Effects of cations on the gelling characteristics of fish mince with added nonionic and ionic gums

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Abstract

The effects of a mixture of added chloride salts (0-1 % NaCl plus 0-1 % KCl plus 0-1 % CaCl₂) on the gelling characteristics of blue whiting muscle containing nonionic (locust bean gum, guar gum) or ionic (xanthan gum, carboxymethylcellulose) gums were studied by response surface methodology (RSM) and factor analysis (principal component analysis). The salts employed did not display any interaction effects altering the gelling properties of the different formulations; only sodium and calcium sometimes exhibited such effects, depending on the hydrocolloid used. The main trends in the variables tested revealed by principal component analysis showed that calcium yielded elastic gels with lower cohesiveness and higher lightness and that this same salt contributed to the factor(s) that explained the largest proportion of the variance. In contrast, addition of the monovalent salts had a lesser effect on the gelling properties, with intermediate concentrations resulting in the largest increases in the properties. No similar trends were observed with addition of the monovalent cations or the divalent cation or with hydrocolloid charge (nonionic or ionic gums).

Key words: cation, locust bean, guar, xanthan, carboxymethylcellulose, fish mince, gelling properties, RSM, factor analysis.

INTRODUCTION

The structure of the gel, according to Ross-Murphy (1995), consists of a network of macro-molecule chains of great size, with lateral chains and with infinite points of union consequence
5 of the balance between the interactions of the different components. The points of union between chains can vary from small regions from contact to great regions between several chains depending on the chemical structure of polymer. Moreover, in the case of fish gels depend also on the functional quality of the myofibrillar protein, the additives used, and processing conditions, as well as such other factors inherent to the medium as ionic strength,
10 pH, etc.

In addition, in aqueous systems polysaccharides undergo disorder-order transitions during heat treatment, such that the presence of cations may alter hydrocolloid function (Miyoshi, Takaya, Williams & Nishinari, 1996; Sudhakar, Singhal & Kulkarni, 1996; Rosett, Kendregan,
15 Gao, Schmidt & Klein, 1996; Ma & Barbosa-Cánovas, 1997; Tang, Tung & Zeng, 1997; Bryant & McClements, 2000). Salt increases the solubility of myofibrillar protein during homogenization and is thus conducive to the subsequent formation of cross-linked myosin heavy chains in fish mince (Niwa, Kanoh & Nakayama, 1986; Wan, Miura & Seki, 1992). Little interaction is presumed to occur between proteins and nonionic hydrocolloids (Imeson,
20 Ledward & Mitchell, 1977; Bernal, Smajda, Smith & Stanley, 1987; Ambjerg-Pedersen & Jørgensen, 1991). Nevertheless, hardly any studies have dealt with the influence of salts on hydrocolloid behavior in complex systems such as myosystems.~~repetido en la ultima frase del parrafo anterior (quitar lo subrayado)~~ The protein-hydrocolloid interaction is mainly electrostatic in nature, between the anionic groups on the hydrocolloid and the positively
25 charged groups on the protein. Behavior in a heterogeneous system like muscle depends on the presence of other components. Moreover, although the water content is high, it is mostly bound by the myofibrillar protein, with little available to hydrate the hydrocolloid. In previous

work, we have focused on the effect of adding different proportions of hydrocolloids on the gelling of mince (Pérez-Mateos & Montero, 2000), but there are no studies dealing with the effect of salts on hydrocolloid behavior in this complex systems as myosystems are.

5 The object of the present study was to determine the possible influence of certain salts, singly or in combination, on gelling when added to blue whiting mince containing a thickening hydrocolloid such as locust bean gum, guar gum, xanthan gum, or sodium carboxymethylcellulose. Experimental design used response surface methodology to determine possible interactions between the cations added, and in addition principal
10 component analysis was used to relate the properties of the gels for each of the hydrocolloids used according to the effects of the cations.

MATERIALS AND METHODS

15 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 (%) of mince was performed according to AOAC procedures (1984) and crude fat by Bligh and Dyer (1959): 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
20 added cryoprotectant (4.2 %).

Fish were headed, gutted and washed. Skin and bones were removed with a deboning machine (Baader 694, Lübeck, Germany). The resulting mince was washed in a solution of NaCl (0.2 %) at 0-3 °C, proportion 3:1 (solution:minced muscle), first with constant stirring for
25 10 min then without stirring for another 10 min. After draining, excess water was removed using a screw press (Baader 523, Lübeck, Germany). Sorbitol (4 %) and tripolyphosphate (0.2

%) were added as cryoprotectants. The mince was immediately vacuum-packed in bags (Cryovac BB-1, Grace, Spain) and frozen in a plate-freezer (Sabroe SMC, Denmark). The bags were stored at - 80 °C in a freezer cabinet (Revco ULT, Giralt, Revco Scientific, Inc., Asheville, N.C., USA) in order to minimize alteration during frozen storage up to gel preparation.

Gel preparation. Frozen, washed blue whiting mince was tempered at room temperature for one hour 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 [locust bean gum (Viscogum™ BE), guar gum (FFH-200), xanthan gum (Satiaxane™ CX90) or sodium carboxymethylcellulose (Tylopur™ C10.000) was added in an amount of 0.5 % and 1 % in the case of locust bean gum (all from SKW Biosystems, Rubí, Spain). The molecular weight of hydrocolloids are: 700 KDa for locust bean gum, about 1000 KDa for guar gum; 3000 KDa for xanthan gum; and about 2000-3000 KDa for sodium carboxymethylcellulose;. The average values for 100 grams of different ions included in the galactomanas (locust bean and guar gum) are 10 mg sodium, 330 mg of potassium, 95 mg of calcium and 40 mg of magnesium; in xanthan: 2700 mg of sodium, 300 mg of potassium, 85 mg of calcium and in sodium carboxymethylcellulose, the maximum content of salts (NaCl) 0.5 %. Mixtures of salts (0-1 % NaCl plus 0-1 % KCl plus 0-1 % plus CaCl₂) were also added according to the experimental design (response surface methodology) at concentrations of 0, 0.2, 0.5, 0.8 and 1 %, along with crushed ice to give the required final gel moisture (80 %). The homogenate was beaten slowly for 6 min under vacuum, 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.

Samples were removed from their casings, cut (3.5 cm diameter, 3 cm height) and tempered at 20 °C. The analyses were determined as described in Pérez-Mateos and Montero (2000) made at least six replications: folding test resistance of a slice (3.5 cm diameter, 3 mm height) folded over twice (score 1-5); puncture test (breaking deformation, breaking force, work of penetration) with a round-ended stainless steel plunger ($\varnothing=5$ mm) at a speed of 10 mm / min using a load-cell of 100 N; Texture Profile Analysis (hardness, adhesiveness, cohesiveness) at a deformation rate of 50 mm / min using a cylindrical plunger ($\varnothing = 58$ mm) adapted to a load-cell of 5 kN compressed to 60 %; and stress-relaxation test after 1 min of relaxation (elasticity). Also colour (L^* , a^* , b^*) using the CIE Lab scale (D65/10°) at least six replications were performed and water holding capacity determined by centrifuge method at least in triplicate.

Statistical analysis. Response Surface Methodology (RSM) was used to study the simultaneous effect of three independent variables (NaCl, KCl, CaCl_2) at concentrations of 0, 0.2, 0.5, 0.8, and 1 % for each batter according to a central composite rotatable design, using the Statgraphics statistical program (STSC Inc., Rockville, MD, USA). 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.

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

RESULTS

Gels made from mince with added locust bean gum

All the gels made from the muscle protein plus locust bean gum attained the maximum folding test scores (Table 1), irrespective of the proportion and type of added cations, even though the added salts brought about a slight decrease in the final protein concentration (13.80 % without added cations as opposed to 11.36 % with the highest quantity of added cation mixture 3 %). No significant differences were observed in the breaking force values with the added cations. The addition of CaCl_2 resulted in a significant decrease in the breaking deformation and work of penetration values (Table 2) and, conversely, the addition of NaCl resulted in a slight increase in breaking deformation (Figure 1.A). Added potassium did not significantly alter the mechanical properties measured using the puncture test.

The properties measured by the compression test (Table 3) underscored the effect of the

interaction between the monovalent cations (Na^+ and K^+), giving rise to softer gels ($p \leq 0.05$). Addition of the different types of cation considered had an antagonistic effect on the adhesiveness of the gels (Table 3). Thus, the addition of the monovalent salts (Na^+ , K^+) had a tendency to lower the adhesiveness, while the addition of Ca^{2+} tended to increase it ($p \leq 0.01$, linear component). The experimental analysis presented a rather high correlation coefficient ($r = 0.95$) for cohesiveness (Table 4). Cohesiveness increased slightly with the addition of NaCl ($p \leq 0.05$, linear component) and with the addition of the combination of NaCl and KCl ($p \leq 0.05$) but decreased sharply with the addition of the CaCl_2 ($p \leq 0.01$, linear component). Elasticity (Table 4) did not change significantly with the addition of either sodium or potassium, but it increased with the addition of Ca^{2+} ($p \leq 0.01$, linear component).

The water holding capacity of the gels containing locust bean gum (Table 1) increased appreciably with the addition of potassium or calcium cations ($p \leq 0.05$ and $p \leq 0.01$, respectively, quadratic component). The color readings (Table 5) showed that the lightness (L^*) and redness (a^*) values increased with CaCl_2 concentration ($p \leq 0.05$ and $p \leq 0.01$, linear component), although there was a tendency to decrease at high concentrations ($p \leq 0.01$, quadratic component) and in the combined presence of Ca^{2+} and K^+ ($p \leq 0.05$). On the other hand, the yellowness (b^*) value displayed a tendency to increase slightly with the addition of Na^+ and Ca^{2+} together ($p \leq 0.05$).

Exploratory factor analysis using the correlation matrix identified five principal components which explained over 89.7 % of the variance in the data. Table 6 presents the contribution of the 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 those variables assigned to different factors tended to be less correlated. Factor 1 explained 30.81 % of the total variance and included CaCl_2 but not the other two salts. This salt had a negative effect on cohesiveness

and a positive effect on elasticity. Although calcium chloride was the main contributor (with a high factor loading) to the factor 1, it also contributed to factor 2. Factor 2 explained 24.65 % of the total variance and was composed mainly of the variables breaking strength and work of penetration. NaCl was the only salt in factor 3, which explained 20.45 % of the variance and had a strong negative effect on the yellowness (b^*) value and water holding capacity, along with a less pronounced negative effect on hardness and adhesiveness. The potassium salt contributed to factor 4, which explained 15.82 % of the variance and exhibited a negative effect on hardness, adhesiveness, and the redness (a^*) value while positively affecting water holding capacity. Factor 5 (8.27 % of the variance) mainly comprised the folding test.

Gels made from mince with added guar gum

The effect of cations on the folding test of the blue whiting muscle mince with added guar gum is also summarized in Table 1. The addition of calcium cations (Ca^{2+}) lowered the gelling capacity, reflected by a decrease in the score ($p \leq 0.05$, linear component). Also, as shown by the RSM plots (Figure 1.B), addition of the salts had a negative effect on breaking deformation ($r = 0.95$), namely, at concentrations of Na^+ and K^+ higher than 0.5 % ($p \leq 0.01$ and $p \leq 0.05$, respectively, quadratic component) and at all concentrations of Ca^{2+} ($p \leq 0.01$, linear component). In contrast, calcium displayed two effects on breaking strength (Table 2): this added salt tended to increase gel strength as reflected by the quadratic component ($p \leq 0.05$), whereas it tended to bring about a decrease as reflected by the linear component ($p \leq 0.01$). The values for work of penetration ($r = 0.94$) were only affected negatively by the addition of increasing concentrations of divalent cations (Ca^{2+}) ($p \leq 0.01$, linear component). Mixtures adding a combination of cations did not cause any significant effect on the characteristics of the gel (Table 2).

Table 3 gives the results of the compression tests and indicates that the addition of extra salts did not significantly affect the hardness of the gels made using guar gum. However, the interaction of calcium and sodium ions did affect adhesiveness ($p \leq 0.05$). As in the case of the added locust bean gum, added calcium ($p \leq 0.01$, linear component) yielded less cohesive gels (Table 4). Addition of KCl ($p \leq 0.05$, quadratic component) lowered the elasticity (Table 4).

The results for water holding capacity (Table 1) showed increases for those formulations with concentrations of NaCl or CaCl_2 greater than 0.5 % ($p \leq 0.01$, quadratic component). The results of the color measurements (Table 5) showed that for the range of salt concentrations tested, addition of concentrations of potassium and sodium cations higher than 0.5 % ($p \leq 0.01$ and $p \leq 0.05$, respectively, quadratic component) tended to increase lightness ($r = 0.94$). This effect was particularly marked for CaCl_2 . The redness value as measured by the parameter a^* ($r = 0.95$) tended to increase with the addition of Ca^{2+} ($p \leq 0.01$, linear and quadratic components) and the addition of concentrations of the monovalent cations greater than 0.5 % ($p \leq 0.01$, quadratic component). Still, as reflected by the quadratic component, the a^* values decreased as a result of the effect of the interaction of the combination of cations (Na^+ and K^+). The yellowness (b^*) value tended to decrease with the addition of Ca^{2+} ($p \leq 0.05$, linear component).

Principal component analysis (Table 7) showed that 90.1 % of the variance could be explained by five factors. Breaking deformation, work of penetration, breaking strength, and color (L^* , a^*) were affected solely by calcium salt concentration (Factor 1: 47.66 %), all having high factor loadings. Cohesiveness was affected by calcium (Factor 1) and also, to a lesser extent, by sodium (Factor 5: 9.81 %), which had a major effect on adhesiveness. The potassium salt contributed to factor 4, which explained 11.12 % of the total variance. KCl exerted an

appreciable negative effect on elasticity. None of the salts contributed to the other factors; gel lightness (L^*) was the major contributor to factor 2 (16.47 %), and hardness to factor 3 (14.44 %).

5 **Gels made from mince with added xanthan gum**

Addition of sodium cations to the blue whiting mince with added xanthan gum resulted in a slight increase ($p \leq 0.05$, linear component) in resistance to the folding test (Table 1). On the other hand, addition of the potassium and calcium cations ($p \leq 0.05$, quadratic component and $p \leq 0.01$, linear component, respectively) tended to lower the resistance to folding. Figure 1.C depicts the RSM plots for breaking deformation and shows a profile that is quite similar to the profile for the gels made with locust bean gum (Figure 1.A). In both cases addition of sodium yielded gels that were more deformable, while addition of calcium ($p \leq 0.01$, linear component) had the opposite effect. Addition of any of the chloride salts at concentrations higher than 0.5 % tended to decrease the breaking strength as reflected by the quadratic component; calcium exerted that effect over the entire range of concentrations tested (Table 2). Addition of the combination of Na^+ and Ca^{2+} accentuated that effect. Addition of sodium increased the work of penetration values (Table 2). On the other hand, as for the rest of the hydrocolloids considered, addition of calcium had a negative effect. Nevertheless, at concentrations of sodium and potassium higher than 0.5 %, the quadratic component reflected a tendency for work of penetration to decrease. Similarly to the findings for breaking strength, addition of a combination of Na^+ and Ca^{2+} accentuated the negative effect on work of penetration values ($r = 0.97$).

25 Addition of sodium ions tended to increase softness in the gels made from blue whiting muscle homogenate with added xanthan gum, while addition of calcium lowered adhesiveness. Both

those changes were significant as reflected by the quadratic component (Table 3). Cohesiveness (Table 4) increased with added sodium and decreased with added calcium, an effect similar to that described above for the galactomannans (locust bean and guar gums). On the other hand, elasticity of the gels (Table 4) tended to increase with the addition of calcium ($p \leq 0.01$, linear component), as it did in the case of the gels made with locust bean gum. There was also a tendency for elasticity to undergo a slight increase at high concentrations of added sodium chloride salt.

As in the case of the nonionic gums (locust bean and guar gum) described above, addition of calcium ions acted to increase the water holding capacity ($p \leq 0.05$, quadratic component) and lightness ($p \leq 0.01$, linear component) of the gels made with added xanthan gum (Table 1). Addition of calcium also tended to increase the redness value ($p \leq 0.05$) and to decrease the yellowness value ($p \leq 0.01$) as reflected by the linear component.

Factor analysis extracted four factors which explained 89.8 % of the variance in the data (Table 7). CaCl_2 concentration (Factor 1) and NaCl concentration (Factor 3) exerted opposite effects on the characteristics of the gels: addition of calcium increased elasticity, while sodium decreased it; conversely, sodium increased cohesiveness, while calcium decreased it. Work of penetration, elasticity, breaking deformation, breaking strength, cohesiveness, and folding test values were all affected by the addition of calcium and sodium, which generally elicited opposing responses. In contrast, potassium, which contributed to factor 4, did not exert any effect on any of the characteristics considered.

Gels made from mince with added carboxymethylcellulose

In the gels containing carboxymethylcellulose, addition of calcium ($p \leq 0.01$, linear component)

had a negative effect on the folding test values (Table 1), as was also observed for the gels made with added guar gum and xanthan gum, although in those cases the effect was reflected by the quadratic component. Table 2 presents the penetration test results, and Figure 1.D graphically represents the results for breaking deformation. The trend in the breaking deformation values differed from the trends for the preceding gels (Figures 1.A, 1.B, 1.C), in that values tended to increase at concentrations greater than 0.5 %. The linear component for breaking strength ($r = 0.95$) tended to decrease with the addition of any of the salts. The trend for work of penetration ($r = 0.94$) with the addition of salt was rather similar to the trend for breaking deformation but differed in that it was negatively affected by sodium.

The results for the properties of the gels made with added carboxymethylcellulose indicated by the compression test showed that the addition of the chloride salts tended to produce softer gels, though for the potassium salt the quadratic component reflected the opposite effect (Table 3). Furthermore, the addition of any of the salts tested (NaCl, KCl, CaCl_2) tended to decrease adhesiveness ($r = 0.94$). As in the previous cases, the effect of the addition of calcium ions tended to make the gels less cohesive (Table 4) as reflected by the linear component. Gel elasticity ($r = 0.96$) tended to increase on addition of NaCl and CaCl_2 ($p \leq 0.01$, linear component), though the effect of the interaction between Na^+ and Ca^{2+} was negative (Table 4). Moreover, concentrations of KCl of around 0.5 % tended to lower gel elasticity, and the same occurred at very high concentrations of CaCl_2 .

Water holding capacity (Table 1) tended to decrease with the addition of the divalent salt (Ca^{2+}), and at concentrations of the monovalent cations (Na^+ and K^+) higher than 0.5 %; the correlation coefficient obtained was rather high ($r = 0.95$). Gel color (Table 5) was affected by all three chloride salts. In particular, lightness (L^*) tended to increase with the addition of calcium ions, which exerted a similar effect on the gels made with the other hydrocolloids studied. On the other hand, redness (a^*) values decreased with the addition of NaCl, and

yellowness (b^*) values decreased with the addition of CaCl_2 ($p \leq 0.01$, linear component), as was also observed for the gels made with guar gum and xanthan gum, but not for those made with locust bean gum.

- 5 Principal component analysis (Table 9) yielded four factors that explained 91.2 % of the variance. Factor 1 explained 44.07 % of the total variance and was composed primarily of certain properties measured by the penetration test (breaking deformation, breaking strength, work of penetration), elasticity, and lightness (L^*). Factor 2 (22.51 %) comprised KCl, which mainly altered hardness and adhesiveness, having opposite effects on those two parameters.
- 10 Factor 3 (19.29 %) in which the principal contributors were water holding capacity (WHC) and the color properties yellowness (b^*) and redness (a^*), with similar factor loadings, though with opposite signs. Factor 4 (14.13 %) comprised mainly NaCl with a high factor loading, though minor contributions were also made by certain functional properties of the gels. Both deformation and work of penetration contributed only to factor 1, and hence they were not
- 15 affected by either the sodium or the potassium salts, which did not contribute to that factor.

DISCUSSION

- In view of the large number of different variables involved in the gelling process, similar
- 20 processing conditions and formulations were the foundation for the experimental design, with changes only in salt concentration and hydrocolloid type, in order to be able to distinguish more clearly the effect of the salts. In addition, again because of the large number of factors involved, response surface methodology was employed as a screening technique to select the key factors affecting responses.
- 25 otro sitio?

In principle, differing behaviors might be expected for formulations containing ionic gums (xanthan gum and carboxymethylcellulose) and for the formulations containing nonionic gums (locust bean gum and guar gum); nevertheless, similar behaviors were not observed as a result of hydrocolloid charge. Este parrafo es para borrar de aqui, lo pongo al final de dsicusion

Addition of any of the individual salts did not exert any significant effect on the folding test values for the gels that contained locust bean gum; on the other hand, adhesiveness was affected. Addition of any of the salts to the gels that contained guar gum altered gel color (L^* , a^*). In the gels that contained an ionic gum, addition of cations significantly altered the folding test, breaking strength, and work of penetration values for the gels made with xanthan gum and most of the properties analysed (breaking strength, work of penetration, adhesiveness, hardness, elasticity, and water holding capacity) for the gels made with carboxymethylcellulose.

In previous work (Montero, Hurtado & Pérez-Mateos, 2000), it was reported that the anionic hydrocolloids were mixed throughout the protein matrix, probably through interaction with the myofibrillar protein and the neutral hydrocolloids were dispersed throughout the matrix but did not interact with it. It is important consider that at the same percentage of salt, there will be about the double of monovalent ions than divalent ions. Measuring turbidity and rheology, Bryant and McClements (2000) observed that xanthan gum caused a significant increase in the gelation rate of whey protein solutions in the presence of salt (NaCl) due to the formation of xanthan-rich regions embedded in a whey protein gel.

Considering the type of salt added, CaCl_2 exerted the most pronounced effects, significantly altering the cohesiveness, elasticity, color (L^* , a^* , b^*), water holding capacity, and folding test values in all the gels, irrespective of whether they contained a nonionic or an ionic gum

(except folding in the case of locust bean gum). Thus, calcium seemed to have the effect of contributing to the formation of certain types of bonds that are readily broken when subjected to an outside force, giving rise to gels with low cohesiveness, low folding test values, and low deformability but a highly elastic structure, such that the gels are able to recover when the force applied is removed. The increase in lightness (1-2 %) may have been caused in part by the white color of the salt itself and also in part by the gel structure that formed.

Use of a combination of the salts gave rise to certain significant changes, such as the interaction between the sodium and the calcium on the xanthan gum, leading to a decrease in the breaking strength and work of penetration, and on the guar gum, leading to an increase in adhesiveness. The different behaviour of cations, maybe explained for the size of the cation, small ion may be able to interact better with anionic groups located more towards the inside of the molecular structure. Thus, alkaline ions will have more accesability??? due to their smaller size, and in particular potassium compare with sodium in its hydrated state.

The interaction mechanism could attribute to the size of regions non replaced and also to the size or molecular weight, and the charge of lateral groups. For example, guar gum has less short regions without lateral groups than locust bean gum. It is possible that the highest molecular weight of ionic gums (xanthan gum and carboxymetulcellulose) may act modified the network mainly interruntng them; however, the low molecular weight of neutral gums (locust bean and guar gum) probably facilitate the formation of bonds with the cations and association with other macromolecules (protein or hydrocolloid); however lateral chains difficult the ions bonds produce some small cavities inside the matrix no se si es demasiado tajante y si se puede decir en comparacion con la gran cantidad de proteina que hay y el 0.5 % de hidrocoloide??? Quizas mejor decir :lo que pongo en rojo, but not interacct with it. The gums were added in the dry state and the water would be retained mainly by the myofibrillar protein and hence the gums would have little available water in comparison with aqueous system. In

this conditions, xanthan gum would tend to aggregate and coil upon itself; also carboxymethylcellulose requires a lot of water in order to disperse well. Moreover in complex system like fish mince is, 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.

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There have not been many studies dealing with the effect of these salts in gels made from fish muscle – washed mince or surimi- (Gómez-Guillén & Montero, 1996; Gómez-Guillén, Borderías & Montero, 1996). There are large variations according to myofibrillar protein quality, and hence studies of variability within the same myosystem using different formulations (hydrocolloid and salt) hold our interest. Studies of different concentrations of NaCl (1.0 % and 2.5 %) using blue whiting mince showed that low level of salt improved the overall functional properties compared with higher concentration (Pérez-Mateos, Lorenço, Montero & Borderías, 1997).

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Principal component analysis revealed that the variables that made major contributions to the same factor tended to be highly correlated, whereas the variables that contributed to different factors tended to exhibit low correlations. The fact that, on the whole, each of the different salts contributed to a different factor may be indicative that each cation exerted a mostly independent effect on the gelling properties of the muscle protein–gum mixtures. Still, as we have observed in the RSM studies, in some cases there were minor interaction effects between the sodium and the calcium.

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In the formulations made using locust bean gum, elasticity, cohesiveness, redness (a^*), and work of penetration were only affected by the addition of CaCl_2 , whereas other properties, such as gel hardness, were affected by all three salts. In the formulations made with guar gum, the contribution of the calcium concentration to factor 1 was indicative of that salt's effect on most of the mechanical properties (folding test, breaking deformation, breaking strength,

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work of penetration, cohesiveness, adhesiveness) and color (L^* , a^*), either increasing or decreasing the values, whereas sodium only affected adhesiveness and to some extent cohesiveness, and potassium only affected elasticity. In experiments carried out using isolated systems comprising starch-guar combinations, Sudhakar *et al.* (1996) suggested that salts (KCl and CaCl_2) facilitated hydration of the guar gum.

In the gels with xanthan gum, calcium again contributed to factor 1, affecting a large number of properties contributing to that factor (breaking strength, breaking deformation, work of penetration, elasticity, color (L^* , a^* , b^*), cohesiveness, and folding test). In contrast, NaCl and KCl contributed to factors of lesser importance, with sodium exerting a positive effect on breaking strength, breaking deformation, cohesiveness, hardness, and folding test and potassium a slightly negative effect on redness (a^*). In the formulation using carboxymethylcellulose as the gelling adjuvant, elasticity was much more affected by the divalent salt than by the monovalent salts; hardness was affected by all three salts, KCl exerting the greatest effect.

Calcium always contributed to factor 1, explaining more than 50 % of the variance, indicating that the addition of that salt brought about more pronounced changes than the addition of the monovalent salts. According to Tang *et al.* (1997), divalent cations might act differently when inducing cross-linking between polymers, in that because they are more highly charged they are more effective in shielding electrostatic repulsive forces between adjacent carboxyl groups in junction zones and also form direct polyanion-cation-polyanion linkages between polymer chains. Susheelamma, Chand and Rajalakshmi (1994) recorded an increase in the rheological parameters (yield stress and consistency index) with the calcium concentration. Ma and Barbosa-Cánovas (1997) attributed an increase in the viscoelastic properties of xanthan gum to the formation of non-covalent bonds on addition of the sodium and calcium ions. In this respect, Rosett *et al.* (1996) found that anionic substituents of xanthan gum interacted with

sodium by ionic binding, while this was not the case for carboxymethylcellulose, due to the considerably higher endogenous cation content.

According to Niwa, Tsujimoto and Kanoh (1992), the increase in the physical properties of surimi with the increase in the ratio of CaCl_2 was not so remarkable when compared with that induced by addition of polysaccharides. In that respect, in a previous paper Pérez-Mateos and Montero (2000) reported that higher percentages of hydrocolloid (locust bean gum, guar gum, xanthan gum, carboxymethylcellulose) significantly reduced the rheological properties; on the other hand, water holding capacity was lowest at low concentrations, and gel color was virtually unaffected by the presence of different proportions of hydrocolloid in the formulation. Gómez-Guillén and Montero (1996) found that adding NaCl and certain hydrocolloids had differing and sometimes even opposite effects on gelling of mince depending on the functional properties of the mince.

Rosett *et al.* (1996) indicated that if the endogenous cation content of the ionic gums was very high, the binding sites were saturated, hence the behavior of the salts was nearly the same for both ionic and nonionic gums. Therefore, the failure to record large differences between the different salts may be due to the original salt content initially present in the hydrocolloids and also, in part, to the high proportion of salt taken up by the muscle (despite its having been homogenized and solubilized with NaCl beforehand), thus masking the alterations on the hydrocolloid, present in a smaller proportion than the myofibrillar protein.

In principle, differing behaviors might be expected for formulations containing ionic gums (xanthan gum and carboxymethylcellulose) and for the formulations containing nonionic gums (locust bean gum and guar gum); nevertheless, similar behaviors were not observed as a result of hydrocolloid charge. RSM and factor analysis for each of the mince-hydrocolloid formulations revealed the principal functional properties altered by each of the salts and the

possible effect of interactions between the salts, a highly useful finding holding out a broad range of practical possibilities. The range of restructuring possibilities is known to depend not only on the source species for the muscle tissue but also the functional quality of the muscle and the presence of additives. Addition of different cations broadens the range of options for use in producing gels, and in some of the formulations the effects of the added salts followed similar patterns despite the use of different hydrocolloids. Thus, adding calcium yielded elastic but not very cohesive gels with all of the hydrocolloids studied except guar gum, in which case elasticity did not undergo any significant alteration. Adding sodium or potassium increased cohesiveness in the gels made with locust bean gum while decreasing hardness and adhesiveness in the gels made using carboxymethylcellulose.

From technological point of view, the addition of hydrocolloids and ions to blue whiting mince allow to obtain a very wide range of gel properties. The percentage to favour almost of the mince gel properties were about 0.5 % NaCl plus 0.5 % KCl for gels containing locust bean gum and guar gum; 0.7 % NaCl plus 0.5 % KCl for xanthan gum; and 1 % NaCl in the case of carboxymethylcellulose.

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