock surimi suppressed the polymerization of the myosin heavy chain by inhibiting endogenous transglutaminase activity.

The main objective of this study was to study the effect and suitability of adding tetra-sodium pyrophosphate, cystine and lysine to surimi dough to improve the gelation process of low NaCl content surimi gels formed after heating and different setting conditions.

## 2. Materials and methods

### 2.1. Raw materials

Grade KA Alaska Pollock surimi (*Theragra chalcogramma*), supplied by the enterprise Angulas Aguinaga (Guipuzcoa, Spain) in 20-kg frozen blocks, was used to create the gels. Sodium chloride (Panreac, Quimica, S.A.; Barcelona, Spain) was added to solubilize the surimi protein.

The additives tested as gelation enhancers were: tetra-sodium pyrophosphate (Panreac, Quimica, S.A. Barcelona, Spain), cystine (Merck KGaA, Darmstadt, Germany) and lysine (Panreac, Quimica, S.A.; Barcelona, Spain).

### 2.2. Proximate analysis

Composition of raw surimi was determined in quadruplicate. Ash ( $0.52 \pm 0.12\%$ ), fat ( $0.30 \pm 0.01\%$ ), crude protein ( $15.33 \pm 0.53\%$ ) and moisture content ( $75.99 \pm 0.11\%$ ) of Alaska Pollock were determined according to AOAC (2000). Crude protein content was measured by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). The rest, approximately  $7.85 \pm 0.15\%$ , was cryoprotectant (a mixture of sucrose and sorbitol) and polyphosphates that are always added to surimi to protect protein from damage during freezing.

### 2.3. Sample preparation

Alaska Pollock surimi was homogenized under vacuum and refrigeration conditions using a homogenizer at 1500 rpm/10 min (Stephan UMC 5, Stephan Machinery, Germany) with two different NaCl concentrations: 0.3% NaCl (Lot A) and 3% NaCl (Lot B). Different doughs were formed with these two NaCl levels by adding cystine (C), tetra-sodium pyrophosphate (P) and

L-lysine (L) in different proportions as shown in Table 1. Gel moisture was adjusted to 76% and each surimi dough was stuffed into a 35 mm Krehalon casing (Amcor group Flexibles Hispania S.L., Barcelona, Spain).

Different batches were obtained for each ingredient (C, P and L) and were classified according to the treatment applied after being stuffed. Gelation was performed in two ways: by heating at 90 °C/30 min (Lot Q) followed by a setting period at 5 °C/24 h and heating at 90 °C/30 min (Lot SQ). A further two samples were also made: “surimi dough” (SD), i.e. the dough obtained after homogenization with the ingredients and “suwari gels” (SG) obtained after a setting period at 5 °C/24 h. These last two samples were studied to determine the initial state of the protein before heat-induced gelation.

### 2.4. Experimental design

The study was designed as a factorial design (including the additives and the different percentages of each them) and with two different treatments (Q and SQ) for each of the two lots, i.e. Lot A (0.3% NaCl) and B (3% NaCl) as shown in Table 1. The study was based on the hypothesis that different additives can serve as protein gelation enhancers resulting in different gel textures as previously reported by different authors (Lee, Lee, Chung, & Lavery, 1992; Chen et al., 1999; Julavittayanukul et al., 2006).

The mechanical properties of the gels (Table 2) determined by a Puncture test were the initial screening parameters used to select the most convenient concentration of different additives. After statistical analysis (Section 2.11), samples with additives from Lot A were chosen on the basis of a significantly higher value in mechanical properties than their corresponding control samples (AQ or ASQ) (Table 2). In the case of significant values with the same additive, the sample with a lower concentration of the additive was chosen. The physicochemical and rheological properties of the selected gels were then studied. Lot B samples were studied for the purpose of being compared with Lot A samples.

### 2.5. Differential scanning calorimetry

Thermal behavior of the myosin of surimi dough (SD), and suwari gels (SG) after a setting period (5 °C/24 h) was monitored using a differential scanning calorimeter (DSC Q1000, TA Instruments, New Castle, USA). Samples were placed in hermetically sealed aluminum pans. The approximate sample weight was around 10 mg as determined by an electronic balance (Sartorius ME235S, Goettingen, Germany). The samples were scanned in triplicate at 10 °C/min from 5 to 110 °C under a dry nitrogen purge at 50 mL/min. Second scans were recorded after cooling (30 °C/min) down to 5 °C to check

**Table 1**  
Coding of each sample with a different combination of additives, salt content and temperature treatment.

Ingredients	Ingredient (%)	Definitive (Q) (90 °C/30 min)		Definitive after setting (SQ) (5 °C/24 h + 90 °C/30 min)	
		0.3% NaCl (Lot A)	3.0% NaCl (Lot B)	0.3% NaCl (Lot A)	3.0% NaCl (Lot B)
Controls	–	AQ	BQ	ASQ	BSQ
Cystine (C)	0.05	CA005-Q	CB005-Q	CA005-SQ	CB005-SQ
	0.1	CA01-Q	CB01-Q	CA01-SQ	CB01-SQ
	0.2	CA02-Q	CB02-Q	CA02-SQ	CB02-SQ
Tetra-sodium pyrophosphate (P)	0.05	PA005-Q	PB005-Q	PA005-SQ	PB005-SQ
	0.1	PA01-Q	PB01-Q	PA01-SQ	PB01-SQ
	0.2	PA02-Q	PB02-Q	PA02-SQ	PB02-SQ
Lysine (L)	0.05	LA005-Q	LB005-Q	LA005-SQ	LB005-SQ
	0.1	LA01-Q	LB01-Q	LA01-SQ	LB01-SQ
	0.2	LA02-Q	LB02-Q	LA02-SQ	LB02-SQ

**Table 2**

Mechanical properties of the gels subjected to initial screening.

Samples	Breaking force (N)		Breaking deformation (mm)	
	Q	SQ	Q	SQ
A	2.21 ± 0.05	3.47 ± 0.13	6.17 ± 0.41	6.61 ± 0.21
CA005	2.35 ± 0.14	3.75 ± 0.39	6.41 ± 0.42	7.12 ± 0.37
CA01	2.94 ± 0.22 <sup>*</sup> CAQ	4.78 ± 0.29 <sup>*</sup> CASQ	6.91 ± 0.29 <sup>*</sup> CAQ	7.71 ± 0.32 <sup>*</sup> CASQ
CA02	2.30 ± 0.18	4.10 ± 0.51 <sup>*</sup>	6.85 ± 0.38 <sup>*</sup>	7.62 ± 0.44 <sup>*</sup>
PA005	2.50 ± 0.26 <sup>*</sup> PAQ	3.74 ± 0.12 <sup>*</sup> PASQ	6.59 ± 0.19 <sup>*</sup> PAQ	8.63 ± 0.46 <sup>*</sup> PASQ
PA01	2.16 ± 0.12	4.10 ± 0.54 <sup>*</sup>	6.44 ± 0.43	8.05 ± 0.39
PA02	2.09 ± 0.14	3.50 ± 0.15	6.01 ± 0.33	8.16 ± 0.15
LA005	2.55 ± 0.27	3.44 ± 0.15	5.85 ± 0.26	7.28 ± 0.51
LA01	2.87 ± 0.14 <sup>*</sup> LAQ	4.49 ± 0.13 <sup>*</sup> LASQ	6.86 ± 0.22 <sup>*</sup> LAQ	8.13 ± 0.15 <sup>*</sup> LASQ
LA02	2.17 ± 0.17	4.01 ± 0.36 <sup>*</sup>	6.54 ± 0.54	7.48 ± 0.55 <sup>*</sup>
B	1.64 ± 0.13	4.19 ± 0.53	7.07 ± 0.44	10.11 ± 0.33
CB005	1.71 ± 0.13	4.75 ± 0.25	8.11 ± 0.85 <sup>*</sup>	10.65 ± 0.23
CB01	2.72 ± 0.10 <sup>*</sup> CBQ	4.88 ± 0.13 <sup>*</sup> CBSQ	9.42 ± 0.32 <sup>*</sup> CBQ	11.01 ± 0.25 <sup>*</sup> CBSQ
CB02	2.02 ± 0.28 <sup>*</sup>	3.93 ± 0.35	8.95 ± 0.49 <sup>*</sup>	10.30 ± 0.48
PB005	2.03 ± 0.26 <sup>*</sup> PBQ	4.21 ± 0.21 PBSQ	8.69 ± 0.46 <sup>*</sup> PBQ	10.55 ± 0.30 PBSQ
PB01	1.95 ± 0.12 <sup>*</sup>	4.67 ± 0.11	8.35 ± 0.40 <sup>*</sup>	10.65 ± 0.08
PB02	2.05 ± 0.40 <sup>*</sup>	4.49 ± 0.26	8.73 ± 0.31 <sup>*</sup>	10.57 ± 0.19
LB005	2.86 ± 0.16 <sup>*</sup>	5.04 ± 0.26 <sup>*</sup>	8.62 ± 0.32 <sup>*</sup>	10.08 ± 0.15
LB01	2.15 ± 0.52 <sup>*</sup> LBQ	4.00 ± 0.16 LBSQ	8.62 ± 0.29 <sup>*</sup> LBQ	10.18 ± 0.08 LBSQ
LB02	2.04 ± 0.53	4.40 ± 0.19	7.92 ± 0.40	10.45 ± 0.27

<sup>\*</sup> Indicates values significantly higher than the corresponding control, A or B ( $p < 0.05$ ). See Table 1 for samples code.

for residual/new effects. The water content of each individual sample was determined by desiccation at 105 °C to normalize thermal data to dry matter content. Temperature,  $T_{\text{peak}}$  (°C) and enthalpy of transition  $\Delta H$  (J/g) were determined for each sample.

## 2.6. Fourier transform infrared spectroscopy

Changes that occurred in the protein secondary structure of surimi dough (SD) and suwari gels (SG) were examined by Fourier transform infrared spectroscopy. Spectra between 4000 and 650  $\text{cm}^{-1}$  were recorded using a Perkin-Elmer Spectrum 400 Infrared Spectrometer (Perkin-Elmer Inc., Waltham, MA, USA) equipped with an ATR prism crystal accessory. The spectral resolution was 4  $\text{cm}^{-1}$ . Measurements were performed at room temperature using approximately 1 mg of each gel sample, which was placed on the surface of the ATR crystal, and pressed with a flat-tip plunger until spectra till suitable peaks were obtained. All experiments were performed in triplicate on surimi doughs (SD) and suwari gels (SG) after setting, of Lots A and B, in order to discern how protein structure influenced the physicochemical properties of the gels due to changes in their secondary structures. To increase spectral resolution, a second-derivative spectrum was determined, in which the minimum peak corresponds to the maxima intensity region in the original spectrum. Background interference was eliminated using the Spectrum software version 6.3.2 (Perkin-Elmer Inc.).

## 2.7. Determination of sulfhydryl group content

Determination of sulfhydryl groups was carried out on selected gels (Q and SQ) according to the method described by Ellman (1959) using Ellman's buffer (Tris-HCl 50 mM, NaCl 0.6 M, EDTA 6 mM, Urea 8 M, SDS 2% pH 8) and DTNB solution (5,5'-dithiobis-2-nitrobenzoic acid 0.01 M in sodium acetate 50 mM) with small variations as described by Cando, Moreno, Tovar, Herranz, and Borderías (2014). All determinations were carried out in triplicate and the results were expressed in terms of micromoles of sulfhydryl per grams of protein.

## 2.8. Dynamic rheometry measurements

Dynamic oscillatory measurements were carried out immediately after subjected the surimi pastes were subjected to high

hydrostatic pressure (HPH) treatment using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). In order to study the protein gelation profile, approximately 1 g of each surimi paste was placed on the lower plate and the higher plate was set with a gap of 1 mm. Then, the samples were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. Heating was performed from 15 to 85 °C at a scan rate of 1 °C/min using a Peltier element. The frequency was fixed at 0.1 Hz and the strain at  $\gamma = 0.5\%$  (within the LVE range).

Storage ( $G'$ ) and loss ( $G''$ ) moduli data were collected every 2 min during dynamic oscillatory measurements. Each measurement was the mean of three replicates.

Prior to the temperature sweep, stress sweeps were conducted to determine the linear viscoelastic (LVE) region. The stress sweeps were run at 6.28 rad/s and the shear stress ( $\sigma$ ) of the input signal varied from 10 to 3000 Pa at 25 °C.

## 2.9. Water binding capacity (WBC)

Approximately 2 g of each selected surimi gel (Q and SQ) was cut into small pieces and placed in a centrifuge tube ( $\varnothing = 10$  mm) with 2–3 pieces of filter paper as an absorber (Whatman n° 1  $\varnothing = 90$  mm). The samples were centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10 min at 3000g at room temperature. WBC was expressed as percentage of water retained per 100 g water present in the sample prior to centrifuging (Moreno, Cardoso, Solas, & Borderías, 2009). All determinations were carried out in triplicate.

## 2.10. Mechanical properties

The puncture test was carried out at room temperature (25 °C) over Q and SQ selected gels (diameter 35.0 mm, height 30.0 mm) up to breaking point. Puncturing was performed using a 5 mm diameter, cylindrical stainless steel plunger attached to a 50 N cell connected to the crosshead on a TA-XT plus Texture Analyser (Texture Technologies Corp., Scarsdale, NY, USA). From force-deformation curves derived at 1.0  $\text{mm s}^{-1}$  crosshead speed, breaking force (BF) and breaking deformation (BD) were determined. The measurements were carried out in sextuplicate.

### 2.11. Statistical analysis

In order to select the initial samples, one-factor ANOVA analysis was carried out with the SPSS® computer programme (SPSS Inc., Chicago, IL, USA) and differences were evaluated by Tukey Test using a 95% confidence interval. This way, only the samples with significantly higher values than the control samples were selected.

One-factor ANOVA analysis with SPSS® computer programme was also used to evaluate differences between samples by Tukey Test using a 95% confidence interval.

## 3. Results and discussion

### 3.1. Initial screening and selection of the additives for the rest of the study

As mentioned in Section 2.4, mechanical properties of the gels were used to select the most appropriate concentration of the additives in the dough for the creation of low NaCl content gels. Values marked with an asterisk in Table 2 correspond to the gels that contained the lowest concentration of an additive and showed significantly higher values of breaking force (BF) and breaking deformation (BD) when compared with the corresponding control gel without any additives (AQ, ASQ, BQ or BSQ). Samples featuring significantly higher values were: cystine 0.1%, pyrophosphate 0.05%, and lysine 0.1%. Selected samples of Lot B are the same as in Lot A. The objective behind selecting the same samples in Lot B is to have a control, in order to compare low NaCl samples with a sample of regular NaCl concentration with the same formulation. As the results of Table 2 indicated, the ability of increasing BF and/or BD is more evident in Lot B than in Lot A, which is to be expected due to the fact that protein in Lot B is properly solubilized, thus more reactive groups are exposed and able to react both with the additives and within proteins. In both cases, the gel network is reinforced and supports the results (Table 2).

### 3.2. Chemical structure

#### 3.2.1. Differential scanning calorimetry

Changes induced by ingredients can lead to significant conformational changes of myosin inducing protein unfolding, resulting in better functional and gel properties (Park, 2005).

Fig. 1a–d shows the different DSC thermograms of SD and SG samples from Lot A and B. DSC thermograms of heated induced gel samples are not shown because heating totally denatured the myosin. The endothermic peak thermograms showing the thermal transition of protein, and the peak areas representing the enthalpy of this transition, were recorded to monitor protein evolution over a gradient of rising temperature. Thus, a decrease in the peak area or the disappearance of specific peaks implies a loss in protein structural stability (Qiu, Xia, & Jiang, 2014).

In all thermograms, the transition peak appearing at approximately  $50 \pm 2$  °C corresponds to myosin without any significant differences based on NaCl content or ingredients added (Fig. 1a–d).

The denaturation enthalpy of myosin is higher in Lot A than in Lot B (Fig. 1c and d) both for surimi dough (Fig. 1a) and suwari gels (Fig. 1b) except in sample LASG. That fact suggests a more native structure in Lot A due to less solubilized myosin and thus less protein unfolding. Moreover, in both, ASD and ASG samples, the introduction of additives resulted in reduced denaturation enthalpy of myosin when compared with the control sample. This indicates that myosin denaturation or unfolding takes place when ingredients are added because the effect of the NaCl alone is tested in the samples without additives and was reported to be lower compared to Lot B. That last result also supports the fact that stronger

networks were obtained from a rheological point of view when additives were introduced as reported in Section 3.3.4 (Fig. 3a and b). That effect is particularly evident in the suwari sample of Lot A with lysine (Fig. 1c – LASG) in which a clear unfolding of the protein is observed.

In Lot B, proteins were unfolded sufficiently due to the higher NaCl concentration (3.0% NaCl) as indicated Fig. 1b and d. That amount of NaCl has been previously reported to be required in order to solubilize myosin (Park, 2005). According to the results, it seems that unfolding of myosin is mainly due to NaCl. The effect of additives upon myosin solubilization and unfolding should not be underestimated as observed in Lot A, but also as indicated by the results of Table 2, because breaking force and breaking deformation increases by the addition of these additives. So, it appears that the first and more remarkable stage of myosin solubilization is due to NaCl followed by the additives effect. Therefore the effect of the additives in Lot B was not as relevant as in Lot A, but still noteworthy. Moreover, no important differences were detected in the setting process (5 °C/24 h) in Lot B compared to SD and SG possibly because the protein is unfolded due to the NaCl content (Liu, Gao, Ren, & Zhao, 2014). In summary, additives helped to unfold the protein molecules in Lot A in which the low NaCl content did not provide enough ionic strength to unfold the actomyosin by itself.

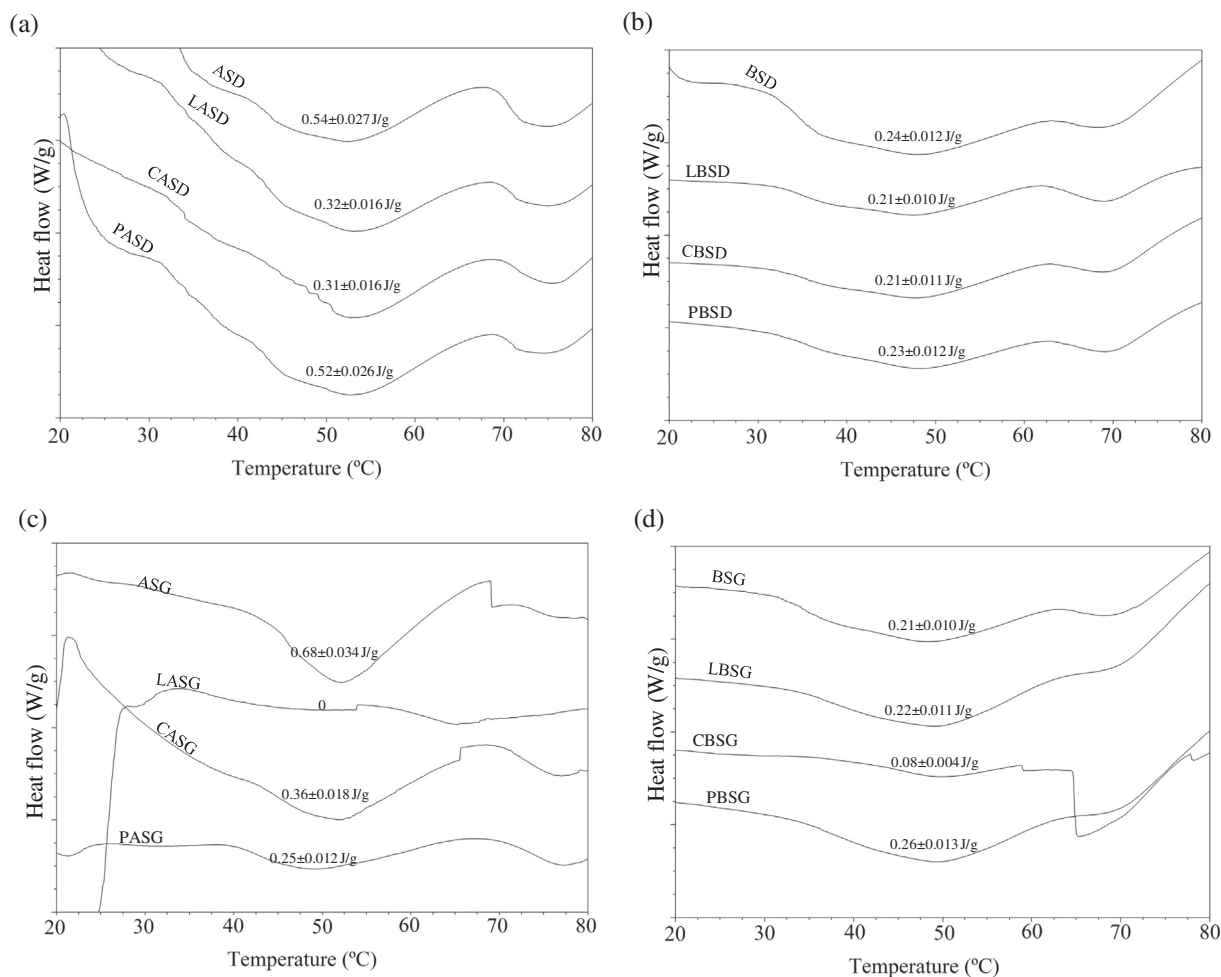
#### 3.2.2. Fourier transform infrared spectroscopy (FTIR)

The study focused on the Amide I region ( $1700\text{--}1600\text{ cm}^{-1}$ ) as this is the most sensitive spectral region for detecting changes in secondary protein structure. Moreover, the second derivative of the spectra (Fig. 2a–d) was calculated in order to enhance spectral resolution and gain insight into changes related to the secondary structure (Kong & Yu, 2007).

As shown in Fig. 2a, Lot A surimi dough spectra are quite similar irrespective of the additive used. This could be due to the fact that the additives did not come into contact with reactive groups of the proteins because of their native structure (less unfolded) as was observed for DSC. In Lot B surimi dough (Fig. 2b), the most unfolded structure is characterized by a loss of definition in  $\alpha$ -helix bands ( $1655 \pm 5\text{ cm}^{-1}$ ) and an increase in definition of  $\beta$ -sheet bands ( $1695 \pm 2$ ,  $1685 \pm 1$ ,  $1630 \pm 2$  and  $1618 \pm 3\text{ cm}^{-1}$ ) particularly when cystine (CBSD) and pyrophosphate (PBSD) were added (Fig. 2b). This would suggest a more unfolded protein structure after the addition of cystine and pyrophosphate, which could indicate an improved predisposition of the proteins to form a good gel because the formation of  $\beta$ -sheets occurred simultaneously with the unfolding of  $\alpha$ -helical structures during the gelation process and  $\beta$ -sheets are involved in the formation of a more ordered network with a higher density of cross-links as previously reported in the thermal gelation profile (Section 3.2.1) (Bouraoui, Nakai, & Li-Chan, 1997; Sánchez-González et al., 2008). Moreover, when comparing Lot A and B surimi dough (Fig. 2a and b), the higher definition of  $\beta$ -sheet bands in Lot B ( $1695 \pm 2$ ,  $1685 \pm 1$ ,  $1630 \pm 2$  and  $1618 \pm 3\text{ cm}^{-1}$ ) as a result of increasing NaCl content, is due to protein solubilization and the unfolding of the protein that brings about an increase in  $\beta$ -sheet components at the expense of  $\alpha$ -helix structure as occurred during protein gelation.

In Lot A suwari samples, ingredients were kept at 5 °C for 24 h and some additives induced modifications in the secondary protein structure (Fig. 2c). The introduction of additives resulted in a markedly lower definition of the  $\alpha$ -helix band ( $1655 \pm 5\text{ cm}^{-1}$ ) indicating protein aggregation when compared with the control sample (ASG). As previously reported (Damodaran, 1996), the  $\alpha$ -helix structure is mainly stabilized by hydrogen bonds between the carbonyl oxygen ( $-\text{CO}$ ) and the amino hydrogen ( $\text{NH}-$ ) of a polypeptide chain. Also, electrostatic interactions occur between amino acids during setting, contributing to structure stability.





**Fig. 1.** Thermograms obtained by differential scanning calorimetry (DSC) of surimi dough of Lot A ((a) ASD: Lot A surimi dough control, LASD: Lot A surimi dough with lysine, CASD: Lot A surimi dough with cystine, PASD: Lot A surimi dough with pyrophosphate) and Lot B ((b) BSD: Lot B surimi dough control, LBSD: Lot B surimi dough with lysine, CBSD: Lot B surimi dough with cystine, PBSD: Lot B surimi dough with pyrophosphate); suwari gels of Lot A ((c) ASG: Lot A suwari gel control, LASG: Lot A suwari gel with lysine, CASG: Lot A suwari gel with cystine, PASG: Lot A suwari gel with pyrophosphate) and Lot B ((d) BSG: Lot B suwari gel control, LBSG: Lot B suwari gel with lysine, CBSG: Lot B suwari gel with cystine, PBSG: Lot B suwari gel with pyrophosphate).

Lot B suwari gels exhibited a similar trend to that observed in Lot A. As was observed in surimi doughs (BSD) (Fig. 2a and b), Lot B suwari gels (BSG) (Fig. 2c and d) also exhibited higher definition of  $\beta$ -sheet bands ( $1695 \pm 2$ ,  $1685 \pm 1$ ,  $1630 \pm 2$  and  $1618 \pm 3 \text{ cm}^{-1}$ ), as a result of increasing NaCl content, likewise due to protein solubilization and subsequent unfolding of the protein.

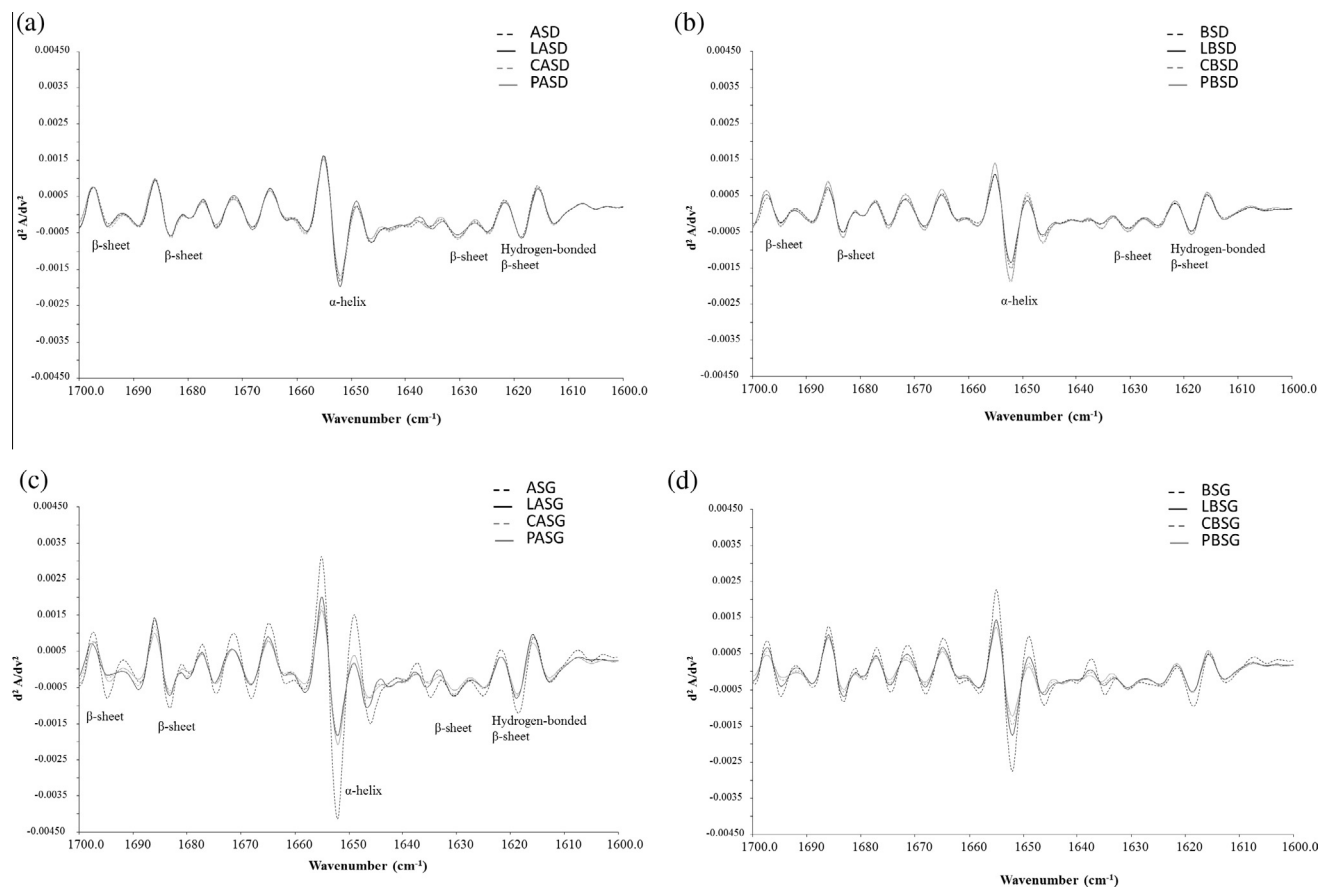
In summary, sufficient setting time is needed to allow the additives to bring about enough denaturation changes in Lot A to induce better gels after the heating process. Denaturation in Lot B, with a more open structure due to higher NaCl content, is quicker and more profound. Moreover, the use of additives when protein is unfolded as in the case of Lot B, results in a higher presence of the  $\beta$ -sheet necessary for proper gelation.

### 3.2.3. Determination of sulfhydryl group content

Sulfhydryl groups (SH) make a significant contribution to the gelation of proteins when, by oxidation, S–S groups are formed both within the same protein and between proteins (cross-linking) (Lund, Heinonen, Baron, & Estévez, 2011). Generally, oxidation is a detrimental process because proteins lose functionality but, on the other hand, during the induced gelation process intermolecular disulfide bonds are formed by the oxidation of two cystine molecules on neighboring protein chains with a reactive

sulfhydryl group (–SH), the result being enhanced gel properties (Park, 2005).

For that reason, sulfhydryl group analysis was performed on heat-induced gels made without setting (Q) and after setting (SQ) of Lots A and B. Table 3 shows the changes in free sulfhydryl content when the different additives were introduced to produce the gels. In Lot A, both in Q and SQ gels, the addition of pyrophosphate and lysine induced a significant decrease in free SH groups when compared with the control samples (AQ and ASQ) indicating a reductant effect of the additives on the proteins inducing oxidation of the latter and formation of the disulfide bonds that stabilized the gel structure (Lin & Park, 1998; Shahidi & Botta, 1994). A peculiar effect was observed following the addition of cystine since its structure contains S–S groups. These groups can be reduced, inducing the oxidation of the proteins and thus releasing two cystine molecules with reactive SH groups. For that reason, the amount of SH dramatically increased when cystine was added (Table 3). The effects of adding the ingredients to Lot B were very similar to those observed in Lot A in the Q group of samples. However, in Lot SQ a significantly higher presence of SH groups was observed as compared to control samples, suggesting that, hypothetically, fewer S–S groups were formed in those samples. The oxidation is a very complex process influenced by different factors. For



**Fig. 2.** Second derivative of FTIR spectra of surimi dough of Lot A ((a) ASD: Lot A surimi dough control, LASD: Lot A surimi dough with lysine, CASD: Lot A surimi dough with cystine, PASD: Lot A surimi dough with pyrophosphate) and Lot B ((b) BSD: Lot B surimi dough control, LBSD: Lot B surimi dough with lysine, CBSD: Lot B surimi dough with cystine, PBSD: Lot B surimi dough with pyrophosphate); suwari gels of Lot A ((c) ASG: Lot A suwari gel control, LASG: Lot A suwari gel with lysine, CASG: Lot A suwari gel with cystine, PASG: Lot A suwari gel with pyrophosphate) and Lot B ((d) BSG: Lot B suwari gel control, LBSG: Lot B suwari gel with lysine, CBSG: Lot B suwari gel with cystine, PBSG: Lot B suwari gel with pyrophosphate).

**Table 3**  
Results of different analyses performed over selected gels.

Samples	Sulphydryl groups content ( $\mu\text{mol}/10^3 \text{ g protein}$ )		WBC (%)		BF (N)		BD (mm)	
	Q	SQ	Q	SQ	Q	SQ	Q	SQ
AAQ/ASQ	$5.04 \pm 0.52^{c,1}$	$2.14 \pm 0.21^{c,2}$	$56.44 \pm 1.38^{d,2}$	$62.82 \pm 1.17^{e,1}$	$2.21 \pm 0.05^{b,2}$	$3.47 \pm 0.13^{d,1}$	$6.17 \pm 0.41^{c,1}$	$6.61 \pm 0.21^{e,1}$
CAQ/CASQ	$7.53 \pm 0.08^{b,1}$	$7.50 \pm 0.11^{b,1}$	$73.97 \pm 1.74^{b,2}$	$79.96 \pm 1.50^{bc,1}$	$2.94 \pm 0.22^{a,2}$	$4.78 \pm 0.29^{a,1}$	$6.71 \pm 0.50^{c,2}$	$7.71 \pm 0.32^{d,1}$
PAQ/PASQ	$2.00 \pm 0.15^{e,1}$	$1.70 \pm 0.05^{d,2}$	$78.05 \pm 1.39^{a,2}$	$85.38 \pm 0.90^{a,1}$	$2.40 \pm 0.26^{ab,2}$	$3.74 \pm 0.12^{c,1}$	$6.59 \pm 0.19^{c,2}$	$8.63 \pm 0.46^{c,1}$
LAQ/LASQ	$2.85 \pm 0.07^{d,1}$	$1.75 \pm 0.12^{d,2}$	$75.36 \pm 1.03^{b,2}$	$81.60 \pm 0.76^{b,1}$	$2.87 \pm 0.14^{a,2}$	$4.49 \pm 0.13^{ab,1}$	$6.56 \pm 0.22^{c,2}$	$8.13 \pm 0.15^{cd,1}$
BQ/BSQ	$1.44 \pm 0.06^{f,1}$	$0.59 \pm 0.04^{f,2}$	$67.56 \pm 1.20^{c,1}$	$68.72 \pm 1.21^{d,1}$	$1.64 \pm 0.13^{c,2}$	$4.19 \pm 0.53^{abc,1}$	$7.07 \pm 0.44^{c,2}$	$10.11 \pm 0.33^{b,1}$
CBQ/CBSQ	$8.33 \pm 0.02^{a,2}$	$8.99 \pm 0.17^{a,1}$	$80.09 \pm 0.51^{a,1}$	$82.11 \pm 2.15^{b,1}$	$2.72 \pm 0.1^{a,2}$	$4.88 \pm 0.13^{a,1}$	$9.42 \pm 0.32^{a,2}$	$11.01 \pm 0.25^{a,1}$
PBQ/PBSQ	$0.75 \pm 0.03^{g,2}$	$0.98 \pm 0.03^{e,1}$	$76.67 \pm 2.04^{ab,2}$	$81.61 \pm 1.80^{b,1}$	$2.03 \pm 0.26^{b,2}$	$4.21 \pm 0.21^{b,1}$	$8.69 \pm 0.46^{b,2}$	$10.55 \pm 0.3^{ab,1}$
LBQ/LBSQ	$1.28 \pm 0.09^{f,1}$	$1.09 \pm 0.09^{e,2}$	$76.23 \pm 2.41^{ab,2}$	$79.10 \pm 1.45^{c,1}$	$2.15 \pm 0.52^{ab,2}$	$4.00 \pm 0.16^{b,1}$	$8.62 \pm 0.29^{b,2}$	$10.18 \pm 0.08^{b,1}$

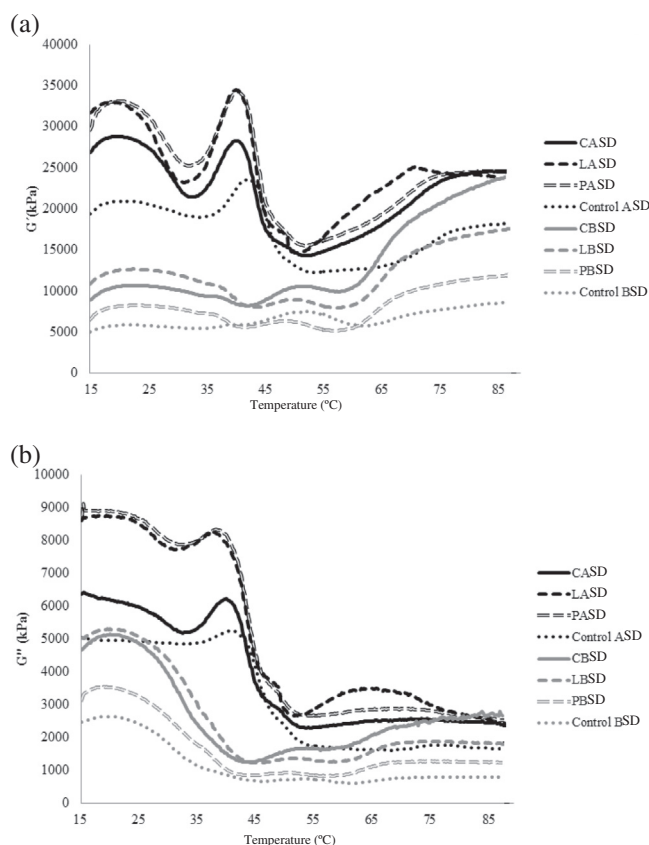
Letters (a–f) indicate significance between different formulations (A, CA, PA, LA, B, CB, PB, LB) for the same kind of gel (Q, SQ). Numbers (1–2) indicate significance between different kinds of gel (Q, SQ) for the same additive ( $p < 0.05$ ). WBC: water binding capacity, BF: breaking force, BD: breaking deformation.

that reason, it is not easy to explain the reason for the increase in SH groups in Lot B. It is hypothesized that the increase might not be correlated with S–S formation but with the greater or lesser exposure of sulphydryl groups, due to the higher solubilization produced by the NaCl addition. Comparison of the presence of SH groups in control samples of Lot A and B showed a higher presence in A than in B which means lower subsequent formation of S–S. The difference between samples from Lots A and B following the introduction of additives is not remarkable, indicating that these additives induce the formation of S–S bonds probably because, as has been previously reported, the protein molecule of Lot B is more

unfolded thus leaving more reactive groups exposed to form new bonds.

### 3.2.4. Dynamic rheometry measurements

Thermal gelation profiles of Lot A and Lot B surimi dough in terms of storage ( $G'$ ) and viscous moduli ( $G''$ ) formed in the 15–85 °C range are important in describing the thermal gelation behavior of the gels (Fig. 3a and b). Both viscoelastic moduli ( $G'$  and  $G''$ ) are parameters that characterize the formation of a gel protein network and its configuration. Storage modulus ( $G'$ ) is a measurement of the energy stored by the protein network, thus



**Fig. 3.** Dynamic rheometry measurements. Thermal gelation profiles from 15 to 85 °C of surimi dough studied by thermal gelation behavior of storage ( $G'$ ) of Lot A and B ((a) ASD: Lot A surimi dough control, LASD: Lot A surimi dough with lysine, CASD: Lot A surimi dough with cystine, PASD: Lot A surimi dough with pyrophosphate) and Lot B (BSD: Lot B surimi dough control, LBSD: Lot B surimi dough with lysine, CBSD: Lot B surimi dough with cystine, PBSD: Lot B surimi dough with pyrophosphate); and viscous moduli ( $G''$ ) of surimi dough of Lot A and B ((b) ASD: Lot A surimi dough control, LASD: Lot A surimi dough with lysine, CASD: Lot A surimi dough with cystine, PASD: Lot A surimi dough with pyrophosphate) and Lot B (BSD: Lot B surimi dough control, LBSD: Lot B surimi dough with lysine, CBSD: Lot B surimi dough with cystine, PBSD: Lot B surimi dough with pyrophosphate).

representing the solid-like nature or network-forming ability, while viscous modulus ( $G''$ ) measures the energy dissipated as heat, thus representing the viscous proportion (Egelandsdal, Martinsen, & Autio, 1995).

Both types of surimi dough from Lot A and Lot B (ASD and BSD) exhibited the characteristic four-stage progression in the thermal gelation profiles of  $G'$  and  $G''$  (Fig. 3a and b, respectively): (1) “softening”, (2) “first heat gelation”, (3) “gel weakening” and (4) “second heat gelation” or “gel strengthening” (Chen & Huang, 2008). It is worth noting that all these stages were much more marked in the dough with the low amount of salt (Lot A) than in Lot B, irrespective of the additive used. This coincides with the results observed by DSC and FTIR analysis indicating more solubilized myosin in Lot B, resulting in a more “open network” because the myosin molecule is less unfolded and/or denatured. Thus, samples of Lot A (0.3% NaCl) showed higher  $G'$  and  $G''$  values than those of Lot B throughout the entire temperature range, especially from 15 to 45 °C owing to the more native protein structure. In the softening stage of dough A,  $G'$  decreased to ~33 °C (Fig. 3a). From that point up to 43 °C,  $G'$  and  $G''$  increased reaching a peak: “first maximum peak”, related to preliminary protein network formation by weak interactions, consisting of mainly hydrogen bonds (Lefèvre, Fuconneau, Ouali, & Culioli, 1998; Qiu, Xia, & Jiang, 2013). The fact that  $G''$  also increased (Fig. 3b) reflects the relatively high fluidity of

this preliminary network and that it is no longer an elastic, well-formed final network. This network fluidity is not observed in samples with higher salt content (Lot B) whose “first heat gelation” stage is characterized by a slight increase of  $G'$  (up to ~50 °C) but with  $G''$  barely changing until the end of the heating. As already mentioned in Section 3.3, this is due to a more unfolded structure in Lot B samples, because of the higher amount of salt leading to a complete unfolding and/or denaturation of the myosin and exposure of its free SH groups and hydrophobic groups and aminoacids, permitting the formation of stronger bonds such as disulfide and non-disulfide covalent bonds and hydrophobic interactions (Ding et al., 2011). Furthermore, the formation of covalent crosslinks could have also taken place between the  $\epsilon$ -amino group and the  $\gamma$ -carboxamide group of glutamyl residues of adjacent proteins as a consequence of endogenous transglutaminase action (Dickinson, 1997). This compact and well-structured network of Lot B as compared to A barely changes at all during the “gel weakening” stage due to the heat-resistant nature of these strong bonds. Moreover, in the low salt content samples (Lot A),  $G'$  (Fig. 3a) and  $G''$  (Fig. 3b) profiles decrease dramatically from ~43 to 55 °C owing to the partial rupture of the hydrogen bonds due to heating (Qiu et al., 2013); the rest of the bonds established in Lot B are not formed in the same way. As commented above, it has been reported that the addition of phosphates to surimi could have a detrimental effect on gel properties due to chelation of the  $\text{Ca}^{2+}$  ion (Julavittayanukul et al., 2006) and L-lysine, suppresses the polymerization of myosin heavy chain by inhibiting endogenous transglutaminase activity during the initial stages of surimi gelation. None of these effects were observed in the present study (Fig. 3a and b).

Lastly, at the gel strengthening stage,  $G'$  of Lot A and B continues increasing from ~56 to 75 °C and after this point (>75 °C)  $G'$  remains stable except for the sample where cystine was added (CBSD) in which  $G'$  continues to increase due to the S–S covalent bonds formed as a consequence of cystine addition, as was also reported in relation to DSC analysis (Section 3.2.1).  $G''$  remains nearly constant from 45 °C in both type of surimi dough irrespective of salt level (Lot A and B), indicating the formation of a definitive three-dimensional protein network formed by an increase in the number of cross-links between protein molecules (Niwa, 1992; Qiu et al., 2013; Xiong, 1997).

The  $G'$  and  $G''$  values increased in both surimi doughs (Lots A and B) in the presence of additives when compared to their respective control gels through all temperature profiles, indicating that stronger networks ( $G'$ ) were obtained, but they did not show better structural configuration ( $G''$ ). In the case of Lot A, all ingredients produced gels with similar rigidity as determined by FTIR analysis (Fig. 2a and c). In Lot B, the dough with cystine (CBSD) produced a gel as strong as those of Lot A irrespective of the additive. This could be due to a higher density of cross-links in this sample (CBSD) arising from the formation of disulfide covalent bonds during the strengthening stage as previously indicated (Section 3.2.1).

In short, thermal profiles showed that additives enhanced myosin gelling capacity especially at low salt content (0.3%); i.e. stronger gels could be formed with respect to those formed at regular salt content (3%).

### 3.3. Functional properties

#### 3.3.1. Water binding capacity (WBC)

The water binding capacity in surimi gels depends primary on protein-water interactions and the amount of these interactions, and gives an idea of how the water is linked in the protein matrix (Lakshmanan, Parkinson, & Puggot, 2007).

As shown in Table 3, all of the ingredients tested in Lot A induced an increase in WBC irrespective of the additive. This is

consistent with the different ways in which these ingredients are able to bind water molecules. In the case of pyrophosphate, water is bonded to the phosphate anions, which also increases the repulsion of protein groups due to the predominance of negative charges on the protein groups, thus opening up protein structures and increasing the number of binding sites available for water (Xiong, 2005). Cystine promotes SH group protein oxidation as previously reported (Table 3), to intermolecular disulfide bonds (S–S), trapping water in the matrix (Itoh, Yoshinaka, & Ikeda, 1979). Lysine is supposed to stimulate endogenous transglutaminase, thus inducing the formation of a protein network that holds water molecules (Han, Zhang, Fei, Xu, & Zhou, 2006). Moreover, the treatment applied to the samples is also an important factor to consider in water binding. This is particularly evident in SQ samples in which the setting period led to the additives' ability to more effectively hold water molecules. Also, the beneficial effect of the setting period was reported in the FTIR analysis (Section 3.2.2) where a higher  $\beta$ -sheet structure in SQ samples was observed.

In Lot B (Table 3) the effect of the ingredients was less prominent. As it has already mentioned several times, this is because the higher salt content was enough to unfold the proteins and expose the reactive groups, which were able to form more bonds by themselves. Similar results were observed when SH groups were determined (Table 3).

When comparing control samples of Lot A and B (AQ vs. BQ and ASQ vs. BSQ) Table 3, it was evident that Lot B showed a significantly higher WBC than their counterparts from Lot A. This could be due to the more unfolded protein structures in Lot B owing to the higher presence of salt as previously observed in DSC and FTIR analysis of the proteins. Furthermore, as some authors have reported, the presence of salt increases WBC due to the ability of chloride ions to bind to myofibrillar proteins and raise the electrostatic repulsion between filaments, thus increasing protein affinity for water (Hamann, 1972; Niwa, 1992).

### 3.3.2. Mechanical properties

Breaking force (BF) and Breaking deformation (BD) data are shown in Table 3.

In both Q and SQ samples of Lot A, the addition of cystine and lysine improved BF compared to the corresponding control samples (AQ, ASQ). This could be due to protein denaturation induced by the additives (Section 3.2.1) and also to the ability of cystine and lysine to form S–S bonds, thus inducing the formation of covalent crosslinks with lysine acting as a substrate of endogenous transglutaminase (Chen et al., 1999; Dickinson, 1997; Roussel & Cheftel, 1990). In contrast, BF was not modified by the addition of pyrophosphate, although the ability of phosphates to dissociate protein complexes enhancing gelation capacity has previously been described (Julavittayanukul et al., 2006; Matsukawa et al., 1995). It should also be noted that SQ gels, regardless of the ingredient added, showed higher BF when compared with Q samples in Lot A. This means that the setting period of 5 °C/24 h is appropriate for the action of the additives, resulting in more pronounced mechanical properties. This is due to the formation of electrostatic interactions between amino acids contributing to structure stability and also to the role of endogenous transglutaminase during the setting period (Zhu, Lanier, Farkas, & Li, 2014). Moreover, as was previously reported by FTIR analysis (Section 3.2.2), the use of additives resulted in a significantly less defined  $\alpha$ -helix band, indicating protein aggregation.

In Lot B the results were very similar to Lot A, suggesting improvement with the addition of the ingredients in Lot A, which is also consistent with the thermal gelation profile of Lot A and the native structure of the protein observed by DSC and FTIR analysis (Figs. 1 and 2).

Regarding breaking deformation (Table 3), in Lot A the highest values also correspond to SQ gels in which the addition of any of the ingredients resulted in gels with higher deformation compared to the control (AQ and ASQ). As has already been mentioned, the setting period is apparently crucial in enabling the ingredients to act before heating, because, during setting, partial actomyosin molecules form regular network structures mainly constituted by  $\epsilon$ -( $\gamma$ -glutamyl) lysine bonds, disulfide bonds and hydrophobic interactions (Roussel & Cheftel, 1990). Concerning Lot B (Table 3), a comparison of Q and SQ gels showed a significantly higher BF in SQ than in Q gels as also reported in Lot A.

BF was very similar in both Lots A and B while breaking deformation tended to be higher in Lot B, possibly due to a better protein network formed as a consequence of the high ionic strength. This was also observed in the thermal gelation profile of the surimi dough (Fig. 3a and b) and in the protein structure observed by DSC and FTIR analysis (Figs. 1 and 2).

## 4. Conclusions

The three additives studied, cystine (0.1%), tetra-sodium pyrophosphate (0.05%) and lysine (0.1%) enhance gelation of Alaska Pollock surimi in the presence of only 0.3% NaCl, the most effective being cystine and lysine. The action of these additives is mainly based on inducing primary protein denaturation or unfolding of myofibrillar proteins facilitating the formation of different types of bonds. The resulting gels containing low NaCl are characterized by a similar breaking force although lower breaking deformation and WBC than those formed with a higher NaCl content (3.0%), characterized by a more compact and well-structured network. Hence, it is possible to create surimi-gels with reduced NaCl content by adding cystine and lysine.

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