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Functional characterisation of muscle and skin collagenous material from hake (*Merluccius merluccius* L.)

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Abstract

The purpose of this paper was to examine the modifications occurring in certain functional hydration properties (protein solubility in 0.5 M acetic acid, apparent viscosity and water-binding capacity) of the collagenous material from skin and muscle of hake (*Merluccius merluccius* L.) in relation to the pH and the ionic strength (expressed as variation in NaCl concentration) of the medium. At the same time, these modifications were compared with those reported for collagenous material from trout in a previous paper. Generally speaking, skin collagenous material showed higher functionality than muscle collagenous material. It was found that protein solubility, apparent viscosity and water-binding capacity presented maximum values at pH levels between 2 and 4, and at concentrations of less than 0.25 M NaCl. Finally, the functional values were found to be higher in collagenous material from hake than from trout, especially in the skin material.

Keywords: Fish; Collagen; Muscle; Skin; Functional properties

1. Introduction

Hake (*Merluccius merluccius* L.) is a species widely processed in Spain for sale in the form of skinned and boned frozen fillets. In other parts of the world where it is less highly prized, it is used in the manufacture of minced products as the great functionality of its muscle makes it suitable for many industrial preparations. These two forms of processing produce large amounts of waste, a major proportion of which consists of skin and muscle connective tissue, both very rich in collagen. Collagenous material from mammals, especially after conversion into gelatin, has been widely studied and also many industrial applications have been reported (Bailey & Light, 1989; Gillet, 1985; Hood, 1985). Functional behaviour of collagenous material from different fish species, in terms of protein solubility in acetic acid, apparent viscosity, as well as water binding, gel forming, emulsifying or foaming capacity, has been reported previously (Montero & Borderías, 1990, 1991; Montero et al., 1991; Montero et al., 1994). In trout samples, skin collagenous material was found to present greater functionality than the muscle (Montero et al., 1991). Borderías et al. (1994) used collagenous material from plaice skin to improve the water-holding ability and the sensory properties of cod mince during frozen storage.

The purpose of this paper is to determine how various functional properties of hydration (solubility, water-binding capacity and viscosity) vary in collagenous material in the face of induced changes in pH and the presence of NaCl. This work further aims to compare these properties with those found in analogous collagenous material from trout (*Salmo irideus Gibb*) treated under identical conditions, and others, in order to know if there are great differences in the behaviour due to species.

2. Materials and methods

The studies were conducted on hake (*M. merluccius*) caught in the autumn off the Galician coast and made up to a lot of 52 kg. Average specimen size was 60.7 cm and individual weight 1.6 kg. These specimens were kept refrigerated for approximately 24 h.

The fish was processed, and the skin and muscle collagenous material (Scm and Mcm, respectively) isolated and purified following the procedures described by Montero et al. (1991).

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Proximate analyses were performed according to AOAC methods (1990).

The functional properties of both Scm and Mcm, that is protein solubility (PS) in 0.5 M acetic acid, apparent viscosity and water-binding capacity (with and without heating treatment: WBCh and WBCc, respectively), at different pHs and ionic strengths (in terms of NaCl concentration) were determined according to the procedures described by Montero et al. (1991). The protein concentration for the determination of PS was 1:40 (w/v, collagenous material/solvent), for apparent viscosity 1:25 (w/v) and for WBC 1:30 (w/v). The solvent used in PS and viscosity was 0.5 M acetic acid, while in WBC it was twice-distilled water (pH 6).

Collagen quantity was determined through hydroxyproline content, which was analysed according to the method of Leach (1960). Total protein was quantified by the method of Kjeldahl using a conversion factor of 5.3.

For statistical analyses, two-way analysis of variance and a Tukey test was used to determine the level of significance between averages. Correlations were established between the different collagenous materials for each functional property as a function of pH and ionic strength. Factor Analysis was performed by determining the Principal Component Analysis (PCA) with Varimax rotation.

3. Results and discussion

The amount of protein present in the muscle collagenous material (Mcm) was less than that in the skin material (Scm), even when expressed in terms of dry matter (Table 1). Proximate analyses, in general, are similar to those reported previously for trout muscle and skin (Montero et al., 1991). However, the skin material from hake shows higher protein content and lower moisture than plaice skin (Montero et al., 1994). Collagen yield, with respect to total protein, was 88.7 ± 2.6 for Mcm and 88.4 ± 3.1 for Scm. The collagenous material extracted was less rich than that obtained from trout (95.7 ± 0.7 and 95.0 ± 3.5, respectively) (Montero et al., 1991).

Table 1
Proximate analysis (%) of muscle collagenous material (Mcm) and skin collagenous material (Scm)

	Mcm	Scm
Moisture	72.0 ± 1.0	64.5 ± 0.2
Ash	1.7 ± 0.1	1.1 ± 0.1
Crude fat	1.2 ± 0.1	2.0 ± 0.3
Crude protein	24.1 ± 1.7	33.5 ± 1.3
CDP*	86.1 ± 1.0	94.2 ± 2.0

Crude protein (CDP) values expressed in terms of dry matter (results are expressed as average ± standard deviation).

The effects of pH variations on protein solubility in 0.5 M acetic acid and on viscosity are shown in Fig. 1. Whereas the solubility of Mcm peaks at pH 3 and declines as the pH rises or falls from there, the Scm solubility is not greatly affected by pH until this reaches pH 5 (Fig. 1a). However, higher pH levels cause the solubility to decline so that, at pH 6, both Mcm and Scm are practically insoluble. Scm shows higher solubility than Mcm in the range of pH between 1 and 5, indicating a lower degree of molecular crosslinking in skin, or the predominance of weaker bonds than in muscle (Montero et al., 1990). A similar behaviour as a function of pH was reported for trout (Montero et al., 1991), although hake samples show lower values than trout at pHs where solubility reached a maximum. This could be related to the higher aggregation observed in hake skin collagenous material (Montero et al., 1990). Similarly, the soluble protein was considerably lower than that reported for plaice skin (Montero et al., 1994). As Fig. 1b shows, variation in apparent viscosity with pH follows a similar pattern to that of protein solubility. Beyond pH 3, apparent viscosity declines progressively until at pH 5 it is zero for both types of collagenous material. Again, viscosity in Scm is

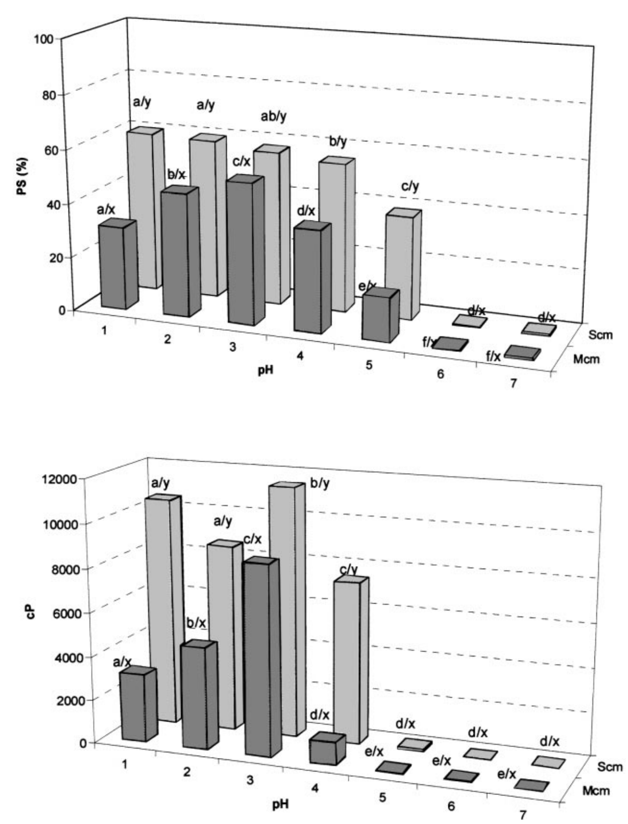

Fig. 1. Protein solubility (PS) and viscosity of muscle (Mcm) and skin (Scm) collagenous materials with reference to variations in pH. Different letters (a,b,c...) indicate significant differences ($p \leq 0.05$) with reference to pH values; different letters (x,y) indicate significant differences ($p \leq 0.05$) between Mcm and Scm.

considerably higher than in Mcm, and this coincides with previous findings in trout (Montero et al., 1991), although in hake material this functional property is considerably greater than in trout. In contrast to protein solubility, the viscosity of plaice skin material was much lower than hake skin (Montero et al., 1994).

The WBC of the collagenous materials was measured with (WBCh) and without (WBCc) a heating treatment (30 min at 60°C). As the pH increases, so does WBCc, until maximum is reached at pH 2–3 (Fig. 2, Table 2), while higher pH levels cause this parameter to decrease, so that at pH 4 very low values are found. Within the interval of maximum WBCc, Scm is found to have greater WBCc than Mcm. Increased pH also brings with it an increase in WBCh until maxima are reached at pH 2 for Mcm and pH 4 for Scm (Fig. 2, Table 2). WBCh in Mcm is not affected by pH above 3, while in Scm is enhanced at pH 7. This effect in Scm is due to gel formation by the neutralization of the medium. Similar findings, but with lower values at pH 1–4, were found in trout skin (Montero et al., 1991). Such values are similar to those reported for plaice skin (Montero et al., 1994). The heat treatment causes the rupturing of bonds and conformational changes of the collagen molecule, leading to the conversion into gelatin and increasing the hydration capacity (Osborne et al., 1990). The reason why it does not occur in Mcm is unclear. It is possible that, given the lower solubility of the Mcm (as an index of a greater degree of molecular crosslinking), the heating treatment is not enough to allow the conversion into gelatin, or perhaps more likely, that it might occur at higher pH than has so far been studied.

The variation in hydration properties with pH can be interpreted in the same way as that described for collagenous material from trout (Montero et al., 1991). As the pH rises, the functionality levels increase as a result of the augmented charge until maximum is reached between 2 and 4. As the pH approaches the isoelectric point, the opposite phenomenon occurs. Functional behaviour at very low pH levels may be attributed to protein denaturation.

Increased ionic strength causes a decrease in protein solubility in 0.5 M acetic acid and in apparent viscosity for both types of collagenous materials (Fig. 3a and b) so that, at 0.51 M NaCl, both functional properties are practically nil. Scm shows higher values than Mcm in viscosity at a NaCl concentration between 0 and 0.25 M, and in protein solubility between 0.17 and 0.25 M, which again takes into account the greater functionality of skin. The decline in functionality with NaCl concentration could be explained in terms of the salting-out phenomenon which occurs in this protein at relatively low molarities (Asghar & Henrickson, 1982), as in the case of collagenous material from trout (Montero et al., 1991), although the latter is less sensitive to the addition of salt than is hake. Montero et al. (1994), comparing different collagen extraction procedures in plaice skin, did not observe significant changes in acid solubility by previous homogenization of skins with solutions of NaCl between 0 and 0.4 M, concluding that the 0.4 M NaCl treatment did not induce any undesirable aggregation of the collagen. Although this is not exactly comparable with the present work, it does suggest a lower degree of salt-induced alteration by the collagenous material from plaice skin than from hake.

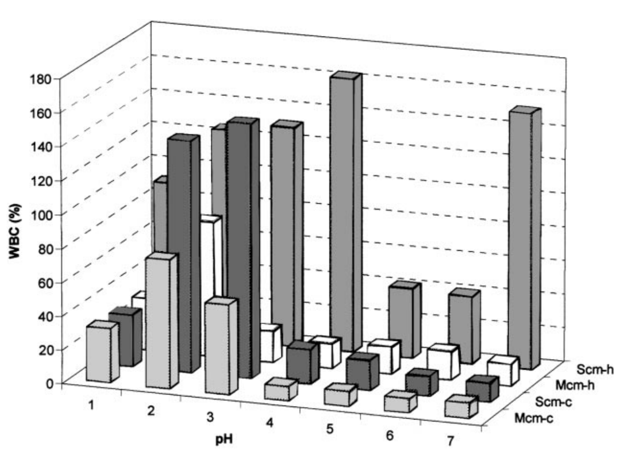

Fig. 2. Water-binding capacity (WBC), with and without a heating treatment (30 min at 60°C), of muscle (Mcm-c [cold] and Mcm-h [heated]) and skin (Scm-c [cold] and Scm-h [heated]) collagenous materials with reference to variations in pH.

Table 2
Two-way analysis of variance for water-binding capacity without heating (WBCc) and with heating (WBCh) with reference to pH and ionic strength

	pH						
	1	2	3	4	5	6	7
WBCc							
Mcm	a/x	b/x	c/x	d/x	d/x	d/x	d/x
Scm	a/x	b/y	c/y	d/y	de/y	e/y	e/y
WBCh							
Mcm	a/x	b/x	c/x	c/x	c/x	c/x	c/x
Scm	a/y	b/y	b/y	c/y	d/y	d/x	c/x

	Ionic strength					
	0	0.17	0.25	0.34	0.51	1
WBCc						
Mcm	a/x	b/x	c/x	bc/x	bc/x	c/x
Scm	a/y	b/y	c/y	cd/y	d/x	d/x
WBCh						
Mcm	a/x	b/x	c/x	ac/x	d/x	d/x
Scm	a/y	b/y	b/y	a/y	a/y	a/y

Different letters (a,b,c ...) in the same row indicate significant differences ($p \leq 0.05$); different letters (x,y) in the same column indicate significant differences.

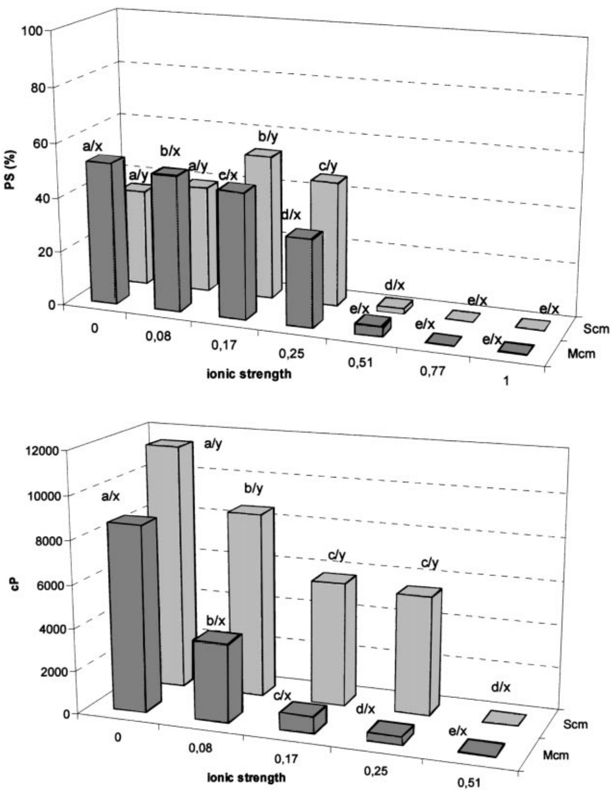

Fig. 3. Protein solubility (PS) and viscosity of muscle (Mcm) and skin (Scm) collagenous materials with reference to variations in ionic strength (as NaCl concentration). Different letters (a,b,c...) indicate significant differences ($p \leq 0.05$) with reference to ionic strength; different letters (x,y) indicate significant differences ($p \leq 0.05$) between Mcm and Scm.

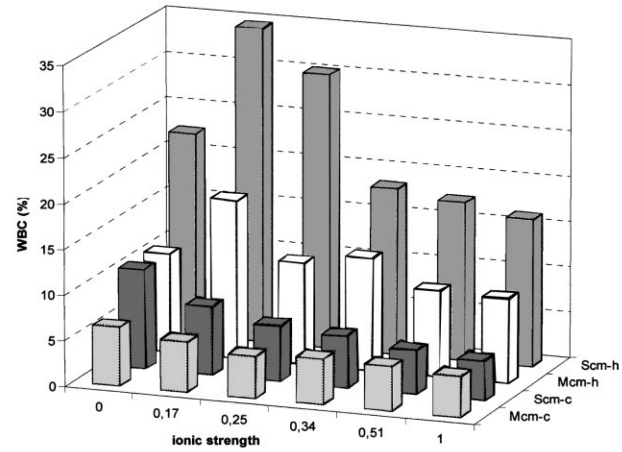

Fig. 4. Water-binding capacity (WBC), with and without a heating treatment (30 min at 60°C), of muscle (Mcm-c [cold] and Mcm-h [heated]) and skin (Scm-c [cold] and Scm-h [heated]) collagenous materials with reference to variations in ionic strength (as NaCl concentration).

Table 3
Correlation established between various hydration properties of skin and muscle collagenous material with reference to the parameters studied

	pH	Ionic strength
PS	0.916*	0.907*
Viscosity	0.799*	0.641*
WBCc	0.925*	0.912*
WBCh	−0.343	0.645*

PS: protein solubility; WBCc: water-binding capacity without heating treatment; WBCh: water-binding capacity with heating treatment.

An increase in ionic strength produces a decline in the WBC of Mcm and Scm without heating treatment (Fig. 4, Table 2). However, very low values are found for this parameter under the experimental conditions and therefore the effect of NaCl concentration is not very great. Similar results were obtained in trout muscle and skin collagenous materials (Montero et al., 1991) although, in the interval between 0 and 0.34 M NaCl, the muscle material from trout presented higher WBC than hake. When collagenous material is subjected to a heating treatment (30 min at 60°C), WBC increases considerably, mainly in Scm, reaching a maximum at 0.17 M NaCl. Similar behaviour was described for skin collagenous material from trout (Montero et al., 1991) although, in that case, higher values were achieved. Hake skin also shows less WBC after heating treatment than plaice skin (Montero et al., 1994), when comparing both species without addition of NaCl. These results are in accordance with the lower protein solubiltiy exhibited by the hake samples.

When correlations are established between Mcm and Scm functional properties with reference to the variables studied (Table 3), it is found that the two types of collagenous material present similar behavioural patterns with respect to pH and ionic strength, as reflected in their degree of significance (99%). In WBCh there is no significant correlation between the collagenous materials in terms of pH changes, due to the appearance of gelation phenomena in Scm, which causes the hydration capacity to increase even when the pH is close to the isoelectric point.

A Factor Analysis was performed to observe the interrelationships among the studied variables (except for WBC with heating treatment) in both skin and muscle material with respect to pH variations. Fig. 5 shows a two-dimensional representation of principal component loadings, and although all the factors would be needed to exactly reproduce the combined structure of the data, only two factors sufficed to account for 94% of total variance. WBC in both skin and muscle correlates strongly with PC1, whereas protein solubility shows a strong correlation with PC2, especially the skin material. This indicates that, although both variables are based on collagen hydration properties and are explained in terms of degree of protein aggregation, they must be defined by other factors, such as protein hydrodynamic properties, density,

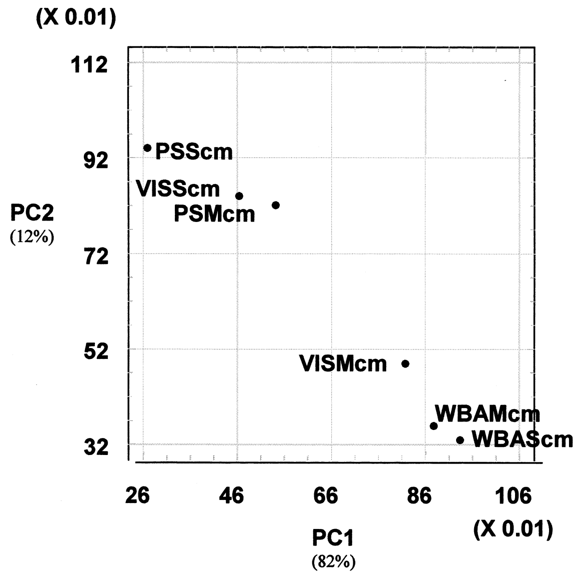

Fig. 5. Principal component loadings with Varimax rotation of the muscle (Mcm) and skin (Scm) collagenous materials for the variables, water-binding capacity (WBC, without heating treatment), protein solubility (PS) and viscosity (VIS). PC: principal component.

volume and shape. The viscosity of Scm does not behave in the same manner as Mcm, the latter being more related to WBC. The different behaviour of viscosity in relation to the type of sample denotes that this functional property depends greatly on the molecular diameter and shape, which are different. In this connection, Montero et al. (1990) reported that the collagenous material in muscle showed a higher degree of aggregation than in skin.

4. Conclusion

From a technological point of view, the collagenous material from hake, especially the skin, shows greater functionality at around pH 3 and concentrations of NaCl less than 0.25 M NaCl, offering a number of industrial aplications. The determination of the apparent viscosity seems to be a suitable method for determining collagenous material functionality. It is also sufficiently sensitive to distinguish between materials from different sources.

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