Effects of Na⁺, K⁺ and Ca²⁺ on gels formed from fish mince containing a carrageenan or alginate

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Abstract

The effects of mixtures of added chloride salts (0-1 % NaCl plus 0-1 % KCl plus 0-1 % CaCl_2) on the characteristics of blue whiting muscle gel containing different added gelation agent (iota-carrageenan, kappa-carrageenan, or sodium alginate) were studied by response surface methodology (RSM) and factor analysis (principal component analysis). The salts exhibited independent effects on gel properties. NaCl mainly altered breaking strength in the gels made with iota-carrageenan, the yellowness (b^*) value in the gels made with kappa-carrageenan, and adhesiveness in the gels made with sodium alginate. KCl mainly affected adhesiveness in the gels made with one of the carrageenans, especially iota-carrageenan; it had no apparent effect on the gels made with sodium alginate. The effect of CaCl_2 in all the formulations was mainly to increase elasticity and lightness and decrease the rest of the properties considered. Combined action of the salts did reveal certain significant interactions, such as the interaction between sodium and calcium which affected the folding test value in the gels made with alginate and breaking strength in the gels made with iota-carrageenan, or the interaction between potassium and sodium which affected the redness (a^*) value in the gels made with iota-carrageenan. Factor analysis showed the main trends in the variables tested, calcium being the salt that exerted the primary influence and contributed to the first two factors in each of the factor analyses. Each of the salts contributed to a separate factor, the only exception being the mixture of CaCl_2 and KCl in the formulations that contained iota-carrageenan, in which both salts contributed to factor 1.

Key words: cation, carrageenan, alginate, rheological properties, gel

INTRODUCTION

Iota and kappa carrageenans can alter the texture and water holding properties of restructured fish products as a result of their gel-forming capacity and their ability to interact with the myofibrillar protein as a result of their anionic nature. The addition of salt (both concentration and type) exerts a considerable influence on gelation of the carrageenans (Hermansson, Eriksson & Jordansson, 1991; Keogh, Lainé & O'Connor, 1995; DeFreitas, Sebranek, Olson & Carr, 1997) and on gelation of mince (Gómez-Guillén & Montero, 1996; Gómez-Guillén, Borderías & Montero, 1996; Pardo, 2000).

Gelation of iota-carrageenan is enhanced mainly by calcium, kappa-carrageenan by potassium. Ohashi, Ura, Ochi, Lida & Ukai (1990) reported that cations ($\text{Ca}^{+2} > \text{K}^+ > \text{Na}^+$) bind to the carrageenans, blocking the sulphate groups and thereby preventing interaction with thaumatin (a basic protein). The cations affect the balance of attractive and repulsive forces between the molecules so that optimum gel strength will occur at certain levels of each cation.

Iota-carrageenan has no specific ion binding sites for monovalent ions, and bonds therefore arise only from electrostatic interactions; hence the possible effects of monovalent ions are attributable to the level of impurities in the kappa-carrageenan (Piculell, 1991). In the case of kappa-carrageenan, alkaline ions bind to the helix of the hydrocolloid, thus partially neutralising the sulfate groups. This gives rise to aggregation of the double helices, increasing gel rigidity. In that respect, da Ponte, Herst, Roozen & Pilnik (1985) reported that at low potassium concentrations, kappa-carrageenan is

difficult to hydrate and thus to gel in minced cod muscle. Shand, Sofos and Schmidt (1994) pointed out that the strongest effects of kappa-carrageenan on structured beef roll texture occurred at salt levels of around 1 %. In its hydrated state, the sodium cation is larger and cannot readily penetrate the helixes, while the calcium cation causes aggregation of the helixes, giving rise to weak, friable gels (Rees, 1969; Piculell, 1991). Both the effect of the salt and the effect of the hydrocolloid will be dependent on mince quality (Gómez-Guillén & Montero, 1996; Gómez-Guillén *et al.*, 1996; Pardo, 2000). In addition, the mince and the hydrocolloids will compete for the added salt and for the available water, such that more salt needs to be added to the batter than the amount that would be required for adequate gelation of the hydrocolloids (iota and kappa-carrageenan and alginate) in an aqueous system, in that a large proportion of the salt will be trapped by the mince.

Alginates can also form heat-reversible and irreversible gels, depending on the concentration of polyvalent cations, particularly calcium, and the length of the uniform regions on the guluronic acid, the main site of cation interaction. Varying the degree of polymerization or changing the ionic environment can yield friable, breakable gels of variable rigidity (Morris, 1986). Pardo (2000) observed that to increase the work of penetration and breaking strength of gels made from washed sardine mince containing 1.5 % sodium alginate, the optimum quantity of salt to be added was 1.5 % NaCl and 0.5 % CaCl₂, concentrations higher than that needed for a pure alginate gel.

A large number of studies have dealt with the effects of individual ingredients on the

functional properties of mince or surimi gels, but relatively few have considered the interaction effects of mixtures of ingredients for complex mince/polysaccharide systems focused to make fish gelled products. Such studies require non-linear statistical processing. The effects of compositional variables, quality parameters, and process variables on the properties of gels are all interrelated, making it difficult to conclude which effects may be attributed to any given ingredient individually. The experiment reported in this paper is intended to elucidate the effects of cation (Na^+ , K^+ , and Ca^{+2}) type and concentration on gelation in gels made from washed fish mince containing different hydrocolloids (iota-carrageenan, kappa-carrageenan, and sodium alginate). Response surface methodology was employed to determine possible interactions among the cations. Factor analysis was performed to relate the effects of the added cations to the properties of the different gel formulations containing the different hydrocolloids.

MATERIALS AND METHODS

The blue whiting (*Micromesistius poutassou* Risso) used in this study was caught in the Bay of Biscay off northern Spain in January. Average size was 24.0 ± 1.5 cm and average weight was 128.4 ± 24.6 g. The proximate composition (%) was: crude protein 14.54 ± 0.04 ; moisture 79.40 ± 0.2 ; crude fat 0.15 ± 0.10 ; and ashes 0.54 ± 0.08 . Analyses do not show added cryoprotectant (4.2 %).

Gel preparation. Frozen, washed blue whiting mince was thawed in a cold room and

ground for 1 min at high speed in a refrigerated vacuum homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Germany). Sodium chloride (1 % w/w) in gel (Panreac, Montplet & Esteban S.A., Barcelona, Spain) was added and homogenized at low speed for 3 min. A hydrocolloid: iota-carrageenan(Satiagel™ RPT25), kappa-carrageenan (Satiagel™ RPT8), or sodium alginate (Satialgine™ S1100) {SKW Biosystems, Rubí, Spain}, was added (0.5 % w/w). The molecular weight of hydrocolloids are: 800 -1000 KDa for iota-carrageenan, 700 KDa for kappa-carrageenan and about 300 KDa for sodium alginate. The impurity of kappa carrageenan in iota and *viceversa* is around maximum of 5 %. The average values for 100 grams of different ions included in the hydrocolloids are 3200 mg sodium, 7100 mg of potassium, 270 mg of calcium and 95 mg of magnesium in iota-carrageenan, 900 mg sodium, 1400 mg of potassium, 190 mg of calcium and 95 mg of magnesium in kappa-carrageenan, 8500 mg of sodium, 500 mg of potassium and 100 mg of calcium in sodium alginate. Mixtures of salts (NaCl, KCl, CaCl₂) were also added according to the experimental design in a range 0 - 1%, along with water/crushed ice to give the required final gel moisture (80 % w/w). The homogenate was beaten slowly under vacuum for 6 min, keeping sample temperature below 10 °C. The resulting batters were stuffed into 40 µm-thick flexible plastic casings (Krehalon Soplaril, Barcelona, Spain) to a diameter of 3.5 cm and subjected to heat treatment at 37 °C for 30 min followed by 90 °C for 50 min. All batters in their casings were immediately cooled in water at 0 °C and stored in a cold room at 4 °C for 24 hours before analysis.

Analyses were performed according to Pérez-Mateos & Montero (2000). Gels were

removed from their casings, cut (3.5 cm diameter, 3 cm height) and tempered at 20 °C. Folding test resistance of a slice (3.5 cm diameter and 3 mm high) folded over twice. Puncture test (breaking deformation, breaking strength, work of penetration): with a round-ended stainless steel plunger ($\varnothing=5$ mm) at a speed of 10 mm/min using a 100 N load-cell. Texture profile analysis (hardness, adhesiveness, cohesiveness): at a deformation rate of 50 mm/min using a cylindrical plunger ($\varnothing=58$ mm) adapted to a 5 kN load-cell and a compression rate of 60 %; and a stress-relaxation test after 1 min of relaxation (elasticity). Color (L^* , a^* , b^*) was determined using the CIE Lab scale (D65/10°), and water holding capacity was measured by the centrifuge method. At least three replications of all determinations were performed.

Statistical analysis. Response surface methodology was used to study the simultaneous effect of three independent variables (NaCl, KCl, CaCl_2). Two variables were plotted and the third variable held constant at its mean level versus each response variable according to a central composite rotatable design, using the STATGRAPHICS statistical program (STSC Inc., Rockville, MD, U.S.A.). Assessment of error was performed by replication of one treatment as considered in the design (Cochran & Cox, 1957). Each parameter was represented by a second-order polynomial expression that used a multiple correlation coefficient (r) to predict the effects of the combinations of independent variables:

$$Y = b_0 + \sum_{i=1}^k b_i x_i + \sum_{i=1}^k b_{ii} x_i^2 + \sum_{i < j}^k b_{ij} x_i x_j$$

where Y was the estimated response; the b values were the equation parameter

estimates (constant: b_0 ; linear term, b_i ; quadratic term, b_{ii} ; interaction term: b_{ij}); x_i and x_j were the variables analysed; and k was the number of variables. The significance of the equation parameters for each response variable was assessed by an F-test in order to check the goodness of the model as a whole.

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Multivariate analysis. Factor analysis (BMDP 4M) was performed using the BMDP computer program (BMDP Statistical Software, Inc., Cork Technology Park, Cork, Ireland) employing principal component analysis (PCA) for all the mean values for the blue whiting muscle gels made with added hydrocolloid and added salts according to the RSM design.

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RESULTS

Gels made from mince with added iota-carrageenan

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In the gels that contained iota-carrageenan, addition of the salts had a strong, significant effect on the folding test value (Figure 1, Table 1). The value declined considerably with the addition of CaCl_2 over the entire range of concentrations studied ($p \leq 0.01$ for the linear component, $p \leq 0.05$ for the quadratic component). In the case of the monovalent salts, there was a slight downward trend for both NaCl and KCl ($p \leq 0.05$, quadratic component) at concentrations above 0.5 %. No significant interaction effect was observed between the salts considered.

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Only calcium exerted an effect on the breaking deformation (Table 2) of the gels ($p \leq 0.01$, linear component); the effect was negative. Figure 2 shows that the addition of extra quantities of any of the salts resulted in a substantial decrease in breaking strength ($p \leq 0.01$, linear component). That effect was greatly accentuated when sodium and calcium were present together ($p \leq 0.05$, interaction effect). The effect on the breaking strength had a major effect on the trend for work of penetration (Table 2), hence work of penetration, as reflected by the linear component, decreased on addition of any of the salts considered here. On the other hand, no significant interaction effect was observed for the combination of sodium and calcium.

Furthermore, these gels tended towards greater elasticity and softness and lower cohesiveness with the addition of the divalent salt (Table 3). Table 3 also shows that the addition of any of the salts brought about a significant decrease in adhesiveness (for sodium and potassium, $p \leq 0.05$, linear component; for calcium, $p \leq 0.01$, quadratic component). No interaction effect between the salts was observed.

The water holding capacity of the gels (Table 4) generally increased over the linear portion of the function with the addition of the monovalent salts (Na^+ and K^+), but for both salts the water holding capacity decreased at concentrations higher than 0.5 % ($p \leq 0.01$ and $p \leq 0.05$, respectively, quadratic component). The addition of calcium tended to bring about a decrease in the water holding capacity ($p \leq 0.01$, linear component). Addition of a combination of the salts did not reveal any significant interaction on this functional property.

Calcium had opposite effects on lightness, depending on the concentration (Table 4). On the one hand, it seemed to bring about an increase in the linear portion of the function ($p \leq 0.01$), though at high concentrations of CaCl_2 lightness values tended to decrease ($p \leq 0.01$, quadratic component). The addition of sodium or potassium likewise increased gel lightness (for these salts the effect was reflected by the linear component at a level of significance of $p \leq 0.01$ and $p \leq 0.05$, respectively). The redness (a^*) value displayed a general tendency to increase with the addition of calcium ($p \leq 0.01$, linear component) but to decrease with the addition of potassium. Addition of calcium together with potassium yielded an interaction effect ($p \leq 0.05$) giving rise to a declining trend (Table 4). The yellowness (b^*) values underwent a slight decrease ($p \leq 0.05$, linear component) only with the addition of calcium cations (Table 4).

Principal component analysis performed using the correlation matrix identified four principal components that explained over 91.1 % of the variance in the data. Table 5 presents the contributions of the different variables to each of the rotated factors, in descending order. Variables with loadings on the same factor tended to be highly correlated with each other, while variables with loadings on different factors tended to be less closely correlated. Factor 1 explained nearly 40 % of the total variance. Contributions to that factor were made by calcium and various dependent variables, chief among them being: elasticity and redness (a^*), which increased on addition of calcium, and water holding capacity and the folding test value, which exhibited the opposite effect in the presence of added calcium. Calcium also contributed appreciably

to factor 2 (23.14 % of the total variance), though the contribution of calcium was smaller than its contribution to factor 1. In both cases the contribution had the same sign. The following gel properties were the major contributors to factor 2: breaking deformation, breaking strength, and lightness (L^*). The main properties contributing to factor 3 (20.48 % of the total variance) were adhesiveness, breaking strength, hardness, and work of penetration. All of which underwent decreases with increasing concentration of added KCl. NaCl contributed to factor 4 (18.37 % of the total variance); this salt tended to decrease gel breaking strength, work of penetration, and the yellowness (b^*) value primarily, though adhesiveness, lightness (L^*), and hardness also contributed to this factor.

Gels made from mince with added kappa-carrageenan

The results for the folding test values (Figure 1) in the gels that contained kappa-carrageenan showed that the addition of CaCl_2 had a negative effect ($p \leq 0.05$, quadratic component) and that, to a lesser extent, the addition of NaCl had the same effect ($p \leq 0.01$, linear component) [Table 1]. The results for breaking deformation were indicative of similar responses to the added cations (Table 2). Breaking deformation values were mainly affected by the addition of calcium (linear component), but also by the addition of the monovalent salts (quadratic component). No interaction effects were observed.

The multiple correlation coefficient value for the fits of the RSM plots to the

experimental results for breaking strength (Figure 2) were very high ($r = 0.96$). Table 2 shows that breaking strength values decreased substantially with the addition of any of the salts considered (for sodium and potassium, $p \leq 0.01$, linear component; for calcium, $p \leq 0.05$, quadratic component). The effect of the addition of the salts on the work of penetration (Table 2) was rather similar to the effect on breaking deformation, in particular the substantial decrease with increasing concentration of the divalent salt (Ca^{2+}) ($p \leq 0.01$, linear component) as well as with the addition of the monovalent salts, especially at concentrations of around 0.5 % ($p \leq 0.05$, quadratic component). No interaction effects were observed in any of the cases.

The values relating to the compression properties of the gels presented in Table 3 show that hardness tended to decrease with the addition of any of the salts (Na^+ : $p \leq 0.05$ and K^+ : $p \leq 0.01$, linear component in each case), particularly with calcium ($p \leq 0.05$, linear component and $p \leq 0.01$, quadratic component). Adhesiveness also decreased with the addition of the cations (Na^+ : $p \leq 0.01$ and K^+ : $p \leq 0.05$, linear component in each case), though this effect was less pronounced for the added calcium ($p \leq 0.01$, quadratic component). The action of calcium was significant ($p \leq 0.01$, linear component) but opposite on both cohesiveness, which decreased, and elasticity, which increased.

The water holding capacity (Table 4) of the gels that contained kappa-carrageenan tended to decrease with the addition of CaCl_2 ($p \leq 0.01$, linear component). Calcium had differing effects on gel lightness and the redness (a^*) value (Table 4). These two properties tended to increase at low concentrations of added calcium ($p \leq 0.01$, linear

component) and to decrease at high concentrations ($p \leq 0.01$, quadratic component). The redness (a^*) and yellowness (b^*) values decreased slightly with the addition of sodium ($p \leq 0.01$, linear component). Otherwise, NaCl and KCl generally had no significant effect or interaction.

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Principal component analysis (Table 6) needed only four factors to explain most of the variance (92.5 %). Factors 1 and 2 explained a high proportion, nearly 65 %, of the total variance (34.91 % and 29.78 %, respectively). The addition of CaCl_2 influenced both factors and increased both gel elasticity and gel whiteness (higher L^* and lower a^*). On the other hand, added calcium decreased cohesiveness, water holding capacity, breaking deformation, breaking strength, work of penetration, the folding test value, and hardness. Factor 3 (20.87 %) was influenced by the addition of NaCl, which negatively affected adhesiveness, breaking strength, hardness, and the yellowness (b^*) value. Addition of KCl was the main contributor to factor 4 (14.43 %) and exerted a negative effect on breaking strength, hardness, and adhesiveness. Work of penetration was affected principally by variations in breaking deformation rather than breaking strength. Breaking deformation, and hence work of penetration, was mainly altered by the addition of calcium, while the addition of the different salts altered the breaking strength in different ways. Elasticity, cohesiveness, water holding capacity, lightness, and the redness (a^*) value were only affected by the addition of calcium.

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Gels made from mince with added sodium alginate

The folding test value also decreased significantly in the gels made with added alginate (Figure 1) at increasing concentrations of the added salts, particularly calcium and also somewhat with the monovalent cations, especially sodium. Addition of the monovalent salts concurrently displayed a negative interaction effect (Table 1). On the other hand, addition of the salts did not exert any significant influence on breaking deformation (Table 2). Conversely, both breaking strength (Figure 2) and work of penetration showed a tendency to decrease with the addition of any of the three salts tested, as reflected by the linear component (Table 2).

Addition of the monovalent salts and the divalent salt in particular exerted a distinct effect on hardness (Table 3), resulting in softer gels. A decrease in adhesiveness was observed (Table 3) with the addition of NaCl ($p \leq 0.01$, linear component) and CaCl₂ ($p \leq 0.01$, linear and quadratic components). Calcium exerted opposite effects on cohesiveness and elasticity, yielding weakly cohesive but highly elastic gels.

Gel water holding capacity (Table 4) tended to decrease with the addition of the divalent salt (Ca²⁺) as reflected by the linear component, though that property also exhibited an opposing effect, with a slight tendency to recover at high concentrations of CaCl₂ or with the addition of the monovalent salt Na⁺ ($p \leq 0.05$, linear component). The gel colour attributes (Table 4) were affected differently by the addition of calcium: lightness tended to undergo a significant increase ($p \leq 0.01$, linear component), while the yellowness (b*) value tended to decrease ($p \leq 0.01$, linear component). The redness (a*) value did not

display any significant trend.

Principal component analysis performed using the correlation matrix needed four factors to explain 92.81 % of the variance. Table 7 sets out the contributions of the different variables to each of the factors. Most variables contributed to factor 1, which explained 52.72 % of the total variance. CaCl_2 was the only salt contributing to that factor. Calcium brought about increases in elasticity and whiteness (L^* , a^*) and decreases in cohesiveness, water holding capacity, the folding test value, hardness, breaking strength, work of penetration, and adhesiveness. Cohesiveness, water holding capacity, elasticity, and the folding test value were affected only by calcium and thus only contributed to factor 1. Breaking strength and work of penetration were negatively affected by the addition of sodium (factor 2: 19.89 %) and potassium (factor 3: 14.51 %). On the other hand, breaking deformation (factor 4: 12.88 %) was not affected by any of the salts. Moreover, the addition of NaCl decreased adhesiveness and hardness, while the addition of KCl lightened gel colour (L^* , a^* , b^*).

DISCUSSION

When preparing restructured, protein gel-based products, maximum values for the mechanical properties are not always desirable, in that each individual product will require a given texture. Addition of a mixture of differing concentrations of salts allows the gel characteristics to be adjusted to fit a wide variety of applications. The results for the folding test, compression test, water holding capacity, and colour values for both the

gels that contained iota-carrageenan and the gels that contained kappa-carrageenan followed very similar trends. In principle, the addition of cations could be expected to produce more changes in the gels made with iota-carrageenan than in those made with kappa-carrageenan on account of the greater negative charge of the iota-carrageenan; on the other hand, only the kappa-carrageenan has specific binding sites for monovalent ions. Probably the biggest quantity of ions – Na^+ and K^+ - in the iota-carrageenan sample are already interacting with the negative charge of the carrageenan and then in proportion it do not have more free sites than kappa-carrageenan. The salts interact with sulphate ions of carrageenans and neutralise the charges. The addition of extra salt, especially calcium chloride, displayed a tendency to bring about a decrease in gel firmness (breaking strength and hardness). The interaction effects for the combinations of salts were minor in the gel formulations that contained iota-carrageenan, in all cases being between the divalent salt and one of the monovalent salts, either sodium or potassium. However, no interaction effects were observed for the monovalent salts. Still, the results suggested that there might be an additive effect between the monovalent salts; that is, in certain cases a certain proportion of each of the monovalent cations appeared to be necessary to achieve a given effect. It is important consider that at the same percentage of salt, there will be about the double of monovalent ions than divalent ions. Also, taking into account moreover the different negative charge of the carrageenan, the higher molecular weight of iota than the kappa-carrageenan. In previous work, it could observed by scanning microscopy that the network formed for both hydrocolloids were quite similar and independent of protein matrix (Pérez-Mateos, et al. 1997), but the optical microscopy

reveal that kappa carrageenan distribution were more expand than iota carrageenan in the gel formed. However, both carrageenan form very dens bodies if there are compared with the formed by neutral and not gelling gums as guar and locust bean (Montero, 2000). These effect seems due to meanwhile the neutral gums don not
5 interact with the myofibrillar protein and catch the water expanding as binders in a cavities inside the matrix of gel, the carrageenan can be interact with the myofibrillar protein by the negative charge, and during the thermal process also the hydrocolloids began to made a proper gel at lower temperature than proteins. Trius, A., Sebranek, J.G., Rust, R.E. & Carr, J.M. (1995) suggested that the different distribution of
10 hydrocolloids along the matrix could be attributed to the different solubility of carrageenan, so iota-carrageenan that is more soluble at low temperatures than kappa, could introduce into the protein matrix before it made gel.

There have not been many studies dealing with the effect of these salts in gels made
15 from fish muscle, be it mince or surimi, with only iota or kappa-carrageenan (da Ponte *et al.*, 1985; Gómez-Guillén & Montero, 1996; Gómez-Guillén *et al.*, 1996; Pardo, 2000) employing different myosystem. There are large variations according to myofibrillar protein quality, and hence studies of variability within the same myosystem using different formulations (hydrocolloid type and salt concentration and type) hold out
20 interest. Studies about chemical interaction and functional properties relationships of blue whiting mince determine that low NaCl level 1.5%) in batter improve the functional properties, overall rheological characteristic, in spite of the chemical bonds -hydrogen, hydrophobic and covalent - formed were not very different Pérez-Mateos, et al. 1997).

In one such study Konstance (1993) found a sharp decrease in sodium caseinate gel hardness on adding KCl together with kappa-carrageenan and iota-carrageenan. The addition of KCl had an effect opposite to the expected effect; the gels were less firm, and the elasticity of calcium caseinate gels with carrageenan was also lower. DeFreitas *et al.* (1997) reported that the addition of KCl affected iota and kappa-carrageenan efficacy, yielding softer pork sausages. Hermansson *et al.* (1991) found structural differences in gel formulations containing kappa-carrageenan depending on the predominant ionic form, i.e., potassium, sodium, or calcium carrageenan, a finding indicative of the importance of the salt used, but this study were in pure hydrocolloids system. In complex system like these, it should be assume that the cations added can interact with the hydrocolloids and with the proteins, and also the hydrocolloids can be interact with the protein.

In other studies (Ipsen, 1997), the addition of NaCl brought about a decrease in the work of penetration and firmness values for gels made from soy and pea protein and added kappa-carrageenan, whereas the addition of CaCl_2 had no influence on the rheological properties. That researcher attributed those results to variation in protein solubility and heat stability and/or to changes in hydrocolloid gelation temperature. In experiments carried out with mixtures of sodium caseinate and carrageenan, it was found that sodium chloride and calcium chloride significantly affected the consistency of kappa-carrageenan gel. However, the effects were dissimilar for the two types of carrageenan, showing that the interaction involved was not of the same importance in

the two carrageenans (Keogh *et al.*, 1995).

The results for water holding capacity showed that concentrations of NaCl or KCl lower than 0.5 % displayed a tendency to increase the binding capacity of the gels that contained iota-carrageenan. However, the addition of calcium decreased water-holding capacity in the gels that contained either of the two carrageenans. It seems that the monovalent ions are bound to the helix of carrageenan and neutralising partiality the sulphite bonds, by this way the double helix are grouping increasing the rigidity of gel and syneresis. With the calcium ions, it caused the helix aggregation producing weak gels and retain more water. These effects have been mention by Piculell (1991). This author also observed that, the impurities of one carrageenan in other may affect the properties that the ions induced in the carrageenan system. In the additives used in the present work the impurities of iota in kappa and viceversa are around 5 %. Trius, Sebranek, Rust & Carr (1994) observed that the addition of KCl resulted in higher cooking losses for sausages with kappa-carrageenan, probably from water expulsion resulting from aggregation of the helixes. Other studies (Defreitas *et al.*, 1997) found that differences in thaw drip between different carrageenans were very slight in the presence of NaCl, but larger differences were observed when KCl was added, with larger water losses compared with the effect of NaCl. Furthermore, Barbut (1994) reported that the addition of monovalent cations helped stabilise meat homogenate, whereas the addition of divalent cations (Ca^{2+}) resulted in high levels of protein aggregation leading to lower water holding capacity. Xiong, Noel & Moody (1999) pointed out that the main advantage of polysaccharide gums seemed to be more to

improve water binding in low-fat beef sausage than to improve texture. Those workers reported that gums and myofibrillar proteins, as charged polymers, may compete for water required to form a viscous sol or a viscoelastic gel, thereby interfering with their structure-forming ability and gel matrix formation.

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On the whole there seem to be differing tendencies according to the concentration of added salts. According to Ambjerg-Pedersen & Jørgensen (1991), the effect of the cations depends on concentration. Thus, at low concentrations they appear to have a stabilising effect, whereas at high concentrations they appear to have the opposite
10 effect. This may mean that saturation of anionic groups occurs at a certain concentration. In the present experiment, the carrageenans were predominantly in the form of potassium carrageenan, and the weak effect of extra quantities of KCl may indicate that the sulphate groups were already over-saturated from processing.

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The effect of the calcium cation on the gels made with alginate was quite similar to that on the gels made with the carrageenans: an increase in elasticity and lightness and a decrease in the folding test value and cohesiveness. Elasticity could be altered by as much as 20-30 % depending on the concentration of the added CaCl_2 . Furthermore, calcium caused considerable softening of the gels, as in the case of the
20 gels that contained iota and kappa-carrageenan. Addition of the monovalent salts also brought about a decrease in hardness in the case of the gels made with the alginate, as it did in the gels that contained kappa-carrageenan. Potassium and sodium cations did not exhibit any appreciable influence on the other properties considered, except for

the effect of NaCl on adhesiveness.

It is known that alginate can be used as a thickening or gelling agent depending on the presence or absence of calcium ions. Non-enhancement of the gelling ability of the alginate in the presence of additional calcium may be due more to alginate structure (the number of binding sites, the strength of the binding sites, and entanglement of the alginate molecules) than to the insufficient concentration of the reticulating cation, preventing complete gelation of the hydrocolloid. In that same sense, Niwa, Tsujimoto & Kanoh (1992) reported that calcium alginate increased the breaking strength of kamaboko gel, whereas sodium alginate did not. In respect of CaCl_2 concentration, in experiments carried out using sardine minced (*Sardina pilchardus*) muscle, Montero, Pardo, Gómez-Guillén & Borderías (1992) and Pardo (2000) recorded the highest work of penetration values in gels made with added CaCl_2 at a concentration of 0.5 % to enhance gelation of the alginate ingredient. Higher and lower concentrations lowered both the folding test value and the work of penetration, although the effect differed according to the setting temperature of the gel. Quantities of sodium chloride higher than 1.5 % of the total formulation also considerably decreased these same mechanical properties. This result was attributed to a negative interaction between the salt and the alginate, because a quantity of 2.5 % NaCl had been found to be optimal for solubilization and gelation of the mince in the absence of added hydrocolloid.

Considering salt concentration, Ustunol, Xiong, Means & Decker (1992) reported that

high levels of NaCl (0.5 M) destabilised the electrostatic interactions between the alginate and pork myofibrillar proteins.

Water holding capacity (Table 4) of the gels that contained alginate tended to decrease
5 with the addition of divalent cations (Ca^{2+}) as reflected by the linear component. But
there was a slight tendency for water holding capacity to make up the loss and recover
at high calcium concentrations or with the addition of monovalent cations like Na^+ ($p \leq$
0.05, linear component). In other words, by changing the salt content of the formulation
the water holding capacity could be altered by up to 20 %. Rourke, T.J.; Clarke, A.D.;
10 Bailey, M.E.; and Hedrick, H.B. (1997) found that ionic bonds -between negative groups
of alginate and positive one of protein - play a very important role in the gelification of
alginate-myofibrillar protein system. In this work seems that the addition of calcium to
sodium alginate decrease almost of gel properties. It could due to that calcium alginate
made a gel than interrupt the protein matrix, meanwhile the sodium, potassium alginate
15 interact with protein and participate in the network made with matrix. By other hand,
attending to molecular weight of sodium alginate (around 300 KD) is smaller than its
aggregate (calcium alginate). As the alginate have certain quantity of ions (Na^+ , K^+ and
 Ca^{++}), mainly sodium, only slight percentage of monovalent ions are necessary to
added to improve the gel properties. Montero, P.; Hurtado, J. and Pérez-Mateos, M.
20 (2000), Pérez-Mateos, M.; Lorenço, H; Montero, P. and Borderías, J. (1997). Pérez-
Mateos, M.; Lorenço, H; Montero, P. and Borderías, J. (1997) observed by optical and
scanning microscopy respectively that gel with sodium alginate participate to form the
matrix, but do not made a proper gel.

On the whole, based on the principal component analysis results, each of the salts contributed to a different factor, indicating that each type of cation exerted a separate effect on the gelling properties of the muscle protein-hydrocolloid mixtures. Calcium was the salt that contributed to the first principle components, the ones explaining the largest proportion of the variance, suggesting that it appreciably altered the variables tested. In the case of the kappa-carrageenan-containing gels, the NaCl mainly influenced the yellowness (b^*) value, with the CaCl_2 affecting elasticity, the redness (a^*) value, and lightness (L^*). In the alginate-containing gels, calcium was also observed to have a clear effect on cohesiveness, elasticity, the folding test value, hardness, and water holding capacity, while the main effect of the NaCl was on gel adhesiveness. On the other hand, in the gels that contained iota-carrageenan, there was an interaction between the KCl and the CaCl_2 on the folding test value, elasticity, water holding capacity, and the redness (a^*) value, apart from the independent effects of each of those salts.

From technological point of view the percentage to favour almost of the mince gel properties are added 0.5 % NaCl and 0.5 % KCl extra in kappa-carrageenan, and lower 0.5 % NaCl and lower 0.5 % KCl extra in iota-carrageenan and sodium alginate. And the addition extra of calcium favour elasticity and lightness of gels. With the addition of hydrocolloids and ions to blue whiting mince is possible to obtain a very wide range of gel properties.

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