Running title: pressure gelling mince – additives

Carrageenans and alginate effects on properties of combined 5 pressure and temperature in fish mince gels

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Abstract

Sulphated polysaccharides (kappa and iota carrageenan) and sodium alginate added to blue whiting mince were subjected to three different pressure/heat treatments in order to determine the functionality of each one in mince gel. The effect of the gelling treatment was largely dependent on the hydrocolloid used. In general, gelation at atmospheric pressure induced gels that were more adhesive, harder (except in the case of iota-carrageenan) and yellow, and less cohesive. Lower pressure conditions (200 MPa, 10 °C, 10 min) produced more cohesive gels with higher breaking deformation and lower elasticity; these gels also had the highest values of work of penetration (especially those containing iota-carrageenan). Higher pressure conditions (375 MPa, 37 °C, 20 min) induced gels with the lowest hardness; all other characteristics were similar in some cases to pressurized gels at lower gelling conditions and in others to heat-induced gels. The carrageenans (iota or kappa) appeared to form a reticular structure in the heat-induced gels, which was not observed with alginate. In the pressurized gels, iota-carrageenan was in globular form, indicating that it had not gelled; kappa-carrageenan, on the other hand, formed small, fine reticular structures. Alginate formed a fine, dense network in the higher treatment conditions, but this was not observed in the lower treatment conditions. From a technological standpoint, the composite system offers new potential, due not only to the added hydrocolloid but also to the treatment applied.

Key words: high pressure, carrageenan, alginate, mechanical properties, structure, mince

INTRODUCTION

Interactions between proteins and polysaccharides have been shown to be an essential element in the study of food texture. The role of hydrocolloids as thickeners or gelling agents in food processing is widely known (Glicksman, 1983; de Ruiter & Rudolph, 1997); however, traditionally only starch, carrageenans and alginate have been used in restructured fish products made by heat treatment. In recent years, there has been some studies on modification of hydrocolloid functionality under high pressure in aqueous systems. In the case of carrageenans (Tsen & King, 1994; Fernandes & Raemy, 1996; Steyer, Béra, Massaux, Sindic, Blecker & Deroanne, 1999), it has been observed that gelation occurs within a pressure range of 200-600 MPa at warm temperatures (30-60 °C) and over varying times (10-120 min). Different pressurizing conditions have also been reported in experiments carried out with sodium alginate. For example, Shioya, Hirano, Tobitani (1994) found gel formation at 900 MPa, whereas Shwertfeger (1999) found that lower pressure (100 MPa) was required for gel homogenization of calcium alginate.

As regards the behaviour of mixed hydrocolloid/protein in aqueous systems under high pressure, Galazka, Smith, Ledward and Dickinson (1999) reported that the complexes of anionic polysaccharide with proteins such as bovine serum albumin appears to protect the protein against pressure-induced aggregation due to disulphide bridge formation during or after high pressure treatment. Recently, Dumay, Laligant, Zasytkin and Cheftel (1999) studied the microstructure of pressure-induced gels of mixed β -lactoglobulin and sodium alginate in an aqueous system. However, the gelling behaviour of hydrocolloids can be very different in an aqueous system as compared to a muscle batter; this is due both to the quality of the protein and to the diversity of the proteins involved and the difference in availability of water. In general, the gelling properties of proteins can be modified or controlled through interactions with polysaccharides; however, little research has been reported on how some polysaccharides

affect the properties of gels prepared by different treatments.

The first aim of this study was to examine the physical properties of gels formed under high pressure with different pressure – time - temperature combinations in a composite system of fish mince and hydrocolloid (carrageenan - iota or kappa - and sodium alginate), something that has never been reported before in this kind of system under high pressure gelling conditions. The second aim was to elucidate the functional role of each hydrocolloid assay in the matrix formed.

MATERIALS AND METHODS

Blue whiting (Micromesistius poutassou Risso) used in this study was caught off the Cantabrian coast in November. Average size was 22.2 ± 1.8 cm and average weight 101.1 ± 22.3 g. The proximate composition (%) was: crude protein 13.25 ± 0.31 , moisture 81.75 ± 0.71 , crude fat 0.16 ± 0.03 and ash 0.40 ± 0.05 (analyses do not show 4.2 % added cryoprotectant) following analyses described in Pérez-Mateos, Lourenço, Montero & Borderías (1997).

Fish were headed, gutted and washed. Skin and bones were removed with a deboning machine (Baader 694, Lübeck, Germany). The resulting mince (3 mm o.d.) was washed in a solution of NaCl (0.2 %) at 0-3 °C, proportion 3:1 (solution:minced muscle), first with constant stirring for 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, Giralte, Revco Scientific, Inc., Asheville, N.C., USA) in order to minimize alteration during frozen storage up to gel preparation.

Gel preparation: Washed blue whiting mince was tempered in a chilled room and placed in a refrigerated vacuum homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Germany). It was ground for 1 min at high speed. Sodium chloride (1 % w/w) in gel (Panreac, Montplet & Esteban S.A., Barcelona, Spain) was added and homogenized for 3 min at slow speed. Then the hydrocolloid [iota-carrageenan as Satiagel™ RPT25, kappa-carrageenan as Satiagel™ RPT8, or sodium alginate as Satiagine™ S1100 (SKW Biosystems, Rubí, Spain)], at a final concentration of 0.5 % for each hydrocolloid, was used with crushed ice to give the required final gel moisture content (80 %); and the extra salts added were NaCl and KCl in light of the results of Pérez-Mateos (1998). The following treatments were applied, as described in Pérez-Mateos and Montero (2000b): 200 MPa, <10 °C, 10 min (gel L) and 375 MPa, 37 °C, 20 min (gel H); which reported characteristics of the gels without gum added using a mince with similar capacity of gelation to the one used in the actual work. The lower pressure conditions will be focus to enhance the myofibrillar gelation and the higher pressure conditions to facilitate the hydrocolloid action. It has been reported that myofibrillar gelation used to be about 200-300 MPa at cold temperature during short times, unlike polysaccharides gelation in aqueous system which was reported to be at higher pressures conditions. The control gel was obtained by traditional gelation at atmospheric pressure under heat treatment: 37 °C, 30 min followed by 90 °C, 50 min (gel T). High –pressure treatments were performed in a high pressure pilot unit (ACB 665, Gec Alsthom, Nantes, France) where the temperature of the immersion medium (water) was controlled via a thermo-couple and kept constant by a temperature control circulation bath. Pressure was increased at 2.5 MPa/s. All the casings were immediately cooled with 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 [2] made at least six replications: folding test resistance of a slice folded over twice (score 1-5); puncture

test [breaking deformation (mm), breaking force (N), work of penetration (N.mm)] 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.

Scanning electron microscopy (SEM): Cubes of 2 to 3 mm were cut from inside the gels for microscopic examination. Samples were fixed in 2 % glutaraldehyde in phosphate buffer (pH 7.3) and dehydrated in increasing series of acetone (from 40 to 100 %). They were then critical-point dried with CO_2 as transition fluid in a dryer (Balzer CPD030, Liechtenstein) and mounted on copper sample holders, followed by sputter-coating with gold in a metallizer (Balzer SCD004). Samples were kept in a dryer until examination by scanning microscope (Jeol, JSM 6400, Japan) at 20 kV. Micrographs were taken of each gel at different magnifications.

Statistical analysis. One-way analysis of variance was carried out using the BMDP computer programme (BMDP Statistical Software, Inc., Cork Technology Park, Cork, Ireland) to determine differences among treatment-dependent characteristics of gels. The difference of means between pairs was resolved by means of confidence intervals using Bonferroni test (BMDP 7D). Level of significance was set for $p \leq 0.05$.

RESULTS

Characteristics of blue whiting gels with added iota-carrageenan were shown in figure 1. The gelling treatment at atmospheric pressure (T) produced gels that were more adhesive, less cohesive, lighter and yellower than pressure-induced gels ($p \leq 0.05$). The pressurized gels differed according to the pressurizing conditions. The pressurized gels at the lower gelling conditions (200 MPa, $<10^\circ\text{C}$, 10 min) presented significantly higher values for the properties determined in the penetration test (breaking deformation, breaking force and work of penetration) and for cohesiveness ($p \leq 0.05$) than the pressure-induced gels at higher treatment conditions (gel H); their elasticity and lightness were also lower ($p \leq 0.05$). Gel H (375 MPa, 37°C , 20 min) was the softest ($p \leq 0.05$); for all other properties, the characteristics were similar to the pressurized gel at lower gelling condition (gel L) and in other cases more like those of the non-pressurized gel (T). Water holding capacity (WHC) was higher in pressurized gels at lower gelling conditions than in non-pressurized gels ($p \leq 0.05$).

Despite the treatment-dependent difference in the rheological behaviour of the gels, at 500 magnifications the structural appearance of the gels containing iota-carrageenan was quite similar (Fig.2.A, B,C). The gels generally presented a fairly compact matrix with scarcely any cavities; however, the heat-induced gels presented more aggregation and porosity. In terms of ultrastructure, the heat-induced gel (T) exhibited a highly dense network composed of short, thin filaments, whereas the pressure-induced gels showed some more or less irregularly shaped globular structures and continuous zones with a somewhat translucent appearance.

Characteristics of gels containing kappa-carrageenan were shown in figure 3. The heat-induced gel (T) presented particularly high hardness and adhesiveness and low cohesiveness. As regards colour, the heat-induced gels were more yellow ($p \leq 0.05$) but had lower water holding

capacity. As in the case of gels containing iota-carrageenan, the pressure-induced gels, and particularly gel L (200 MPa, <10 °C, 10 min), presented notably high breaking deformation and cohesiveness, less elasticity and low lightness ($p \leq 0.05$); on the other hand, the differences in puncture test properties were not as great as in the case of iota-carrageenan.

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The matrix of the heat-induced gel containing kappa-carrageenan presented a more aggregated appearance (Fig.4.A). The pressure-induced gels presented a more compact appearance (Fig 4.B,C), with larger cavities in gel L. At higher magnifications, a reticular structure was visible, composed of small independent filaments, irrespective of the treatment (Fig. 4. E, D).

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Rheological characteristics, water holding capacity and colour of blue whiting gels with added alginate (Fig. 5). The heat-induced gels (37 °C 30 min / 90 °C 50 min) again were harder, more adhesive and less cohesive ($p \leq 0.05$), and more yellow (b^*). Of the pressure-induced gels, those pressurized at lower gelling conditions (gel L: 200 MPa, <10 °C, 10 min) presented high values for breaking deformation, breaking force, work of penetration, cohesiveness and water holding capacity; and lower values for adhesiveness, elasticity and lightness ($p \leq 0.05$). Gel H (375 MPa, 37 °C, 20 min) was the most elastic by a significant margin; the values of all other characteristics were similar to those of other gel types (T and L).

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In the SEM images (Fig. 6.A, B, C), the matrix of the gels containing sodium alginate shows considerable aggregation with no definite orientation. In the heat-induced gel (T), there are homogeneous zones, with small reticular zones dispersed throughout the matrixes (Fig. 6.D). In the pressurized gel at lower gelling condition (gel L: 200 MPa, <10 °C, 10 min) (Fig. 6.E), the hydrocolloid is indistinguishable from the protein matrix; whereas in the lightly-heated pressure gel there is a fine but dense reticular structure with points of connection to the matrix (Fig. 6. F).

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DISCUSSION

There were noticeable treatment-dependent differences in gel characteristics, which seems to suggest that the influencing factor was the myofibrillar protein rather than the hydrocolloid.

5 Irrespective of the hydrocolloid added, the pressurized gel at lower treatment conditions (200 MPa, <10 °C, 10 min) generally presented high values for the penetration test properties (breaking deformation, breaking force and work of penetration). Pressurized gels were generally described as more deformable than heat-induced gels; however, this is not always associated with greater elasticity (Pérez-Mateos & Montero, 2000b). The high pressure conditions used are
10 probably more suitable for inducing protein aggregation in mince than for modifying the functionality of the added hydrocolloid, considering both the water content and the processing conditions (pressure – time - temperature). Our results are consistent with the work carried out in chicken meat batters of Fernández, Cofrades, Solas, Carballo and Colmenero (1998) which indicate that pressure clearly predominated over ingredient effects in high pressure -cooking.
15 However, the behaviour of the hydrocolloid in aqueous systems differs considerably depending on its type (Dumay et al., 1999).

Heat-induced gels were more opaque than pressurized gels at cold temperature (200 MPa, <10 °C, 10 min); and the gel induced at higher conditions (375 MPa, 37 °C, 20 min) showed
20 intermediate characteristics. Regarding the effect of hydrocolloid, pressure-induced gels containing alginate exhibited higher yellowness (b^*) values than gels with carrageenans added. In experiments carried out without hydrocolloid added, pressurized gels were described as glossier, smoother and soft to touch than heat induced gels (Pérez-Mateos, Lourenço, Montero. & Borderías, 1997).

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In heat treatment, the effect of the hydrocolloid is known to depend on the gel-forming capacity of the myofibrillar protein, so that the addition of gelling agents or thickeners detracts from the

product's rheological properties where the muscle presents high gel-forming capacity; on the other hand, these properties are enhanced where the muscle possesses low gel-forming capacity (Lee, Wu & Okada, 1992). Under high pressure treatment, Pérez-Mateos and Montero (1997) reported that the properties of the gels were enhanced more when the mince used had low gel-forming capacity than when a good quality mince was used. Further research would be useful to ascertain the behaviour of the hydrocolloid under high pressure as influenced by mince qualities.

Gel hardness decreased significantly with increased pressure conditions (pressure-time-temperature), especially in gels containing kappa-carrageenan; there were significant differences due to treatments. Generally, compression tests are related best to the sensory data than punch tests; therefore, hardness and elasticity are more correlated with the mouth feel of the samples.

In experiments carried out in solution of iota-carrageenan (3 %), Steyer et al. (1999) found maximum hardness at 200 MPa / 120 min / 60 °C, decreasing with the applied pressure and the temperature, so that if applied at low temperature (20 °C) a gel structure would not even be induced; however no microscopy studies were reported. Also, Schwertfeger (1999) reported that viscosity decreased under pressure higher than 100 MPa due to the increase of repulsive forces and the straight conformation of polymer molecules that prevent entangling of the chains. According to Gekko (1994), pressure induced the destabilization of iota-carrageenan gels due to the release hydration water following polymer-polymer hydrogen bonding.

Unlike the gels with iota-carrageenan (Fig.1), in the case of kappa-carrageenan (Fig. 3) non-pressurized gelling produced gels that were harder but presented lower values for work of penetration. The reason could be that these properties are determined by different tests and may therefore explain different effects. As suggested by Burgarella, Lanier, Hamann and Wu

(1985) and Lee and Chung (1989), the penetration test determines the degree of compacting or density of the network caused by the degree of aggregation, whereas the compression test measures the binding properties of the matrix as a whole. This could suggest that heating promotes more protein unfolding than pressurization; however, it seems that the greater
5 compacting of the matrix in pressure-induced gels is due to the physical effect of the pressure rather than increased protein aggregation (Montero, Pérez-Mateos &, Solas, 1997).

Regarding the way that pressure acts on anionic hydrocolloids in aqueous systems, Fernandes and Raemy (1996) reported that during the application of high pressure (up to 800 MPa at 30 -
10 50 °C), polysaccharides (kappa-carrageenan, xanthan gum and HM-pectin) and whey protein were partially unfolded with a subsequent increase in hydration that could lead to volume exclusion effects originating from incompatibility between unlike biopolymers. Galazka et al. (1999) suggested that the application of pressure probably leads to dissociation of complex protein with sulphated polysaccharide; in this way, the biopolymers would unfold and expose
15 more charged groups during the maintenance of high pressure; then, when pressure was released, interactions would be reformed more strongly and recomplexation would occur, protecting the protein against loss of functionality or aggregation. However, there were no microscopy studies to corroborate these findings at the ultrastructural level.

20 Other studies (Shioya et al., 1994) suggest that pressure may increase the hydration of sodium alginate and the dissociation of calcium salts, so that the carboxyl groups of alginate may react with calcium ions. In our study, no such effect was observed because calcium salt was not added since the type of alginate used was sodium, and no enhancement of gel-forming ability was observed with addition of ions (Pérez-Mateos, 1998). In this connection,
25 Gustin, Bera, Dumont and Mertens (1997) suggested a gelling model based on the redistribution of calcium after high pressure processing, plus the precise water-availability and sufficient polysaccharide concentrations required.

Recent optical microscopy observations by Montero, Hurtado and Pérez-Mateos (2000) showed heat induced gels with iota-carrageenan having small, round cavities and those with kappa-carrageenan having large, elongated cavities. In the heat-induced gel containing iota-carrageenan (Fig.2), there was a clearly observable mesh distributed throughout the matrix; however in the pressure-induced gels, the iota-carrageenan appeared in globular formations suggesting that the hydrocolloid be not gelatinised. In the heat-induced gel containing kappa-carrageenan (Fig.4), there were homogeneous-looking filaments connecting reticular zones, which could have been gelatinised hydrocolloid; in the pressurized gels at lower gelling conditions (gel L; 200 MPa, <10 °C, 10 min), on the other hand, the reticular structure was finer and less well-defined. This could have been because at atmospheric pressure kappa-carrageenan gells at over 70 °C (Glicksman, 1983; de Ruiter & Rudolph, 1997); whereas according to a study carried out by Fernandes and Raemy (1996) in aqueous systems under high pressure (600 MPa for 10 min), it does so at lower temperatures (30 °C). Brighman, Gidley, Hoffmann and Smith (1994) also observed a homogeneous structure of thin and thick fibres in kappa-carrageenan gels, while they were thinner in the case of iota-carrageenan.

The SEM images of the thermal gels with alginate are also consistent with previous results obtained by light microscopy from Montero et al. (2000), which indicate that the alginate was located inside relatively small elongated, crack-like cavities and partially connected to the protein matrix.

Probably because a myosystem is more complex than an aqueous system, our results are not consistent with earlier studies by Dumay et al. (1999) using a mixture of β -lactoglobulin (12 %) and sodium alginate (0.1 %) which revealed microalveoles with a honeycomb structure in pressure-induced gels. According to these authors, it could suggest that pressure induces phase separation, probably depending on the net negative charges carried by the

polysaccharide and on the imparted solution viscosity. They further indicated that the addition of 0.25 % alginate to the solution had the same effect as an increase in protein concentration. However, Pérez-Mateos, Hurtado, Montero and Fernández-Martín (2001) observed by differential scanning calorimeter (DSC) that alginate underwent irreversible exothermic thermal degradation and would therefore present this kind of drawback if used in surimi-based products or other systems requiring high-temperature-setting processes.

From a practical point of view, the results indicate that the textural properties of gels can be modified not only by the addition of ingredients but by different gelling treatments, which change the behaviour of both mince and ingredient. The fact that these hydrocolloids are effective gelling agents / thickeners of the muscular protein network structure suggests that they could be useful in extending the range of gelling properties.

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REFERENCES

- Brigham, J.E, Gidley, M.J., Hoffmann, R.A. & Smith, C.G. (1994). Microscopic imaging of network strands in agar, carrageenan, locust bean gum and kappa cararageenan / locust bean gum gels. Food Hydrocolloids, (3-4):331-344.
- Burgarella, J.C., Lanier, T.C., Hamann, D.D. & Wu, M.C. (1985). Gel strength development during heating of surimi in combination with egg white or whey protein concentrate. Journal of Food Science, 50:1595-1597.
- Dumay, E., Laligant, A., Zasytkin, D. & Cheftel, J.C. (1999). Pressure and heat induced gelation of mixed polysaccharide solutions: scanning electron microscopy of gels. Food Hydrocolloids, 13:339-351.
- Fernandes, P.B. & Raemy, A. (1996). High pressure treatment of whey protein / polysaccharide systems. In R. Hayashi & C. Balny, High Pressure Bioscience and Biotechnology, (pp. 337-342). Tokyo, Japan: Elsevier Sci.
- Fernandez, P., Cofrades, S., Solas. M.T., Carballo, J., Colmenero, F.J. (1998). High pressure-cooking of chicken meat batters with starch, egg white, and iota carrageenan. Journal of Food Science, 63: (2) 267-27.
- Galazka, V.B., Smith, D., Ledward, D.A. & Dickinson, E. (1999). Complexes of bovine serum albumin with sulphated polysaccharides: effects of pH, ionic strength and high pressure treatment. Food Chemistry, 64:303 - 310.
- Gekko, K. (1994). The sol-gel transition of food macromolecules under high pressure. In K.

Nishinari & E. Doi, Food Hydrocolloids: Structures, properties and functions (pp.259-264). New York: Plenum Press.

Glicksman, M. (1983). Red seaweed extracts (Agar, carrageenan, furcellaran). In M. Glicksman, Food Hydrocolloids (Vol 2) (pp.73-113). Boca Raton FL, USA: CRC Press, Inc.

Gustin, D., Bera, F., Dumont de Chassart, Q. & Mertens, B. (1997). Pectin gelification under HHP: gel properties and formation mechanisms. In K. Heremans, High Pressure Research in the Biosciences and Biotechnology. Leuven, Belgium: Leuven Univ. Press.

Lee, C.M. & Chung, K.H. (1989). Analysis of surimi gel properties by compression and penetration test. Journal of Texture Studies, 20:363-377.

Lee, C.M. ; Wu, M.C. & Okada, M. (1992). Ingredient and formulation technology for surimi-based products. In T. Lanier & C. Lee, Surimi Technology (pp.:273-302). New York: Marcel Dekker, Inc.

Montero, P., Pérez-Mateos, M. & Solas, T. (1997). Comparison of different gelation methods using sardine (Sardina pilchardus) mince: effect of temperature and pressure. Journal of Agricultural and Food Chemistry, (45):4612-4618

Montero, P., Hurtado, J.L. & Pérez-Mateos, M. (2000). Microstructural behaviour and gelling characteristics of miosystems protein gels interacting with hydrocolloids. Food Hydrocolloids, (14) 455-461.

Pérez-Mateos, M. (1998). Estudio de hidrocoloides en la gelificación del músculo de bacaladilla (Micromesistius poutassou, Risso) inducida térmicamente y por alta presión. Doctoral Thesis,

University of Alcalá (Madrid), Spain.

Pérez - Mateos, M. & Montero, P. (1997). High pressure induced gel of sardine (Sardina pilchardus) washed mince as affected by pressure - time - temperature. Journal of Food Science, 62(6):1183 - 1188.

Pérez-Mateos, M. & Montero, P. (2000a). Contribution of hydrocolloids to gelling properties of blue whiting muscle. European Food Research and Technology, 210:383-390.

Pérez-Mateos, M. & Montero, P. (2000b). Response surface methodology multivariate analysis of properties of high-pressure-induced fish mince gel. European Food Research and Technology, 211:79-85.

Pérez-Mateos, M., Lourenço, H., Montero, P. & Borderías, A.J. (1997). Rheological and chemical studies on high pressure - induced gels from blue whiting (Micromesistius poutassou) muscle proteins. Journal of Agricultural and Food Chemistry, 45:44 - 49.

Pérez-Mateos, M., Hurtado, J.L., Montero, P. & Fernández-Martín, F. (2001). Interactions of kappa-carrageenan plus other gums in fish myosystem gels. Journal of Food Science 66 (5) (in press)

de Ruiter, G.A. & Rudolph, B. (1997). Carrageenan biotechnology. Trends in Food Science Technology, 8:89-95.

Schwertfeger, M. (1999). Alterations of calcium alginate gel dispersions by high pressure treatment. In: H. Ludwig, Advances in High Pressure Bioscience and Biotechnology (pp. 337-340). Heidelberg, Germany: Springer.

Shioya, T., Hirano, R. & Tobitani, A. (1994). Utilization of high hydrostatic pressure to make alginate gels. In K. Nishinari & E. Doi, Food Hydrocolloids: Structures, Properties, and Functions (pp. 265-268). New York: Plenum Press.

5

Steyer, B., Béra, F., Massaux, C., Sindic, M., Blecker, Ch. & Deroanne, C. (1999). Carrageenan gelification under high hydrostatic pressure: preparation and processing of solutions and analysis of gels obtained. In H. Ludwing, Advances in High Pressure Bioscience and Biotechnology (pp.353-356). Heidelberg, Germany: Springer.

10

Tsen, J.H. & King, V.A.E. (1994). Studies on high-pressure-induced gelation of carrageenan using response surface methodology. Journal of the Chinese Agricultural Chemical Society, (in Japanese with abstract in English) 32(5):543-552.

Figure 1.- Mechanical properties, water holding capacity and colour of blue whiting gels with 0.5 % iota – carrageenan added. Where T means 37 °C, 30 min / 90 °C 50 min at atmospheric pressure; L: 200 MPa, <10 °C, 10 min; H: 375 MPa, 37 °C, 20 min.

5 **Figure 2.-** SEM images of mince gels with 0.5 % iota – carrageenan added. A, B, C: 500 magnifications; and D, E, F: 1000 magnifications. Where A, D means 37 °C, 30 min / 90 °C 50 min at atmospheric pressure; B, E: 200 MPa, 7 °C, 10 min; and C, F: 375 MPa, 37 °C, 20 min.

Figure 3.- Mechanical properties, water holding capacity and colour of blue whiting gels with 0.5
10 % kappa – carrageenan added where T means 37 °C, 30 min / 90 °C 50 min at atmospheric pressure; L: 200 MPa, <10 °C, 10 min; H: 375 MPa, 37 °C, 20 min.

Figure 4.- SEM images of mince gels with 0.5 % kappa – carrageenan added. A, B, C: 500 magnifications; D: 3000 magnifications and E: 1000 magnifications. Where A, D means 37 °C,
15 30 min / 90 °C 50 min at atmospheric pressure; B, E: 200 MPa, 7 °C, 10 min; and C: 375 MPa, 37 °C, 20 min.

Figure 5.- Mechanical properties, water holding capacity and colour of blue whiting gels with 0.5
20 % sodium alginate added where T means 37 °C, 30 min / 90 °C 50 min at atmospheric pressure; L: 200 MPa, <10 °C, 10 min; H: 375 MPa, 37 °C, 20 min.

Figure 6.- SEM images of mince gels with 0.5 % sodium alginate added. A, B, C: 500 magnifications; and D, E, F: 1000 magnifications. Where A, D means 37 °C, 30 min / 90 °C 50 min at atmospheric pressure; B, E: 200 MPa, 7 °C, 10 min; and C, F: 375 MPa, 37 °C, 20 min.

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