Physico-chemical properties of tuna-skin and bovine-hide gelatin films with added aqueous oregano and rosemary extract

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ABSTRACT

The properties of edible, gelatin-based films with added oregano or rosemary extract (two different concentrations) were studied. Gelatins from different sources (bovine hides and tuna skins) were employed with a view to elucidating how inherent gelatin characteristics may affect interaction of the gelatin with the polyphenols in the added extract and hence the properties of the resulting films. The bovine-hide gelatin reacted only slightly with the polyphenols in the extracts as shown by the electrophoretic profile and analysis of the dynamic viscoelastic properties, and consequently the attributes (mechanical properties, water solubility, water vapour permeability) of the films were practically unchanged compared with the film made without any added plant extract. The tuna-skin gelatin did evidence some interactions with the polyphenols in both the oregano and the rosemary extracts, especially for the more concentrated of the two extracts tested, thereby altering the attributes of the corresponding films, namely, a higher glass transition temperature, decreased deformability, and, in

particular, increased water solubility. Opacity increased irrespective of gelatin origin and plant extract type and concentration.

Key words: bovine-hide gelatin, tuna-skin gelatin, polyphenols, films, physico-chemical properties

INTRODUCTION

Considerable efforts are being expended lately to develop new biodegradable packaging materials from natural polymers because of environmental concerns relating to synthetic plastic packaging waste. Gelatin is a protein with a broad range of functional properties and applications, including film-forming ability, and is obtained by hydrolyzing collagen. Collagen is composed of three α-chains (two α_1 and one α_2) intertwined in the collagen triple helix (Gómez-Guillén, Turnay, Fernández-Díaz, Ulmo, Lizarbe, & Montero, 2002). Extraction yields a pool of α-chains (both α_1 and α_2), β components (two covalently linked α-chains), and γ components (three covalently linked α-chains), along with high-molecular-weight aggregates (different covalently linked α-chains, β components, and γ components) and peptide fractions with molecular weights of <100 kDa. The properties and film-forming ability of gelatins are directly related to the molecular weight, i.e., the higher the average molecular weight, the better the quality of the gelatin. The molecular weight distribution depends mainly on the degree of collagen cross-linking and the extraction procedure. However, the physical properties of gelatins are related not only to the molecular weight distribution but also to the amino acid composition. Mammalian gelatins commonly have better physical properties and

thermostability than most fish gelatins (Ledward, 1986), and this has been related mainly to their higher imino acid content (Norland, 1990), which promotes refolding into the triple-helix configuration at low temperature (Gómez-Guillén et al., 2002). Various studies have dealt with the physical and chemical properties of mammalian gelatin films (Menegalli, Sobral, Roques, & Laurent, 1999; Sobral, Menegalli, Hubinger, & Roques, 2001; Simon-Lukasik & Ludescher, 2004; Bertan, Tanada-Palmu, Siani, & Grosso, 2005). Recently attention has focused on the properties of films made from fish gelatins (Muyonga, Cole, & Duodu, 2004; Thomazine, Carvalho, & Sobral, 2005; Avena-Bustillos et al., 2006; Jongjareonrak, Benjakul, Visessanguan & Tanaka, 2006a, 2006b; Gómez-Guillén, Ihl, Bifani, Silva, & Montero, 2007; Pérez-Mateos, Montero, & Gómez-Guillén, 2008; Carvalho et al., 2008).

Film coatings improve storage, mainly by acting as barriers to water, oxygen, and light (Gennadios, Hanna, & Kurth, 1997). Furthermore, active substances, e.g., plant extracts, have also been included in formulations of edible films to afford enhanced antioxidant and/or antimicrobial properties (Zivanovic, Chi, & Draughon, 2005; Kim et al., 2006; Seydim & Sarikus, 2006; Gómez-Guillén et al., 2007) and thus improve the quality and stability of foods during storage (Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004; Gómez-Estaca, Montero, Giménez, & Gómez-Guillén, 2007). However, adding such extracts may alter the mechanical and barrier properties of the gelatin films (Gómez-Guillén et al., 2007) and affect the release of active components (Gómez-Estaca, Bravo, Gómez-Guillén, Alemán, & Montero, 2009) as a consequence of polyphenol-protein interactions, but little information is available in this regard to date.

Because of its intrinsic open structure, gelatin is generally agreed to be more prone to interact with polyphenols than globular proteins (Frazier, Papadopoulou, Mueller-Harvey, Kissoon, & Green, 2003; Naczk, Grant, Zadernowski, & Barre, 2006). Nevertheless, exactly how the intrinsic characteristics of gelatins from different sources may act on these interactions is unknown. Frazier et al. (2003) used isothermal titration microcalorimetry to investigate protein-tannin interactions and found that tannins bound to gelatin by a two-stage mechanism, namely, a first stage of cooperative binding of tannins to the protein, followed by a second stage of gradual saturation of binding sites. Naczk et al. (2006) studied the protein precipitating capacity of phenolics from wild blueberry leaves and fruits and found that the tannin-rich fractions of the extracts were more effective gelatin precipitants than the fractions comprising low-molecular-weight compounds (monomers, dimers, and trimers) only.

The object of this experiment was therefore to evaluate changes in the physical properties of gelatin films on adding aqueous extracts of oregano and rosemary, taking into consideration differences related to gelatin source, i.e., either tuna skins or bovine hides.

MATERIALS AND METHODS

Preparation of the antioxidant extracts

Freeze-dried oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) leaves were purchased at a local market. Quantities of 5 and 20 g,

101 respectively, were mixed with 100 mL of distilled water that had been pre-
102 warmed to 45 ºC, the mixture was then extracted by continuous stirring in a
103 warm water bath at 45 ºC for 10 min. The aqueous extract obtained was filtered
104 through Whatman no. 1 filter paper. The total phenolic content of the aqueous
105 extracts as determined by the Folin-Ciocalteau method according to Montreau
106 (1972) was 2 080 ± 23 µg of caffeic acid/mL for the oregano extract and 665 ±
107 11 µg of caffeic acid/mL for the rosemary extract.
108
109 **Formulation of the film-forming solutions (FFSs) and film formation**
110 The FFSs were prepared using gelatin made from tuna skins [obtained
111 according to the method described by Gómez-Guillén & Montero (2001)] or
112 bovine hides (Bloom 200/220 from Sancho de Borja S.L., Saragossa, Spain) at
113 a concentration of 4 g/100 mL of distilled water. Based on the results of
114 Thomazine et al. (2005), a mixture of sorbitol (0.15 g/g gelatin) and glycerol
115 (0.15 g/g gelatin) was employed as plasticizer. The oregano (OE) and rosemary
116 (RE) extracts were added to the FFSs in the proportion of 6.25 mL OE/100 mL
117 FFS (batch O-L, theoretical phenol content of 130 µg caffeic acid/mL FFS);
118 25 mL OE/100 mL FFS (batch O-H, theoretical phenol content of 520 µg caffeic
119 acid/mL FFS); 12.5 mL RE/100 mL FFS (batch R-L, theoretical phenol content
120 of 83 µg caffeic acid/mL FFS); and 100 mL RE/100 mL FFS (batch R-H,
121 theoretical phenol content of 665 µg caffeic acid/mL FFS). Distilled water was
122 employed to prepare the extract/FFS dilutions, except for batch R-H, which was
123 not diluted. For comparative purposes an FFS with no added extract was also
124 made up (batch C). All mixtures were warmed and stirred at 40 ºC for 15 min to
125 obtain a good blend, and the films were prepared by casting an amount of 40 ml

on 12 cm x 12 cm-square plates and drying in a forced-air oven at 45 ºC for 15 h to obtain a uniform thickness (100 µm; $p < 0.05$) in all cases. Before performing the determinations, the films were conditioned in desiccators over a saturated solution of NaBr at 22 ºC for 2 days.

Electrophoretic profile of the gelatins dissolved in the aqueous extracts

The molecular weight distributions of the solutions of both the bovine-hide and tuna-skin gelatins with the oregano and rosemary extracts at the two concentrations employed were determined by polyacrylamide gel electrophoresis in the presence of SDS, and the SDS-PAGE profiles of were examined to assess the interactions among the aqueous oregano and rosemary extract components and the gelatins. The gelatin solutions were mixed with loading buffer (2 % SDS, 5 % mercaptoethanol, and 0.002 % bromophenol blue) in a proportion of 1:4, resulting in a final protein concentration of 5 mg/mL. Samples were heat-denatured at 90 ºC for 5 min and analysed according to Laemmli (1970) using 4% stacking gels and 6% resolving gels in a Mini Protean II unit (Bio-Rad Laboratories, Hercules, CA, USA) at 25 mA/gel. Loading volume was 15 µL in all lanes. Protein bands were stained with Coomassie brilliant Blue R250. Type I collagen from fetal calf was used as a marker for α-chain and β-component mobilities. A molecular weight standard composed of myosin (212 kDa), α_2-macroglobulin (170 kDa), β-galactosidase (116 kDa), transferrin (76 kDa) and glutamic dehydrogenase (53 kDa) (Amersham Pharmacia Biotech, Buckinghamshire, UK) was also employed.

FFS analysis

Dynamic viscoelastic properties

Dynamic viscoelastic analysis of the film-forming solutions was carried out using a Bohlin CSR-10 rheometer rotary viscometer (Bohlin Instruments Ltd., Gloucestershire, UK) using a cone-plate geometry (cone angle=4º, gap= 0.15 mm) as described in Gómez-Guillén et al. (2007). Cooling and heating from 40 to 6 ºC and back to 40 ºC took place at a scan rate of 1 ºC/min, a frequency of 1 Hz, and a target strain of 0.2 mm. The elastic modulus (G'; Pa), viscous modulus (G"; Pa) and phase angle (º) were determined as functions of temperature. Two determinations were performed for each sample, with an experimental error of less than 6% in all cases.

Gel strength

The film-forming solutions were poured into glasses 2.3 cm in diameter by 3.6 cm in height and left to mature in a refrigerator at 2 ºC for 16-18 h. Gel strength at 8-9 ºC was determined on an Instron model 4501 Universal Testing Machine (Instron Co., Canton, MA, USA) with a 100 N load cell, a cross-head speed of 60 mm/min, and a flat-faced cylindrical plunger 1.27 cm in diameter. The maximum force (g) was determined when the plunger had penetrated 4 mm into the gelatin gels.

Film analysis

Mechanical properties

A puncture test was performed to determine the breaking force and breaking deformation of films. Films were placed in a cell 5.6 cm in diameter and perforated to the breaking point using an Instron model 4501 Universal Testing

Machine (Instron Co., Canton, MA, USA) with a rounded stainless-steel plunger 3 mm in diameter at a cross-head speed of 60 mm/min and a 100-N load cell. The puncture force was expressed in N and breaking deformation in percent, as per Sobral et al. (2001). All determinations are the means of at least five measurements.

Thermal properties

Calorimetric analysis was performed using a model TA-Q1000 differential scanning calorimeter (DSC) [TA Instruments, New Castle, DE, USA] previously calibrated by running high purity indium (melting point: 156.4 ºC; enthalpy of melting: 28.44 W/g). Samples of approximately 10 mg (± 0.002 mg) were weighed out using a model ME235S electronic balance (Sartorious, Goettingen, Germany) and were tightly encapsulated in aluminium pans and scanned under dry nitrogen purge (50 mL/min). Freshly conditioned films were rapidly cooled to 0 ºC and scanned at between 0 and 90 ºC at a heating rate of 10 ºC/min. Glass transition temperatures, Tg (ºC), were determined only on the first heating scans, the values obtained on the second scans being deemed insufficiently reliable because of the virtual impossibility, in practice, of reproducing the original film conditioning. The glass transition temperature was estimated as the midpoint of the line between the temperature at the intersection of the initial tangent with the tangent through the inflection point of the trace and the temperature of the intersection of the tangent through the inflection point with the final tangent. Tg data have been reported as the mean values of at least duplicate samples of each film, usually within ± 1 ºC.

Water solubility

Film portions of 4 cm^2 were placed in aluminium capsules with 15 ml of distilled water and shaken gently at 22 ºC for 15 h. The solution was then filtered through Whatman no. 1 filter paper to recover the remaining undissolved film, which was desiccated at 105 ºC for 24 h. Film solubility was calculated using the equation $FS\ (\%) = [(W_o - W_f)/W_o] \cdot 100$, where W_o was the initial weight of the film expressed as dry matter and W_f was the weight of the undissolved desiccated film residue. All tests were carried out in triplicate.

Water vapour permeability

Water vapour permeability was determined according to the gravimetric method described by Sobral et al. (2001). Films were attached over the openings of cells (permeation area = 15.9 cm^2) containing desiccated silica gel, and the cells were placed in desiccators with distilled water at 22 ºC. The cells were weighed daily for 7 d. Water vapour permeability was calculated using the equation $WVP = w \cdot x \cdot t^{-1} \cdot A^{-1} \cdot \Delta P^{-1}$, where w was weight gain (g), x film thickness (mm), t elapsed time at weight gain (h), A permeation area, and ΔP partial vapour pressure difference between the dry atmosphere and pure water (2 642 Pa at 22 ºC). Results have been expressed as $g \cdot mm \cdot h^{-1} \cdot cm^{-2} \cdot Pa^{-1}$. All tests were carried out in duplicate.

Opacity

Film portions of 0.8 cm x 4 cm were placed in a spectrophotometer test cell, and absorbance was measured at 600 nm using a UV-1601 spectrophotometer (Model CPS-240, Shimadzu, Kyoto, Japan). The opacity index (O) was

calculated as the quotient of the absorbance value at 600 nm divided by film thickness in mm.

Statistical analysis

Statistical tests were performed using the SPSS® computer program (SPSS Statistical Software, Inc., Chicago, IL, USA). One-way and two-way analyses of variance were carried out. Differences between pairs of means were resolved by means of confidence intervals using a Tukey-b test. The level of significance was set for $p < 0.05$.

RESULTS AND DISCUSION

Electrophoretic profile of the gelatins dissolved in the aqueous extracts

The molecular weight distributions of the blends of the bovine-hide and tuna-skin gelatins with the aqueous oregano and rosemary extracts at the different concentrations studied are presented in Figure 1. The presence of aqueous oregano and rosemary extracts in the bovine-hide gelatin solution had hardly any effect on the electrophoretic profiles as compared to the control gelatin solution (batch C), irrespective of extract concentration. In contrast, the tuna-skin gelatin with both concentrations of added oregano extract registered appreciable decreases in β-components and high-molecular-weight aggregates (HMW-a) and a certain increase of polypeptides with molecular weights below 100 kDa. The increase in hydrolyzed fractions was more intense in the batch with the more concentrated oregano extract (batch O-H). These results suggested a high degree of interaction between the phenolic substances in the oregano extract and the fish-gelatin polypeptides, giving rise to cleavage or

disruption of the covalently associated α-chains (β-components and HMW-a). Adding rosemary extract to the tuna-skin gelatin also brought about a decrease in the amount of HMW-a present, but in this case without any appreciable rise in the hydrolyzed low-molecular-weight fractions, although the new protein fractions could be located in the >200 kDa region. This difference with respect to the samples with the added oregano extract could be attributed to qualitative differences in the two extracts as previously observed using confocal laser scanning microscopy and HPLC analysis (Gómez-Estaca et al., 2009). In that study, rosmarinic acid was found to be the most abundant compound in both extracts, but the oregano extract also contained gallic acid and protocatechuic acid while the rosemary extract contained chlorogenic acid. There were also appreciable amounts of a series of other compounds that could not be identified, though based on their absorption spectra they may have been hydroxybenzoic acid derivatives, caffeic acid derivatives, and various flavonoids (primarily flavone derivatives). The stronger polyphenol-protein interaction observed for the tuna-skin gelatin compared with the bovine-hide gelatin can be ascribed to differences in gelatin characteristics. According to Frazier et al. (2003) and Naczk et al. (2006), proteins with an open structure are more prone to interact with polyphenols. Although gelatin is a clear example of a protein with an open structure subsequent to collagen denaturation, there are certain differences that could affect the degree of interaction depending on gelatin origin. Mammalian gelatins are well known to have higher imino acid (Pro+Hyp) contents than fish gelatins (Norland, 1990; Avena-Bustillos et al., 2006), which is conducive to intra and interchain interactions. In addition, the collagenous material in fish skins has low levels of intra and interchain covalent cross-

linking, mainly involving lysine and hydroxylysine residues and aldehyde derivatives (Montero, Borderías, Turnay, & Leyzarbe, 1990). Thus, the presumably higher protein-protein interactions in the bovine-hide gelatin may interfere with the ability of the polyphenols to interact with the protein chains compared with the tuna-skin gelatin.

FFS analysis

Possible interactions between the extract components and the gelatin molecules were also investigated by following the changes in the dynamic viscoelastic properties of the different FFSs upon cooling and subsequent heating (Figure 2 for bovine-hide FFS, Figure 3 for tuna-skin FFS). Addition of the antioxidant extracts did not substantially alter the *G′* curves or the thermal transition points for either gelatin type, indicative of minor interference with triple helical structure formation. However, the viscous modulus (*G″*) value increased in tuna-skin gelatin batches O-H and R-H, suggesting the presence of certain peptide fractions that did not participate directly in the protein gel matrix upon cold renaturation, probably because of interactions with the phenolic compounds. This would be consistent with the electrophoretic profiles of the phenolic-containing fish-gelatin samples, which exhibited an accumulation of hydrolyzed peptide fractions resulting from cleavage of covalently linked peptide chains. Similarly, interference with gelatin polypeptide chains by polyphenolics upon cold renaturation and subsequent melting was also reported in tuna-fish gelatin film-forming solutions with added *murta* extracts (Gómez-Guillén et al., 2007). In that study the increase in the *G″* values was also proportional to the amount of polyphenolics added to the FFSs.

The gel strength of the FFSs was also determined (Figure 4) to shed further light on the possible interactions or interference between the polyphenolic compounds and the protein matrix during cold maturation of the gels, during which triple helix growth takes place by association of different α-chains via hydrogen bonding. The addition of aqueous oregano or rosemary extract brought about an increase in gel strength irrespective of gelatin origin, with minor variations depending on extract type and concentration in the FFSs. This gel strengthening effect could be attributable to non-covalent polyphenol-protein interactions during cold maturation of the gelatins overnight, which may be conducive to the formation of side-by-side associations of gelatin chains without disturbing triple helix formation and growth. According to Oh, Hoff, Armstrong & Haff (1980) and Haslam (1996), the polyphenol-protein interaction initially results from hydrophobic interactions and is subsequently augmented by the possible formation of hydrogen bonds between the polyphenol –OH groups and the protein –COOH groups. Frazier et al. (2003) found that multidentate tannin ligands formed intermolecular cross-links between binding sites on adjacent gelatin molecules, contributing to protein precipitation. In this regard, Naczk et al. (2006) reported that gelatin precipitation due to phenolic compounds from blueberry leaves and fruits was due mainly to the condensed tannin fraction. The aqueous oregano and rosemary extracts employed in the present experiment did not contain these high-molecular-weight polyphenol complexes (Gómez-Estaca et al., 2009), and therefore no protein precipitation was apparent. However, the low-molecular-weight polyphenols present exerted a

strengthening effect, most probably by promoting interactions among adjacent gelatin chains.

Film analysis

The breaking force and breaking deformation values for the resulting films are set out in Table 1. Breaking force values for the control films were similar ($p > 0.05$), but addition of the polyphenolic extracts resulted in differences according to gelatin type. Still, the added plant extracts did not significantly ($p > 0.05$) alter the breaking force for any of the gelatin admixtures compared to the control batches. Similarly, no differences were observed for breaking deformation on adding the plant extracts, except for the tuna-skin gelatin film with the more concentrated rosemary extract (batch R-H), which had a significantly lower breaking deformation value. Other workers have reported lower breaking strength and breaking deformation on adding antioxidant extracts to films made either from soya or from gelatin (Kim et al., 2006; Gómez-Guillén et al., 2007). This behaviour was explained by Orliac, Rouilly, Silvestre, & Rigal (2002) by a weakening of the interactions that stabilize the protein matrix on adding polyphenolic antioxidants, especially higher-molecular-weight polyphenols. The general absence of significant differences in the mechanical property values of the films studied here when the plant extracts were added, in contrast to other published work, is largely ascribed to the characteristics of the phenolic composition of the aqueous oregano and rosemary extracts, which had a predominance of low-molecular-weight compounds (Gómez-Estaca et al., 2009).

Figure 5 plots the DSC traces. Despite encapsulation in non-hermetically sealed pans, samples shrank only by ~1-2% by weight after DSC scanning, indicating good water retaining capacity of the films. However, this produced a certain bending in the trace from water vaporization, which distorted the DSC profiles somewhat at the glass transition exit. Additionally, devitrification overlapped completely with a relaxation effect that introduced considerable uncertainty into the Tg determinations. The films did not exhibit any melting event attributable to crystallization of the gelatins. The glycerol plus sorbitol (Sobral et al., 2001) and the low water contents used yielded wholly amorphous films. It is generally accepted that, in addition to their plasticizing effects, polyols present at low moisture levels may inhibit crystalline structures by constraining molecular mobility (Cheng, Karim, & Seow, 2006). The tuna-skin gelatin batches exhibited Tg values slightly lower than but similar to the corresponding bovine-hide gelatin batches (Table 2). Adding the herb extracts raised the glass transition temperatures in both the bovine-hide and tuna-skin gelatin films. The increase was relatively higher as the amount of added extract rose, with the bovine-hide gelatin being slightly more sensitive than the tuna-skin gelatin. Furthermore, the rosemary extract seemed to bring about a larger increase in the Tg than the oregano extract, despite the similar phenolic contents. However, compared with the respective tuna-skin gelatin batches, the bovine-hide gelatin films displayed a saturation effect with rosemary extract concentration, in that batch R-H resulted in a considerably lower increase than would at first be expected based on the previous effect of batch R-L. Thus, the bending sites for polyphenols in the bovine-hide gelatin appeared to be saturated, and the rosemary extract

374 appeared to be incorporated into the bovine-film matrix partly in the manner of a
375 filler.
376
377 Addition of the antioxidant extracts to the tuna-skin gelatin brought about a
378 pronounced increase in film solubility (Table 3) in the case of batches O-H and
379 R-H, as well as batch O-L. Electrophoretic analysis indicated that the higher
380 solubility could be due to the cleavage or degradation of covalently linked α-
381 chains (β-components) or even HMW-a induced by the phenolic compounds
382 present. This effect observed in the electrophoretic profile was more apparent in
383 the films containing the more concentrated oregano extract, but adding the
384 more concentrated rosemary extract also exerted an influence, reducing the
385 quantities of HMW-a present to some extent. The differences in water solubility
386 observed between batches R-L and O-L could be due to qualitative differences
387 in the polyphenolic composition, as indicated in a previous paper (Gómez-
388 Estaca et al., 2009). Kim et al. (2006) also reported increased film solubility on
389 adding green tea extract to soya-protein films. However, the presence of the
390 phenolic extracts did not produce any significant differences in the solubility of
391 the bovine-hide gelatin films. This finding was in consonance with the
392 corresponding electrophoretic profiles, which likewise were barely affected by
393 the different blends.
394
395 Table 3 also sets out the water vapour permeability (WVP) of the films. All the
396 tuna-skin gelatin films had significantly lower WVP values than the bovine-hide
397 gelatin films. According to Avena-Bustillos et al. (2006), this could be related to
398 higher levels of hydrophobicity resulting from the lower amounts of proline and

hydroxyproline present in gelatins from cold-water fish species as compared to mammalian gelatins. Adding the plant extracts did not significantly ($p > 0.05$) alter the water vapour permeability in either the bovine-hide or the tuna-skin gelatin films. Gómez-Guillén et al. (2007) recorded a decrease in the WVP on adding murta extract to tuna-fish gelatin films when the extract from the murta ecotype with the higher phenolic content was added.

To the unaided eye all the films were quite transparent, though tinted by the natural colour of the oregano and rosemary extracts. Adding the plant extracts increased the *O* values of both types of gelatin, especially for the more highly concentrated oregano extract (Table 4). The higher film opacity on adding plant extracts is directly ascribed to enrichment of the films with polyphenols and to some extent probably also to polyphenol-protein interactions. Similar results were reported for tuna-fish gelatin films with added murta extract (Gómez-Guillén et al., 2007).

CONCLUSIONS

The physical and chemical properties of the bovine-hide and tuna-skin gelatins determine their reactivity with the polyphenols in added plant extracts and hence the properties of the corresponding composite films. The bovine-hide gelatin thus did not react with the polyphenols to the same extent as the tuna-skin gelatin. In consequence, the properties of bovine-hide gelatin films with added oregano or rosemary extract were similar to the properties of the control film with no added extract. In contrast, the tuna-skin gelatin interacted more readily with the polyphenols, appreciably increasing the water solubility of the

films. Opacity increased irrespective of gelatin origin and added plant extract type and concentration.

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Figure captions

Figure 1. Electrophoretic profiles of the bovine-hide and the tuna-skin gelatins with added oregano or rosemary extract. C: no added extract (control batch); O-L: a low concentration of added oregano extract; O-H: a high concentration of added oregano extract; R-L: a low concentration of added rosemary extract; R-H: a high concentration of added rosemary extract. HMW-a: high-molecular-weight aggregates.

Figure 2. Dynamic viscoelastic properties of the FFSs prepared from bovine-hide gelatin with added oregano or rosemary extract (batch designations as in Figure 1) during cooling (a) and subsequent heating (b) ramps.

Figure 3. Dynamic viscoelastic properties of FFSs prepared from tuna-skin gelatin with added oregano or rosemary extract (batch designations as in Figure 1) during cooling (a) and subsequent heating (b) ramps.

Figure 4. Gel strength of FFSs (batch designations as in Figure 1) after cold maturation. Different letters (a, b, c) indicate significant differences between the formulations prepared using the different gelatin types. Different letters (x, y) indicate significant differences between the two gelatin types for each formulation.

Figure 5. DSC traces for the bovine-hide (a) and tuna-skin (b) gelatin films (batch designations as in Figure 1) at the Tg point.

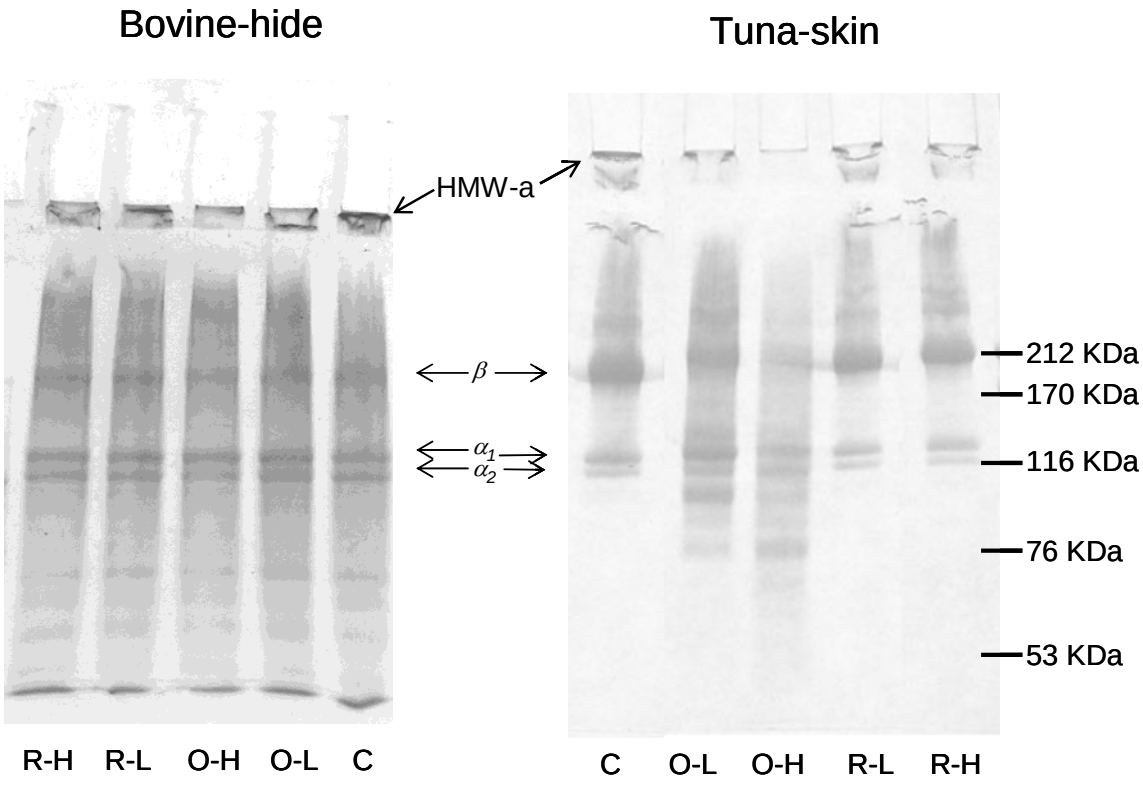
Bovine-hide
Tuna-skin
HMW-a
β
α1
α2
212 KDa
170 KDa
116 KDa
76 KDa
53 KDa
R-H
R-L
O-H
O-L
C
C
O-L
O-H
R-L
R-H

Figure 1

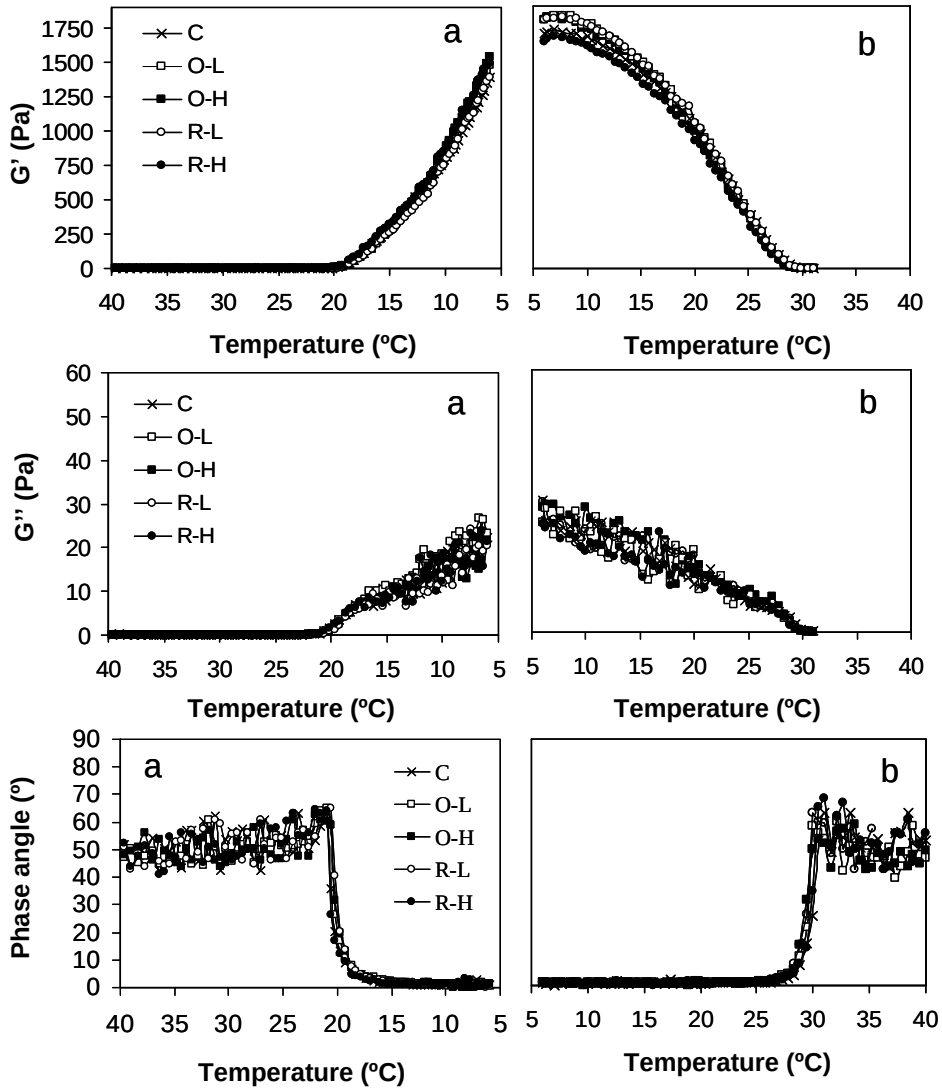

Figure 2

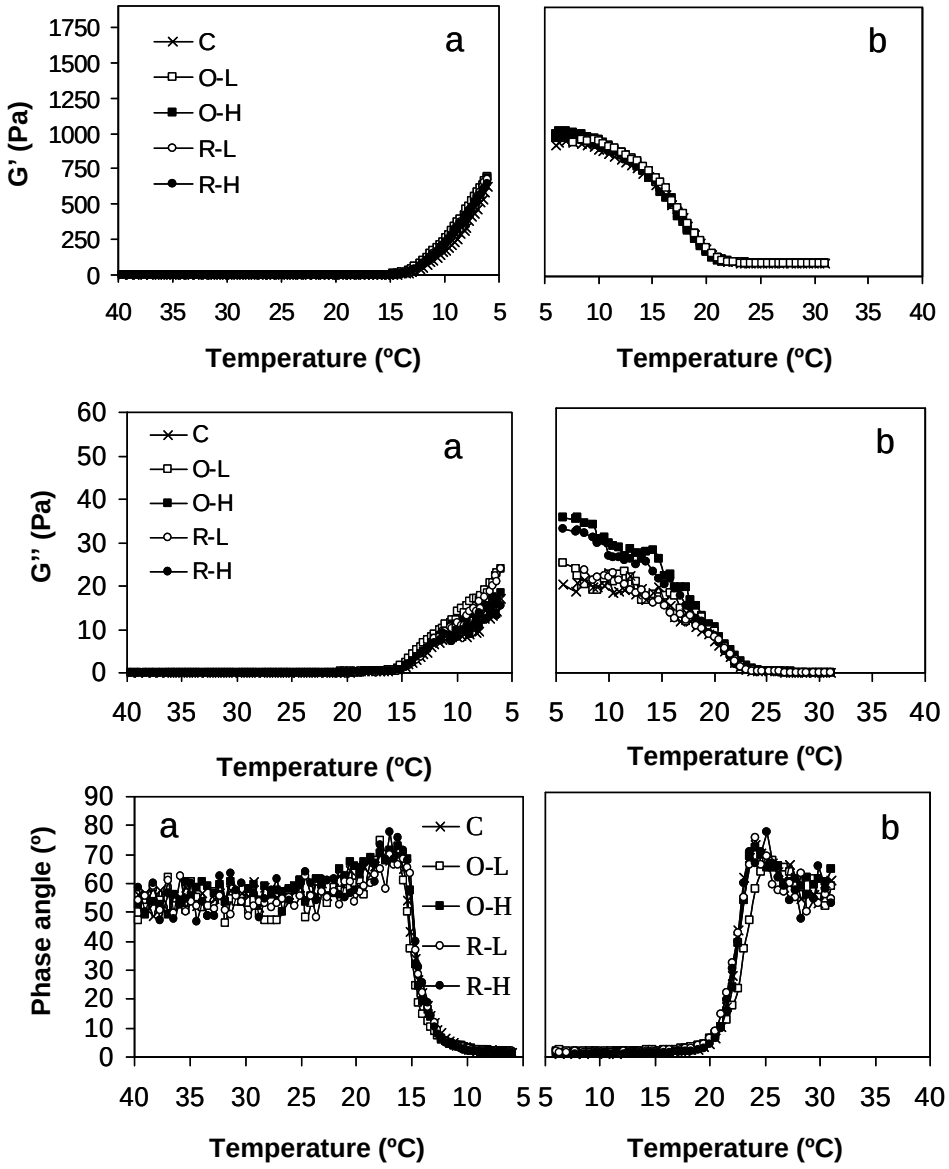

Figure 3

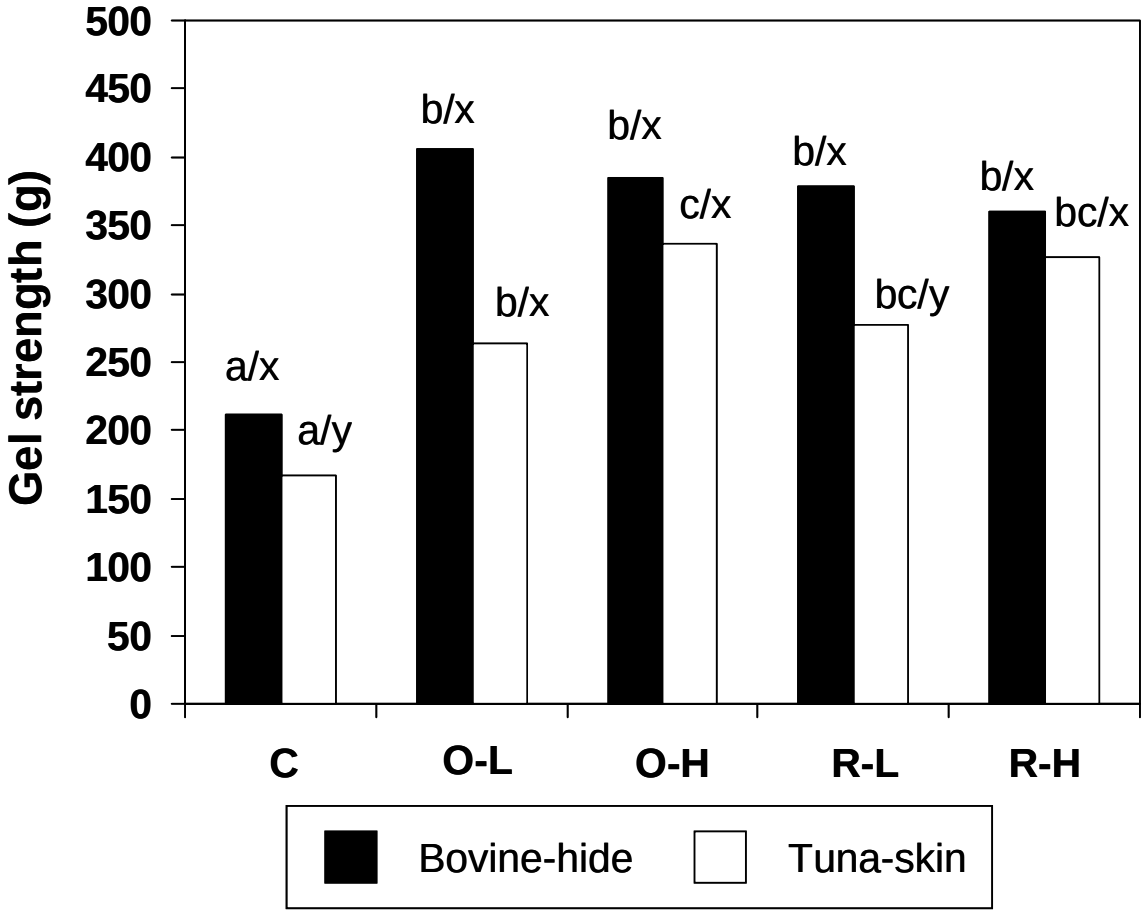
Gel strength (g)
500
450
400
350
300
250
200
150
100
50
0
a/x
a/y
b/x
b/x
b/x
c/x
b/x
bc/y
b/x
bc/x
C
O-L
O-H
R-L
R-H
Bovine-hide
Tuna-skin

Figure 4

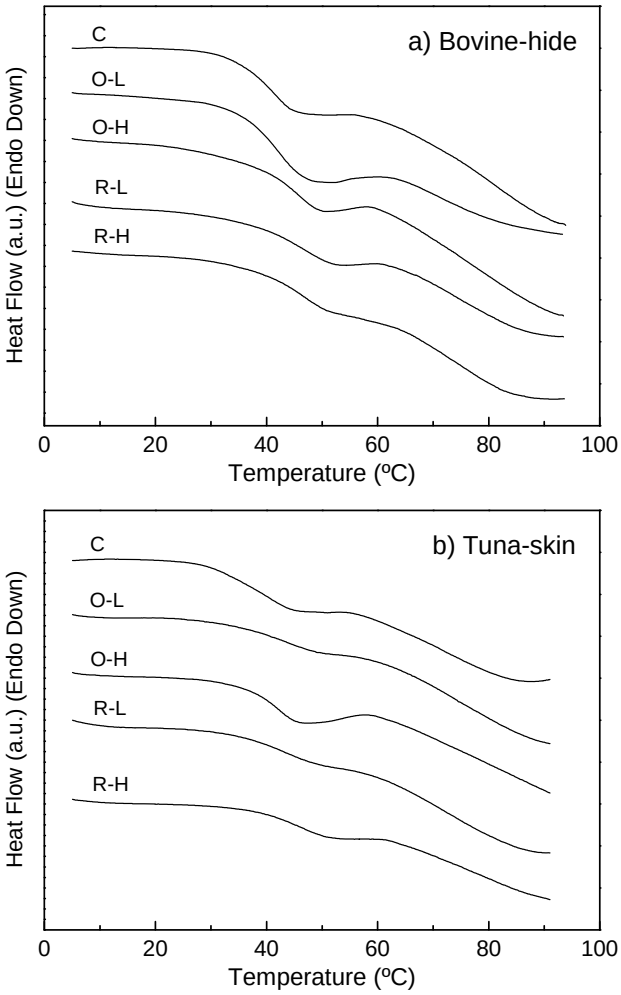
a) Bovine-hide
C
O-L
O-H
R-L
R-H
Heat Flow (a.u.) (Endo Down)
0
20
40
60
80
100
Temperature (ºC)
b) Tuna-skin
C
O-L
O-H
R-L
R-H
Heat Flow (a.u.) (Endo Down)
0
20
40
60
80
100
Temperature (ºC)

Figure 5

Table 1. Mechanical properties of the films (batch designations as in Figure 1). Results have been expressed as the mean value ± standard deviation. Different letters (a, b, c) within the columns indicate significant differences between formulations with/without extract. Different letters (x, y) within the rows indicate significant differences between gelatine types.

Batch	Puncture force (N)		Breaking deformation (%)	
	Bovine-hide	Tuna-skin	Bovine-hide	Tuna-skin
C	10.7 ± 2.2 ax	8.5 ± 1.6 ax	14.1 ± 5.0 ax	154 ± 35 cy
O-L	10.2 ± 1.3 ax	5.2 ± 1.7 ay	14.1 ± 4.7 ax	116 ± 26 cy
O-H	8.8 ± 0.8 ax	6.1 ± 1.0 ay	19.4 ± 4.2 ax	132 ± 14 bcy
R-L	9.9 ± 1.3 ax	6.2 ± 1.3 ax	14.9 ± 6.9 ax	147 ± 38 cy
R-H	12.4 ± 0.7 ax	5.6 ± 1.0 ay	11.6 ± 0.9 ax	87 ± 9 aby

Table 2. Tg values (ºC) of the films (batch designations as in Figure 1). Results are the mean values of at least two samples of each film, usually within ± 1 ºC.

Batch	Tg (ºC)	
	Bovine-hide	Tuna-skin
C	41.5	40.7
O-L	42.6	41.2
O-H	44.3	42.5
R-L	45.3	42.9
R-H	47.4	47.0

Table 3. Water solubility and water vapour permeability of the films (batch designations as in Figure 1). Results have been expressed as the mean value ± standard deviation. Different letters (a, b, c) within the columns indicate significant differences between formulations with/without extract. Different letters (x, y) within the rows indicate significant differences between gelatine types.

Batch	Water solubility (%)		Water vapour permeability (10^{-8}·g·mm·h^{-1}·cm^{-2}·Pa^{-1})	
	Bovine-hide	Tuna-skin	Bovine-hide	Tuna-skin
C	34.3 ± 0.6 ax	39.9 ± 1.3 ax	2.20 ± 0.11 bcx	1.65 ± 0.39 ay
O-L	35.7 ± 1.5 ax	76.6 ± 0.1 by	2.13 ± 0.08 abcx	1.59 ± 0.08 ay
O-H	38.6 ± 1.4 ax	83.7 ± 3.6 by	2.40 ± 0.03 cx	1.35 ± 0.06 ay
R-L	34.8 ± 1.6 ax	36.6 ± 0.6 ax	2.17 ± 0.08 bcx	1.30 ± 0.59 ay
R-H	34.4 ± 0.7 ax	82.6 ± 8.1 by	1.89 ± 0.07 abx	1.42 ± 0.26 ay

Table 4. Opacity index values for the films (batch designations as in Figure 1).

Batch	Opacity index	
	Bovine-hide	Tuna-skin
C	0.461 ± 0.001	0.377 ± 0.000
O-L	0.542 ± 0.000	0.546 ± 0.001
O-H	0.725 ± 0.002	0.655 ± 0.003
R-L	0.530 ± 0.001	0.411 ± 0.001
R-H	0.684 ±0.002	0.557 ± 0.003