5 **Interaction of kappa-carrageenan plus other gums in
fish myosystem gels**

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ABSTRACT: Mixtures of kappa-carrageenan plus another hydrocolloid (locust bean, guar, xanthan, iota-carrageenan, carboxymethylcellulose, alginate) were examined for their effects on the mechanical, color and water holding properties of heat-induced gels made from washed blue whiting mince. No synergistic effect was detectable except for the mixtures of kappa-carrageenan with locust bean. Light microscopy revealed that locust bean mixed with kappa-carrageenan and with xanthan only occasionally. Kappa-carrageenan and xanthan seemed to present interactions with the protein matrix, which were more discernible in the first case. Differential scanning calorimetry revealed faint interactions for the mixtures of kappa-carrageenan with locust bean and xanthan. The blend with alginate exhibited strong interactions but thermal degradation of alginate occurred.

Key words: Blue whiting gels, hydrocolloid blends, mechanical properties, structure, DSC

Introduction

Certain non-muscle proteins and certain hydrocolloids (iota-carrageenan, starch) have been used in the manufacture of restructured fish products (Lee and others 1992; Gómez-Guillén and Montero 1997); however, there are many other hydrocolloids and gums that can be used to impart new functional properties or to manipulate texture. Furthermore, hydrocolloid blends can be attractive when developing new additives because the effects of the individual ingredients can be modified, thereby extending the range of applications.

Protein-hydrocolloid interactions are mainly electrostatic, between the anionic groups on the hydrocolloid and the positively charged groups on the protein, which are dependent upon hydrocolloid concentration and the proportion of the hydrocolloids used (Tolstoguzov 1986).

Some previous studies on hydrocolloid-hydrocolloid interactions have been carried out for aqueous systems and even for meat muscle, but little work has been carried out on fish. The findings reported for studies performed using model systems are not of much help in studying the behavior of myosystems, because processing conditions differ considerably. For instance, guar gum is compatible with alginate in aqueous systems; but in myosystems, it interferes with alginate network structure (Shand and others 1993). Some of the works that study the interactions in aqueous systems are kappa - carrageenan plus galactomannans (Fernandes et al., 1994), xanthan gum plus galactomannans (Doublier and Llamas, 1991; Williams et al., 1991a; Casas and García - Ochoa, 1999), and xanthan gum plus konjac mannan (Williams et al., 1991b).

Hydrocolloids are ordinarily added to myosystems in the form of an unhydrated, dry powder, since water is a limiting factor affecting the texture of the final product. Addition of prehydrated or thermally-activated hydrocolloids (Filipi and Lee 1998) may structurally interfere with the cross linking required for the protein gel network formation giving rise to gel weakening. Another difference with respect to aqueous systems is the interaction with

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proteins, lipids, and other components in myosystems. In addition, there are important differences between myosystems, even though they all consist of myofibrillar proteins. For instance, restructured meats usually have high fat content, whereas in fish products the fat content is much lower and the sarcoplasmic proteins are partially depleted. Further, the gelling capacity of myofibrillar proteins differs greatly according to the physiological condition of the fish and seasonal factors. Studies involving restructured meats have mainly focused on blends of locust bean gum and iota-carrageenan (Troy and others 1999); kappa-carrageenan and iota-carrageenan or xanthan gum in low-fat frankfurters (Bloukas and others 1997); kappa-carrageenan/locust bean mixture (Nielsen and others 1996), and carboxymethylcellulose (Shand and others 1993), the two last cases in alginate restructured beef. Only a few researchers have considered the possibility of adding other types of hydrocolloids or binary mixtures in the gelling of fish muscle (Lee and others 1992).

The object of this study was to determine the behavior of binary mixtures of kappa-carrageenan plus another gum used in combination with minced blue whiting muscle to expand the available options for restructuring the fish muscle. Evaluations of such different functional characteristics as mechanical properties, color, and water holding capacity were carried out. In addition, light microscopy and calorimetric methods (DSC) were employed to detect molecular interactions.

Materials and Methods

Sample provenance

The blue whiting (*Micromesistius poutassou* Risso) used in this study was caught off the coast of northern Spain in the Bay of Biscay in May. Average size was 23.4 ± 1.2 cm and average weight 77.8 ± 12.3 g. Washed mince was prepared, frozen stored, thawed and then analyzed as described elsewhere (Pérez-Mateos and Montero 1999). The proximate composition (%) was: crude protein 12.34 ± 0.32 ; moisture 82.18 ± 0.16 ; crude fat 0.47 ± 0.04 ; and ash 0.63 ± 0.01 (% cryoprotectant not included).

The hydrocolloids added (in powder blend) were locust bean gum (ViscogumTM BE), J.Food Sci. 2001;66(6):838-843

guar gum (FFH-200), xanthan gum (Satiaxane™ CX90), iota-carrageenan (Satiagel™ RPT25), kappa-carrageenan (Satiagel™ RPT8), sodium carboxymethylcellulose (CMC) (Tylopur™ C10.000), and sodium alginate (Satialgine™ S1100) (all from SKW Biosystems, Rubí, Barcelona, Spain) to a final concentration of 0.5 % for each hydrocolloid used (1 % in the case of locust bean gum) with crushed ice to give the required final gel moisture content (80 %); and the extra salts added were NaCl and KCl in accordance with the results of Pérez-Mateos (1998). Gel preparation was carried out as previously described (Pérez-Mateos and Montero 1999).

Textural properties and color

The analyses carried out were folding test, puncture test (breaking deformation, breaking force, work of penetration), Texture Profile Analysis (TPA) (hardness, adhesiveness, cohesiveness) and stress-relaxation test (elasticity), color (L^* , a^* , and b^*) and water holding capacity (WHC), according to methods described elsewhere (Pérez-Mateos and Montero 1999).

Light microscopy

Samples were fixed in 10 % formaldehyde, dried in an increasing series of ethanol (50, 70, 96, 100 %) followed by toluene, and then embedded in paraffin at 56-60 °C. The paraffin blocks were then sliced into 8 µm-thick sections using a microtome (1130/Biocut, Reichert-Jung, Germany) and the sections were fixed on slides coated with a 1:1 solution of albumin and glycerine. The sections were then hydrated by addition of xylol, followed by a succession of decreasing dilutions of ethanol (100, 96, 70 %). Gels containing a blend of an anionic and a non-ionic hydrocolloid were stained using the following procedure: first, staining with Alcian blue; next, staining using Schiff's reagent (Hotchkiss 1948; Martoja and Martoja-Pierson 1970); and finally, picocarmine was added as contrast. All the sections were dried in increasing concentrations of ethanol (70, 96, 100 %) and then xylol. Photomicrographs were taken under a light microscope (Nikon Optiphot, model AFX-IIA, Japan). Anionic hydrocolloids stained blue and non-ionic ones stained pink.

Differential scanning calorimetry (DSC)

Thermal denaturation of proteins and sol-gel-sol transitions of the hydrocolloids were monitored by DSC using a previously calibrated Perkin Elmer DSC7/TAC7DX/PC differential scanning calorimeter (The Perkin-Elmer Corporation, Norwalk, CT, USA). Both recently prepared mixtures of washed fish mince plus the hydrocolloid(s) as well as previously gelled samples were used. The samples, weighing ~50 mg ($\pm$ 0.01 mg) as measured by a Perkin-Elmer AD4 electronic balance were encapsulated in hermetically sealed, large volume, aluminum volatile pans (PE part no. BO014-3021). The samples (3-4 in each case) were scanned at 10 °C/min over the temperature range 5-90 °C under a dry nitrogen purge at 30 mL/min. Cooling curves were subsequently recorded at the same scanning rate to observe the corresponding sol-gel transition. A second cycle of heating-cooling scans was then run in all cases to record hydrocolloid gel-sol reversibility. The water content of each individually encapsulated (pinhole in the lid) sample was determined by desiccation at 105 °C to constant weight, for thermal data normalization to a dry basis. Temperatures $t(^{\circ}\text{C})$ and enthalpies of transition $\Delta H(\text{J/g, dry basis hereinafter})$ are given within 0.5 °C and 5 %, respectively.

Statistical analysis

One-way analysis of variance was run using the Statgraphics computer program (STSC Inc., Rockville, MD, USA). The differences between means pairs were compared using the Bonferroni test, setting the level of significance at $p \leq 0.05$.

Results

Figure 1 shows the characteristics of the blue whiting gels with added blends of kappa-carrageenan and another hydrocolloid, and compares the results to those gels with added kappa-carrageenan alone (KC). Differences in protein concentration were small (Table 1) and seems not to likely justify any differential behavior.

Folding test

On the whole, the binary mixtures composed of kappa-carrageenan plus each of the

other gums achieved non-significantly different scores from kappa-carrageenan alone (Figure 1, Folding test), except for the blends containing guar (KC-GU) and xanthan (KC-XA) gums, for which the scores decreased significantly.

Puncture test

5 Breaking deformation (Figure 1, Breaking deformation es necesario
repetir????queda reiterativo), breaking force (Figure 1, Breaking force), and work of
penetration (Figure 1, Work of penetration) increased with the addition of locust bean gum
(KC-LB). When the mixture of kappa-carrageenan and guar (KC-GU) or xanthan (KC-XA)
gum were added, these measures of penetration exhibited a tendency to decrease, the
10 decrease being significant for work of penetration. Similarly to folding, the rest of the
mixtures behaved very closely to the gel with added kappa-carrageenan alone.

Texture Profile Analysis (TPA)

Binary combinations of kappa-carrageenan and the other polysaccharides yielded
differing results for the hardness values (Figure 1, Hardness). When mixed with guar gum
15 (KC-GU), xanthan gum (KC-XA), or iota-carrageenan (KC-IC), softening occurred ($p \leq 0.05$).
Conversely, hardness values increased slightly ($p \leq 0.05$) when blends with locust bean gum
(KC-LB) and with carboxymethylcellulose (KC-CMC) were used.

Most of the hydrocolloid combinations yielded gels with lower adhesiveness values
($p \leq 0.05$) than when only kappa-carrageenan was added alone (Figure 1, Adhesiveness),
20 the exception being the combinations of kappa-carrageenan with locust bean gum (KC-LB)
and with CMC (KC-CMC), which yielded values similar to the gel with added kappa-
carrageenan alone ($p \leq 0.05$).

Gel cohesiveness was hardly affected by the different hydrocolloid blends added
(Figure 1, Cohesiveness). Only the mixture containing locust bean gum (KC-LB) yielded
25 higher cohesiveness values ($p \leq 0.05$). This can probably be ascribed to the greater
cohesiveness of locust bean gum and the higher concentration of that gum employed.

Pérez-Mateos and Montero (1999) reported that cohesiveness values for blue whiting mince
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gels increased with increasing concentrations of locust bean gum ranging from 0.5 to 2 %, whereas cohesiveness values were barely altered by the addition of kappa-carrageenan. (nota.- este comentario tendria que ir a la parte de discusion???)

Elasticity

5 No significant differences (Figure 1, Elasticity) were observed for the gels containing binary mixtures of hydrocolloids as compared to the gel with kappa-carrageenan as the sole hydrocolloid ($p \leq 0.05$), except for the blend of kappa-carrageenan and iota-carrageenan (KC-IC), which yielded slightly more elastic gels ($p \leq 0.05$).

WHC

10 Water holding capacity (Figure 1, WHC) increased slightly ($p \leq 0.05$) when the kappa-carrageenan was combined with locust bean gum (KC-LB), but for the rest of the combinations no significant variations were recorded with respect to the gel containing added kappa-carrageenan alone.

Color

15 The different mixtures of hydrocolloids added to the blue whiting mince gels did result in slight alterations in gel color. There was no significant change in luminosity or lightness (Figure 1, L^*) in the gel containing added kappa-carrageenan alone. Redness (Figure 1, a^*) increased slightly in the gels from most of the hydrocolloid blends ($p \leq 0.05$). Addition of guar gum (KC-GU), CMC (KC-CMC), and alginate (KC-AL) also resulted in a
20 significant increase in yellowness (Figure 1, b^*), because of the somewhat yellow color of the added hydrocolloids themselves.

Microscopy

Because of the differential staining, only gels containing mixtures of an anionic hydrocolloid plus a non-ionic hydrocolloid were used (KC-LB, LB-XA) to ascertain the
25 distribution of the hydrocolloids in the muscle protein matrix and observe possible interactions. Figure 2 A, B, C (H100, H200, and H400 respectively) shows that the gels containing mixtures of kappa-carrageenan and locust bean gum (KC-LB) presented the

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hydrocolloids distributed separately in two types of cavities (hydrocolloid swelling by water) with no apparent sign of interaction between them (A, B). The kappa-carrageenan was in round cavities intensely stained by the Alcian blue and presented marked continuity with the protein matrix (C2). The locust bean gum (non-ionic, pink) was distributed in large round
 5 cavities with no areas of interaction or contact between the other hydrocolloid and the protein matrix (C1).

Fish mince gels with locust bean gum in mixture with xanthan gum (anionic, blue) (LB-XA) are shown in Figure 2 D, E, F. The hydrocolloids were mainly located in separated cavities (D), and practically did not present continuity with the protein matrix (E, F). Pérez-
 10 Mateos (1998) described on blue whiting gels with added xanthan gum, an array structure that stained blue inside the cavities, showing few areas of contact with the protein matrix.

Small zones stained blue were detected inside pink cavities in both systems. It seems that, in these non-equilibrium conditions, some kind of interaction between the corresponding couple of hydrocolloids is produced in the locust bean-rich domain.

15 DSC

Figure 3 shows the normalized DSC traces for the different samples. Curve M was the curve for the washed mince (basically depleted of sarcoplasmic and stromal proteins), which presented two principal endothermic zones centred at ~44 (myosin) and 71 °C (actin), respectively. The total enthalpy of thermal denaturation was ~13.5 J/g, in good agreement
 20 with previous results when the compositional differences are taken into account (Fernández-Martín and others 1998).

The corresponding hydrocolloid gel-sol transition was investigated by a second heating-scan (suffix h in the notation) (by the first scan on samples previously cooked) devoid of the protein thermal denaturation signals. This was clearly observed in the case of
 25 kappa-carrageenan, either alone or in combination with locust bean, xanthan or alginate. Kappa-carrageenan yielded a small, broad event (Figure 3, KC_h) between 50-70 °C with a maximum at ~63 °C (Goycoolea and others 1994), which did not significantly vary with the
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addition of locust bean or xanthan gum (not shown). In combination with alginate (Figure 3, KC-AL_h), the kappa-carrageenan trace was more symmetrical in shape, and shifted to higher temperatures (65-85 °C, centering at ~77 °C. Alginate used as the only hydrocolloid yielded a DSC trace with a large exothermic zone (not shown). This was consistent with a

5 gas-producing and hence irreversible reaction that implied a permanent reduction in the heat capacity of the system. The effect was delayed by hermetic encapsulation, which was obviously greater the smaller the head space over the sample. This effect was thus not only observable starting at temperatures as low as 50 °C but was also shifted towards nearly 100 °C.

10 In consonance with heating scans, because of subcooling corresponding cooling DSC traces (suffix c in the notation) recorded the sol-gel transition of the gel with added kappa-carrageenan alone (Figure 3, KC_c) at ~38 °C. Unlike the heating scans, the cooling DSC traces revealed a small temperature shifting in the combinations of locust bean (Figure 3, KC-LB_c) and xanthan (Figure 3, KC-XA_c) gums with kappa-carrageenan (~39 and 40 °C,

15 respectively). The transition was considerably shifted upwards (~52 °C) in the blend with alginate (Figure 3, KC-AL_c).

When combined, non-gelling locust bean and xanthan gums (LB-XA) yielded a very smooth event in both the heating (45-60 °C) and cooling (45-35 °C) scans (not shown).

Discussion

20 **YO volveria a nuestra version anadiendo sus nuevos comentarios (si es que hay alguno en nuestra parte) y pegaria su trozo de DSC !!hacer y deshacer y volver a rehacer!!!!!!! Ha quitado un monton de discusion y de citas de otros autores!!!! No discute nada de folding test, engloba a BD< BF Y WP todos juntos como si fuera una**

25 **conclusion!!!, no dice nada de color!!!!!!**

Functional properties

The mixture of kappa-carrageenan with locust bean gum produced gels with

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significantly higher penetration values, appreciable increase in hardness, and cohesiveness. Guar gum however induced significant reduction in folding test scores, breaking deformation and work of penetration, as well as in TPA values of hardness and adhesiveness, but an increase in elasticity. The quite different, opposing behaviors of these two galactomannans is attributable to their different galactose/mannose ratio content, which is much higher in guar gum. Thus cross-linking of kappa-carrageenan double helix aggregates to the unsubstituted regions of the mannose chain is then somewhat more difficult on guar than in locust bean gum. Pérez-Mateos and Montero (1999) observed that 1 % locust bean gum gave rise to high deformation values and could even result in an excessively rubbery texture of blue whiting mince gels. Conversely, addition of locust bean gum mixed with kappa-carrageenan yielded characteristics intermediate to those results obtained by each gum separately.

Combinations of kappa-carrageenan with xanthan or iota-carrageenan gum yielded lower working of penetration and hardness values than gels with added kappa-carrageenan alone. Use of those mixtures yielded lower breaking deformation values than iota-carrageenan alone in frankfurters (Bloukas and others 1997). Even though the two carrageenans are similar in structure, the additional sulphate groups of the iota-carrageenan molecules give rise to internal and external repulsion forces on the blend, thus preventing formation of a compact gel.

Elasticity data was not very sensitive to the addition of a second hydrocolloid, only moderate but significant increases were detected in the mixture with *iota*-carrageenan. The *iota*-carrageenan may itself increase elasticity, especially when added at concentrations greater than 2 % in mince fish gel (Pérez-Mateos and Montero, 1999).

While the added hydrocolloids had high water holding capacities in themselves, the amounts added were too low to bring about any appreciable variation, except for a significant increase in the mixtures of kappa-carrageenan with locust bean.

Microscopy

Optical microscopy did not detect any interactions between the kappa-carrageenan and the locust bean gum. Lee and Kim (1985) postulated that the failure to observe any synergistic effect could be attributed to strong competition for water between the hydrocolloids and the myofibrillar protein. Kappa-carrageenan showed some interaction with the protein matrix, probably through electrostatic bonds with its sulphate groups. Xanthan gum presented similar but lower effects, probably attributable to the presence of side chains on the xanthan molecules as well as their anionic character enhance the hydration.

DSC

Variations in the DSC area ranged from 4 to 7 % with respect to the original mince and were additive in nature (protein denaturation plus hydrocolloid gelling). Addition of 0.5-1 % of any hydrocolloid (1-1.5 % in binary mixture) to sample M did not significantly alter the resulting DSC (protein denaturation) trace at the temperatures considered. This was indicative of practically no interaction between the hydrocolloids and the proteins, in consonance with the general notion that interactions only appear at concentrations greater than 2 % (DeFreitas and others 1997).

Gel-sol transitions revealed faint synergistic effects of kappa-carrageenan in mixture with locust bean or xanthan gums. Both sol-gel and gel-sol transitions on fish gels containing the mixture of locust bean and xanthan gums were indicative of a weakly synergistic gelling effect known to occur between non-gelling galactomannans and glucomannans (Williams and others 1991).

The mixture of kappa-carrageenan with alginate exhibited a strong association, and the poor thermal stability of alginate was thus considerably improved. Regarding this, worth mentioning is that Oates and Ledward (1991) described what they called an "unusual and previously unreported phenomenon" observed in gellifying a soya S7 globulin plus an alginate (mannuronic-rich) system. It consisted in a reduction of the heat capacity of the system at post-transitional (thermal protein denaturation) temperatures ($>90^{\circ}\text{C}$). Those researchers speculated that covalent bonding of protein and hydrocolloid might be the

cause. As described above, however, the permanent decrease in the specific heat of the system was really an outcome of the irreversible exothermic thermal degradation of the alginate.

Conclusions

5 The anionic hydrocolloids kappa-carrageenan and, to a lesser extent, xanthan gum exhibited continuity with the protein matrix suggestive of ionic interactions between those hydrocolloids and the myofibrillar proteins. Interactions among the hydrocolloids were not a general situation; limited synergistic effects on the gel functional properties were detectable on kappa-carrageenan mixtures with locust bean and xanthan gums. Despite a strong
10 association (DSC) detected between kappa-carrageenan and alginate, the blend did not impart any particular improvement on the mechanical and water holding properties of the blue whiting washed mince gel. It was attributed to alginate thermal decomposition. Though partially stabilized by the mixture, alginate gum would present this kind of drawback if used as a gelling ingredient in surimi-based products or other systems requiring high-
15 temperature-setting processes.

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