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Addition of hydrocolloids and non-muscle proteins to sardine (*Sardina pilchardus*) mince gels

Effect of salt concentration

M. C. Gómez-Guillén & P. Montero

Departamento de Ciencia y Tecnología de Carne y Pescado, Instituto del Frío, Ciudad Universitaria, 28040-Madrid, Spain

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This paper examines the effect of adding hydrocolloids (iota-carrageenan and starch) alone and hydrocolloids with non-muscle protein (egg-white, soy protein, casein, gluten) on the texture and water-holding ability of gels made with sardine mince of two different qualities and salt contents (2.5% and 1.5%). Addition of hydrocolloids or hydrocolloids and non-muscle proteins to a mince of high gel-forming capacity with 2.5% NaCl caused a significant drop in gel strength and hardness but not in elasticity or cohesiveness. In low-salt gels, addition of these ingredients significantly improved folding test scores with respect to control. Low-salt gels with hydrocolloids proved harder but less elastic and cohesive than high-salt gels. Where hydrocolloids were added along with non-muscle proteins in low-salt samples, gels exhibited the same or less hardness, elasticity and cohesiveness as high-salt samples. In a muscle of low gel-forming capacity, addition of hydrocolloids and combinations of hydrocolloids and non-muscle proteins increased hardness, elasticity and cohesiveness of both low- and high-salt samples. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

By adding biopolymers possessing gel-forming or binding capacity, it has been possible to develop a large variety of analogues based upon modification of the functional and textural properties of surimi (Okada, 1963; Ikeuchi, 1964; Akahane *et al.*, 1984; Iso *et al.*, 1985; Kim & Lee, 1987; Niwa *et al.*, 1988; Chung & Lee, 1988, 1990). For some products in which neither colour nor flavour are impediments and no great gel-forming capacity is required, unrefined muscle mince can be used with only cursory washing. The base product thus gives greater yield than surimi. Starch is the 'filler' most commonly used in the fabrication of surimi- or fish mince-based products, as it increases firmness and gel strength (Suzuki, 1981; Lee, 1984; Wu *et al.*, 1985*a*,*b*; Lee *et al.*, 1992). Using carrageenan, the gel-forming capacity of Alaska pollack surimi was found to be improved by the addition of iota- and kappa-carrageenans due to the interaction of carrageenan sulphate groups and fish protein (Llanto *et al.*, 1990). Montero *et al.* (1992*a*) and Nakayama *et al.* (1988) have also reported increased gel strength upon adding iota-carrageenan to sardine muscle mince gels. In addition, carrageenans may interact with starch (Gómez-Guillén *et al.*, 1992; Montero *et al.*, 1992*b*; Tye, 1988) and some non-muscle proteins (Bullens *et al.*, 1990; Elfak *et al.*, 1979).

Considering that, unlike surimi, minced sardine muscle does not always gel properly, this research was intended to improve gelation by addition of hydrocolloids, either on their own or combined with non-muscle proteins, with a view to the fabrication of high- and low-salt analogues (2.5% or 1.5%) using two different sardine mince samples.

MATERIALS AND METHODS

The fish used in these experiments were sardine of the species *Sardina pilchardus* (Walbaum), caught in two different seasons: October (S1) and June (S2). Fish mince was prepared as follows. Sardines were headed, gutted and washed. Skin and bones were removed with a Baader Model 694 deboning machine (Nordischer Maschinenbau Rud Baader, Lübeck, Germany). Muscle was minced and held for 10 min at 0–3°C in an aqueous solution of 0.5% bicarbonate (solution:minced muscle 3:1, w/w), with constant stirring. Solutions were left for 10 min. Excess water was then removed using a screw press to reach the same moisture as muscle. As

cryoprotectants, 4% sorbitol and 0.2% sodium tripolyphosphate were added. The mince was immediately vacuum-packed in Cryovac BB-1 bags and frozen in a plate-freezer (Frigoscandia Model R13b; AGA Frigoscandia, Helsingborg, Sweden) (−40°C setting) until the thermocouple registered that the thermal centre had reached −20°C. The various lots were air-freighted with solid CO_2 to our laboratory, where they were stored at −80°C in a REVCO vertical freezer cabinet to minimize alteration during frozen storage for the duration of the experiment.

NaCl was supplied by Panreac, Montplet and Esteban (Barcelona, Spain). Clearam CH 20 starch (made by Roquette Frères) was supplied by Levantina Agrícola Industrial (Barcelona, Spain). Iota-carrageenan was supplied by Litex (Denmark) under the product name Gelcarin XP 8009. Spray-dried egg-white was supplied by Sanofi. Soy protein was a soy isolate supplied by Protein Technologies International under product name PP 500 E. Spray-dried sodium caseinate was supplied by 'La Pilarica'. Wheat gluten was supplied by Levantina Agrícola Industrial, under the product name Vital 'L' Wheat Gluten.

Proximate analysis and protein functionality

To characterize the two washed mince types (S1 and S2), moisture, ash, crude fat and crude protein were determined by methods of the Association of Official Analytical Chemists (1975). Results were mean values of three determinations and expressed as percentage muscle mince. Apparent viscosity (Borderías *et al.*, 1985) and protein solubility (Ironside & Love, 1958) were determined as an index of protein functionality.

Gel preparation

Washed sardine mince was semi-thawed and placed in a refrigerated vacuum homogenizer (Stephan Model UM5; Stephan & Söhne, Germany). Muscle was ground for 1 min (rotor angular velocity 3000 rpm). NaCl and water were added to adjust the final moisture content to 75% in each gel and the mixture was blended for 5 min at 1500 rpm under vacuum. Following this, ingredients were added to each sample as required, in the proportions 2% of iota-carrageenan, 8% of starch, 2% of non-muscle protein, and the mixture was blended again for 5–7 min. The batters were packed into stainless steel cylinders (3 cm height, 3 cm ID) with hermetic screw-on caps and rubber gaskets. The cylinders were heated in a water bath at 37°C for 30 min and then at 90°C for 50 min. Finished gels were kept overnight at 4°C.

Puncture test

This was performed on samples (30 mm height×30 mm diameter), tempered to about 20°C. The gel was penetrated to breaking point using an Instron Model 4501 texturometer (Instron Engineering Corp., Canton, MA), with 5 mm diameter metal probe with rounded head, speed setting of 10 mm min^{-1}, and load cell 100 N. Gel strength was determined by multiplying breaking force (N) by breaking deformation (mm). All determinations were performed in at least quadruplicate.

Compression tests

Both texture profile and compression–relaxation tests were as described by Bourne (1976). This was performed on samples (30 mm height×30 mm diameter) tempered to about 20°C.

For texture profile analysis (TPA), samples were placed on the flat plate of the Instron texturometer. Compression was applied by a cylindrical plunger (diameter 36 mm) adapted to a 5 kN load cell at a deformation rate of 50 mm min^{-1}. On the basis of previous trials to establish a compression limit that would ensure no cracking and recoverability of most samples, it was decided to compress samples to 50% of height. In the test, each sample was compressed twice and hardness (N) and cohesiveness $[(A_2/A_1)\times b]$ were determined.

For the compression–relaxation test, the compression procedure was as in TPA, except that the sample was compressed once only for 1 min, and the force exerted on the sample was recorded. Percentage relaxation was calculated as $Y_T = 100 \times (F_0 - F_1)/F_0$, where F_0 is the force registered at the onset of relaxation immediately after sample compression and F_1 is the force registered after relaxation for 1 min. Thus, $(100 - Y_T)$ is taken as an index of elasticity and is expressed as percentage elasticity of the gel.

All determinations were carried out in at least quadruplicate.

Water-holding ability

This was a modification of the method of Roussel & Cheftel (1990). Chopped sample (1.5 g) was placed in a centrifuge tube together with a Gilson Pipetman pipette filter and centrifuged in a Sorvall RT60008 centrifuge (Du Pont, Wilmington, DE) at 4000***g*** for 10 min at ambient temperature. Water-holding ability (WHA) was expressed as percentage water retained per 100 g of water present in the gel prior to centrifuging. All determinations were carried out in quadruplicate.

Statistical analysis of data

One-way analysis of variance was carried out for the different samples. The computer program used was STATGRAPHICS (STSC, Inc., Rockville, MD). The difference of means between pairs was resolved by confidence intervals using a least significant difference range test. Level of significance was set for $P \leqslant 0.05$.

RESULTS AND DISCUSSION

Proximate analysis and functional properties of the minces

The two types of mince (S1 and S2) differed essentially in moisture and fat content. S2 contained significantly

Table 1. Water-holding ability and analysis of variance of rheological parameters of gels made from S1 and S2 minces, at both salt concentrations, with added iota-carrageenan and starch

Formula	Water-holding ability (%)	Gel strength	Breaking force	Breaking deformation	Hardness	Elasticity	Cohesiveness
Mince S1							
2.5%	87.77 ± 3.27a	a	a	a	a	a	a
1.5%	75.85 ± 4.94b	–	–	–	–	–	–
iCR–2.5%	94.54 ± 1.35cd	b	b	bc	b	a	a
iCR–1.5%	93.02 ± 0.54c	b	c	b	c	b	b
iCR–ST–2.5%	97.86 ± 0.22d	b	bc	c	d	a	c
iCR–ST–1.5%	97.91 ± 0.21d	c	d	d	e	b	d
Mince S2							
2.5%	88.45 ± 2.38a	a	a	a	a	a	a
1.5%	77.48 ± 4.27b	b	b	a	b	b	b
iCR–2.5%	95.86 ± 1.58c	bc	c	ab	c	c	a
iCR–1.5%	97.43 ± 0.55c	ac	ad	b	d	c	a
iCR–ST–2.5%	98.41 ± 0.15c	bc	c	ab	e	d	c
iCR–ST–1.5%	98.20 ± 0.39c	c	cd	ab	c	d	c

iCR, iota-carrageenan; ST, starch.
Different letters in the same column indicate significant differences ($P \leqslant 0.05$).

more fat (9.85 ± 0.13%) and less moisture (72.00 ± 0.06%) than S1 (4.32 ± 0.34% fat, 76.47 ± 1.59% moisture). Fat is an undesirable element in gel-making, as it interferes with the formation of a protein matrix and hence hinders gelation. Crude protein contents of minces were 14.7 ± 0.02% and 14.0 ± 0.01% for S1 and S2, respectively. Ash was 0.61 ± 0.01% for S1 and 0.68 ± 0.01% for S2. S1 exhibited considerably higher protein solubility (62.6 ± 1.15%) and apparent viscosity (3140 ± 34.64 cP) than did S2 (49.8 ± 0.01% and 2323 ± 35.11 cP). Mince S1 may therefore be considered to be of high functional quality and S2 of low functional quality.

Effect of hydrocolloids

High-quality mince (S1)

Gels made from high-quality muscle (S1) with 2.5% NaCl and added iota-carrageenan, or iota-carrageenan plus starch, exhibited significantly lower gel strength than the control (gel without hydrocolloids) (Table 1), as a consequence of reduced breaking force and breaking deformation (Fig. 1). Both gels, however, scored maximum points in the folding test. Burgarella *et al.* (1985) suggested that reduced gel strength upon addition of ingredients might be caused by 'dilution' of the myofibrillar protein, which is the main agent of gel formation. Addition of hydrocolloids reduced muscle protein concentration from 14.0% (control) to 12.1% (carrageenan only) and 7.82% (carrageenan plus starch). Despite the major difference in concentration between the two experimental gels, there was no difference in gel strength, which suggests that the two hydrocolloids might interact positively to compensate for the lower muscle protein concentration (Gómez-Guillén *et al.*, 1992; Montero *et al.*, 1992*b*; Tye, 1988). In low-salt (1.5%) gels, addition of hydrocolloids produced a noticeable improvement in folding test scores of mince S1 with 1.5% NaCl, which lacked gel-forming capacity on its own (Fig. 1).

There are a number of theories as to the mechanism whereby hydrocolloids improve texture parameters. According to Takagi & Shimidu (1972), hydrocolloids act as network fillers and strengtheners. Their high WHA capacity causes them to swell and augment elasticity by reducing the moisture content of the mesh (Niwa *et al.*, 1988; Iso *et al.*, 1985) and increasing the density of the surrounding protein matrix (Niwa *et al.*, 1989). With regard to the influence of salt, only when starch was added together with carrageenan gel were strength, breaking force and breaking deformation all lower in low-salt gels ($P \leqslant 0.05$).

Compression tests (texture profile analysis and compression–relaxation test) (Fig. 2) showed that high-salt gels with added hydrocolloids were significantly less hard than controls, particularly in samples with both carrageenan and starch. Elasticity, on the other hand, was no different from the control. Cohesiveness in gels containing both carrageenan and starch was significantly higher than in the control or in gels with carrageenan only (Table 1). Lee & Kim (1986) have also reported greater cohesiveness in gels with added starch. In the present case, unlike the puncture test, hardness clearly decreased in the gel containing carrageenan and starch, in which muscle protein concentration was considerably lower than in the control or the gel with carrageenan only. This was not, however, the case with elasticity and cohesiveness. According to Lanier (1986), cohesiveness is the most sensitive indicator of quality or functionality in surimi proteins. Moreover, cohesiveness and hardness can vary independently (Hamann & MacDonald, 1992). When salt content was reduced (Fig. 2), gels containing carrageenan and starch exhibited less hardness, equal elasticity and greater cohesiveness than those containing carrageenan only. With one or two hydrocolloids, low-salt

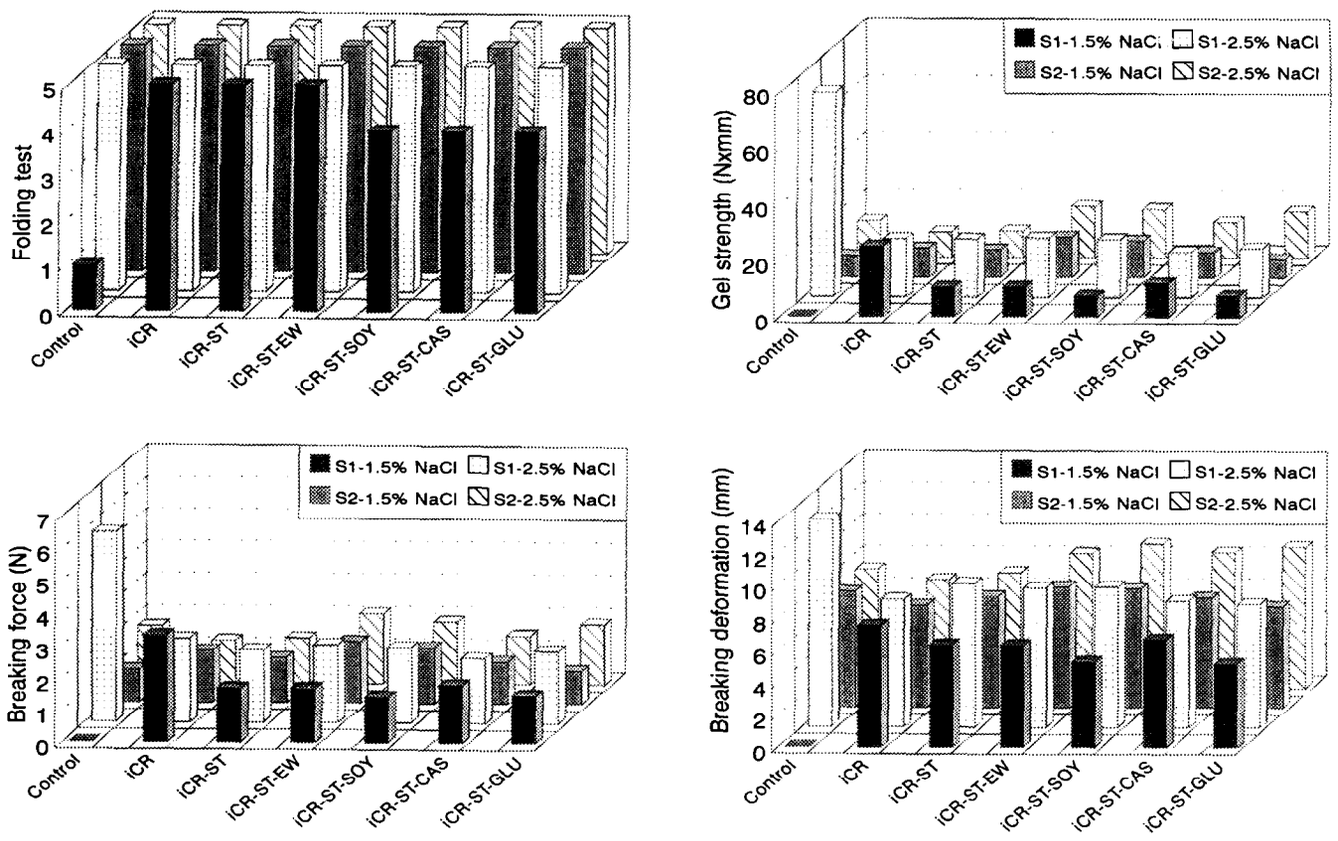

Fig. 1. Folding test, gel strength, breaking force and breaking deformation of gels made from S1 and S2 minces, with addition of hydrocolloids and non-muscle proteins, at both salt concentrations. iCR, iota-carrageenan; ST, starch; EW, egg white; SOY, soy protein; CAS, casein; GLU, gluten.

gels were harder but less elastic and cohesive than high-salt gels (Table 1).

Addition of iota-carrageenan at either salt level produced a very pronounced increase in WHA of gels over their respective controls. The increase was slightly greater when starch was added as well, particularly in the gel with 1.5% NaCl (Table 1).

Low-quality mince (S2)

At both salt concentrations, gels with added hydrocolloids achieved the maximum folding test score (Fig. 1). Gel strength was similar in gels with carrageenan only and with carrageenan plus starch; with 2.5% NaCl they were slightly but significantly ($P \leqslant 0.05$) lower than the control, while with 1.5% NaCl they were significantly higher than the control. Gels did not differ significantly in breaking force or breaking deformation (Table 1) with respect to either hydrocolloid content or salt content.

In the compression tests, gels containing hydrocolloids were significantly harder, more elastic and more cohesive than the controls (Fig. 2 and Table 1). Despite the fact that muscle protein concentration with both hydrocolloids (6.23%) was considerably lower than in either the gel with carrageenan only (10.2%) or the control (11.2%), the 'diluting' effect of this seems to be offset by the action of the added ingredients. Tye (1988) describes a synergistic effect in starch and carrageenan systems. In the present case, however, gels with carrageenan only were slightly harder but less elastic and cohesive than those with both hydrocolloids. Montero *et al.* (1992*a*), Llanto *et al.* (1990), Nakayama *et al.* (1988) and Da Ponte *et al.* (1985) have reported good textures in fish gels with added iota-carrageenan. With regard to the influence of salt content, low-salt gels proved harder than high-salt gels with either hydrocolloid combination. Elasticity and cohesiveness, on the other hand, did not differ significantly with salt concentration (Table 1).

WHA increased significantly with addition of carrageenan or carrageenan plus starch, attaining comparable values to those found in gels made from high-quality muscle with added hydrocolloids (Table 1). Here again, there were no significant differences with salt concentration. In view of the results, addition of hydrocolloids to sardine mince would appear to be crucial for the WHA of the gel, which increased more than 95% irrespective of salt concentration.

Effect of hydrocolloids and non-muscle protein

High-quality mince (S1)

High-salt gels with added hydrocolloids and a non-muscle protein achieved maximum folding test scores (Fig. 1). Gel strength was considerably lower than control in all cases, with no significant differences due to the type of protein added (Table 2). Lee & Kim (1986) found no synergy between hydrocolloids and egg-white

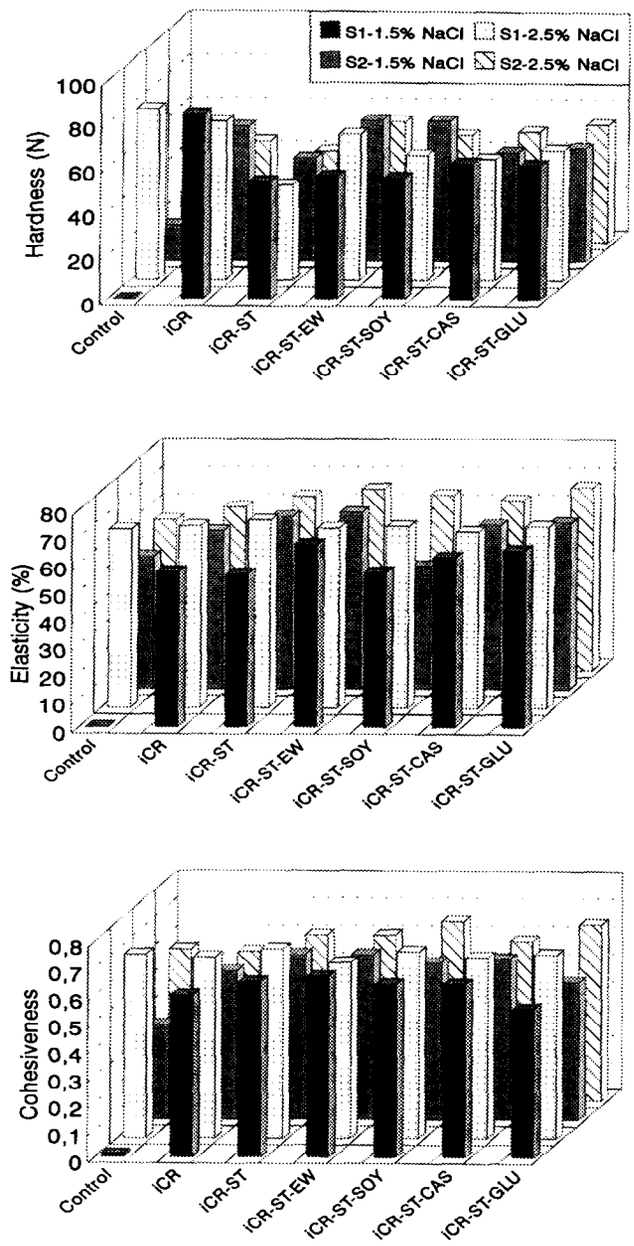

Fig. 2. Hardness, elasticity and cohesiveness of gels made from S1 and S2 minces, with addition of hydrocolloids and non-muscle proteins, at both salt concentrations. iCR, iota-carrageenan; ST, starch; EW, egg white; SOY, soy protein; CAS, casein; GLU, gluten.

as regards modification of surimi gel texture. When a mixture of non-muscle protein and starch is added to a surimi, protein and hydrocolloid compete for the water, thus depleting gel strength (Chung & Lee, 1988). It should also be remembered that muscle protein concentration in gels with hydrocolloids and non-muscle proteins is considerably lower than in the control. It is interesting to note, however, that gel strength was higher—if only slightly—in gels where hydrocolloids and casein were combined than in gels with hydrocolloids only. This effect has been reported in other gels made with added casein (Gómez-Guillén, 1994). This suggests that carrageenan and casein interact to some extent (Xu *et al.*, 1992; Elfak *et al.*, 1979; Snören *et al.*, 1975). Bullens *et al.* (1990) also reported synergy between carrageenan and serum protein in surimi. At the lower salt concentration (Fig. 1), addition of hydrocolloids to any of the experimental non-muscle proteins considerably improved folding test scores with respect to controls, which, as noted earlier, would not gel. However, only the sample with egg-white achieved the maximum score. There were no significant differences in gel strength with respect to type of protein added (Table 2).

Compression test results (Fig. 2) indicated less hardness than control in the high-salt gel with carrageenan, starch and non-muscle protein; gels with egg-white were slightly but significantly harder than the rest. Gels did not differ significantly in elasticity or cohesiveness, which were similar to the control, with the exception of gels containing egg-white, which were significantly ($P \leqslant 0.05$) less cohesive than the rest (Table 2). Low-salt gels with casein or gluten were a little harder than gels with egg-white or soy protein (Fig. 2). On the other hand, gels with egg-white were more elastic and cohesive than the rest. Gels with soy protein were the least elastic, and gels with gluten the least cohesive. With regard to the influence of salt concentration, low-salt gels with egg-white or casein were less hard; where soy protein or gluten was used, there were no significant differences in hardness between high-salt and low-salt samples (Table 2). Elasticity and cohesiveness were significantly less in low-salt than in high-salt gels, except for gels with egg-white where there was virtually no difference.

Addition of hydrocolloids along with a non-muscle protein produced gels with very high WHA (Table 2). However, values were not higher than when only hydrocolloids were added. There were no significant differences ($P \leqslant 0.05$) with respect to the type of non-muscle protein added, so that these would not appear to interfere with the WHA of hydrocolloids. Again, WHA did not vary significantly with the reduction of salt concentration in the gel.

Low-quality mince (S2)

Gels made from S2 muscle with added iota-carrageenan, starch and non-muscle protein achieved maximum folding test scores (Fig. 1). With egg-white or soy protein, gel strengths were slightly higher than the control (Table 2). Changes in gel strength depend more on breaking force than on breaking deformation. In low-salt gels, gel strength, breaking force and breaking deformation were lower than in high-salt gels.

In compression tests, where hydrocolloids and a non-muscle protein were added, gels were significantly harder, more elastic and more cohesive than the control except in elasticity when soy protein with 1.5% NaCl was added (Fig. 2 and Table 2). High-salt gels with vegetable protein were much more cohesive than gels with egg-white or casein. With regard to salt concentration, gels with egg-white or soy protein were harder at the

Table 2. Water-holding ability and analysis of variance of rheological parameters of gels made from S1 and S2 minces, at both salt concentrations, with added iota-carrageenan, starch and non-muscle protein

Formula	Water-holding ability (%)	Gel strength	Breaking force	Breaking deformation	Hardness	Elasticity	Cohesiveness
Mince S1							
2.5%	87.77 ± 3.27a	a	a	a	a	ab	a
1.5%	75.85 ± 4.94b	—	—	—	—	—	—
iCR–ST–EW–2.5%	97.68 ± 0.19c	b	b	b	bc	bc	bc
iCR–ST–EW–1.5%	97.81 ± 0.14c	c	cd	c	d	d	ac
iCR–ST–SOY–2.5%	98.24 ± 0.29c	bd	be	b	cd	cd	a
iCR–ST–SOY–1.5%	98.09 ± 0.19c	c	c	d	e	e	b
iCR–ST–CAS–2.5%	98.22 ± 0.20c	de	ef	e	a	a	ac
iCR–ST–CAS–1.5%	98.38 ± 0.14c	ec	df	c	f	f	b
iCR–ST–GLU–2.5%	98.31 ± 0.08c	bd	be	e	cd	cd	a
iCR–ST–GLU–1.5%	98.24 ± 0.30c	c	c	d	a	a	d
Mince S2							
2.5%	88.45 ± 2.38a	a	abc	a	a	a	a
1.5%	77.48 ± 4.27b	b	d	a	b	b	b
iCR–ST–EW–2.5%	98.09 ± 0.05c	c	e	b	c	c	c
iCR–ST–EW–1.5%	97.95 ± 0.15c	a	ab	a	d	cd	c
iCR–ST–SOY–2.5%	97.66 ± 0.08c	c	ab	b	e	cde	d
iCR–ST–SOY–1.5%	98.20 ± 0.16c	a	ab	a	d	b	ae
iCR–ST–CAS–2.5%	98.21 ± 0.08c	a	bc	b	e	def	ce
iCR–ST–CAS–1.5%	98.25 ± 0.10c	b	cd	ac	e	f	ce
iCR–ST–GLU–2.5%	98.25 ± 0.30c	c	a	b	cf	c	d
iCR–ST–GLU–1.5%	98.00 ± 0.18c	b	d	c	ef	ef	f

iCR, iota-carrageenan; ST, starch; EW, egg-white; SOY, soy protein; CAS, casein; GLU, gluten.
Different letters in the same column indicate significant differences ($P \leq 0.05$).

low-salt level, while gels with casein or gluten did not differ significantly in this respect. Elasticity and cohesiveness of gels with soy protein or gluten decreased when salt content was reduced, whereas gels with egg-white or casein did not vary significantly in this respect (Table 2). Lee *et al.* (1992) likewise reported increased gel hardness and elasticity when starch or non-muscle proteins were added to low-quality surimi.

WHA also increased considerably with the addition of hydrocolloids and non-muscle protein together, attaining values comparable to those obtained with S1 mince under the same conditions, although, here again, WHA was no lower when the non-muscle protein was left out (Table 2). Again, WHA did not vary with respect to the kind of protein added or to salt concentration.

CONCLUSIONS

Addition of hydrocolloids, or hydrocolloids plus non-muscle proteins, to a muscle of high gel-forming capacity with 2.5% salt caused a significant decrease in gel strength and hardness but not elasticity or cohesiveness. In low-salt gels, addition of these ingredients appreciably improved folding test scores with respect to the control. Then again, gels with hydrocolloids were harder but less elastic and cohesive than equivalent high-salt gels. Where hydrocolloids and non-muscle proteins were added together at a low salt concentration, gel hardness, elasticity and cohesiveness were the same as or lower than with a high-salt concentration. However, where a muscle of low gel-forming capacity was used, addition of hydrocolloids alone or hydrocolloids plus non-muscle proteins increased hardness, elasticity and cohesiveness at either salt concentration.

Addition of hydrocolloids considerably increased WHA irrespective of the quality of the muscle protein.

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