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Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae* Turcz)

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Abstract

The aqueous extract of two ecotypes of murta (*Ugni molinae* Turcz) leaves (Soloyo Grande "SG" and Soloyo Chico "SC"), were analysed for their antioxidant capacity, SC extract exhibiting a higher antioxidant capacity than SG extract. This difference affects physical properties of tuna-fish (*Thunnus tynnus*) gelatin-based edible films with incorporated murta leaves extract. Dynamic viscoelastic studies, scanning electron microscopy (Cryo-SEM) and SDS-PAGE denoted a certain degree of interference of polyphenolic compounds in the arrangement of gelatine molecules. The addition of the murta extracts leads to transparent films with increased protection against UV light as well as antioxidant capacity. The puncture test showed no significant differences ($p > 0.05$) between the Control film and the SG film, whereas puncture force and puncture deformation were significantly lower for SC ecotype. Water vapour permeability of tuna-fish skin gelatin films with SG was 2.87×10^{-8} g mm h^{-1} cm^{-2} Pa^{-1}, significantly different ($p \leqslant 0.05$) to 1.83×10^{-8} g mm h^{-1} cm^{-2} Pa^{-1} of the film with SC.

Keywords: Fish skin gelatin; Leaves extracts; Murta ecotypes; Edible films; Water vapour permeability; Antioxidant properties

1. Introduction

Soluble gelatin, obtained industrially from collagen present in bones or skins, is extensively used as an additive to enhance the elasticity, consistency, and stability of food products. It may also be used as an outer film, to protect against drying, light, and oxygen. Gelatin has the property of forming a reversible gel in cold, because of the partial renaturalization of the single collagen chains into triple helical structures, which upon heating, melts from helix to coil transition (Ledward, 1986). The quality of a food grade gelatin depends to a large extent on its rheological properties (mainly gel strength and viscosity), but it is also determined by other characteristics, particularly transparency, absence of colour and flavour, and easy dissolution. Because of all these properties, gelatin has been one of the first materials used as matrix for bioactive components, and this biopolymer remains still of interest because of its abundance, relatively low price and excellent functional properties. There has been recently an increasing interest related to the filmogenic capacity and applicability as food packaging (Arvanitoyannis, 2002). The type of packaging is an important factor to enhance the conservation and protection of perishable foods, specifically in those cases where oxidative and microbiological deterioration occurs. The majority of the packaging materials used to be of synthetic origin, but nowadays, because of environmental motivations, there is an increasing effort in finding biodegradable edible materials, when possible coming from recycling industrial wastes or from renewable resources (Tharanathan, 2003). This same sensitivity can be seen in the recent scientific literature focused to edible and/or biodegradable films, with numerous references related to gelatine, either pure or mixed with other biopolymers (Arvanitoyannis & Biliaderis, 1998; Bertan, Tanada-Palmu, Siani, & Grosso, 2005; Menegalli, Sobral, Roques, & Laurent, 1999; Simon-Lukasik & Ludescher, 2004; Sobral, Menegalli, Hubinger, & Roques, 2001). Nevertheless all these studies have been done on commercial mammalian

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gelatin. There are various reasons for considering the marine gelatin as an alternative to the terrestrian mammals gelatine (bovine and pigs); among them, the possibility of bovine gelatine to transmit infectious illness like spongy-form encephalopathy, or from a social-cultural approach, some cultures reject it because of their religious beliefs. Othersides, the main problem of marine gelatins are their inferior rheological properties, which may limit their application field (Leuenberger, 1991; Norland, 1990). There has been an increased interest these last years on the knowledge focused on fish gelatin (Gómez-Guillén & Montero, 2001; Gudmundsson & Hafsteinsson, 1997; Haug, Draget, & Smidsrod, 2004; Muyonga, Cole, & Duodu, 2004). However, there is not enough information available about the application on films (Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006) and no information at all about antioxidant-fish gelatin active films. In general in foods, development of rancid flavor and undesirable chemical compounds are the results of lipid oxidation, leading to quality deterioration and reduction of shelf-life. Marine proteins, due to their high content on polyunsaturated fatty acids, are especially vulnerable in this aspect.

There is a growing interest to identify antioxidative properties in many natural sources of polyphenolic compounds for food preservation (Peschel et al., 2006). Recently, the germoplasm of murta or murtilla (*Ugni molinae* Turcz), a wild shrub growing in the South of Chile, has been collected and characterised (Seguel, Peñalosa, Gaete, Montenegro, & Torres, 2000) and the leaves extracts obtained using methanol, ethanol or water as solvents, has been reported as a source of antioxidant polyphenols (Rubilar et al., 2006) and physiological benefits (Montenegro, 2002). Therefore, in the last years there has been an increasing interest towards the commercial use of this plant as application for foods and cosmetics. Bifani et al. (2006), using the aqueous extracts of two different ecotypes of murta leaves, and applying them to edible films of carboxymethylcellulose, found distinguished permeabilities between the films, related to water vapour, CO_2 and O_2, because of different quantities of the flavonols myricetin and quercetin found in both ecotypes.

The objective of the present work was to obtain and characterize edible films with antioxidant capacity based on tuna-fish gelatin with extracts of two ecotypes grown in Pumalal, near Temuco, whose different flavonols content was studied before (Bifani et al., 2006).

2. Materials and methods

2.1. Quantification of polyphenols

Quantification of polyphenols was carried out in aqueous extracts of murta leaves. Extractable polyphenols were analysed with the Folin-Ciocalteu method and expressed as μg gallic acid/mL extract.

2.2. Antioxidant activity measurement

Leaf extracts, previously air dried until 10% wet base (w.b.), were obtained as described before (Bifani et al., 2006).

(a) DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity, based on the bleaching of this stable radical: the hydrogen-donating ability of the crude extract was determined by the method described by Hatano, Kagawa, Yasuhara, and Okuda (1988). An aliquot of the extract (100 μL of 1:100 dilution) was added to ethanol 80% (final volume 900 ul), shaked and 2.7 mL of DPPH (final concentration 2.0×10^{-4} M) added. After vigorous shaking, the mixture was left to stand for 20 min and the absorbance measured at 520 nm. A control with 100 μL water in 900 μL ethanol 80%, and a standard curve of the water soluble vitamin E analogue Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid from Aldrich), with 20–80 μL Trolox solution of 2.5 mg/10 mL and 880–820 μL ethanol 80%, respectively) were performed simultaneously. Antioxidant capacity was expressed as mg Trolox/g leaves (w.b.).

(b) *SDS–linoleic acid*. While most of the synthetic antioxidants designed for stabilisation of polymers and other organic materials are lipid soluble, the majority of natural antioxidants are water soluble; to test natural water-soluble antioxidants, microheterogeneous systems like micelles, are used. The SDS–linoleic acid method was chosen here to determine the reduction of lipid oxidation. The conjugated diene hydroperoxides are products of linoleic acid peroxidation induced into the micellar phase by a radical initiator AAPH (2,2′-azo-bis(2-amidinopropanedihydrochloride), which on thermolysis, provides radicals at constant rate. These hydroperoxides in the micelles of SDS in buffer solution at pH 7.4 have strong UV absorption with a maximum at 234 nm, as described by Counet, Callemien, and Collin (2006), Foti, Piattelli, Baratta, and Ruberto (1996), Roginsky and Lissi (2005), Sánchez-Moreno and Larrauri (1998): just before measurement, the reaction mixture of 2.6 mM linoleic acid in 0.1 M SDS solution, made in 10 mM phosphate buffer pH 7.4, 2% of AAPH, and the solution of 0.25 mg Trolox in 8 mL of ethanol + 2 mL distiled water, were prepared. To 4.0 mL of reaction mixture, 20 μL of APPH were added, dissolved and 100, 200 and 300 μL of 1:100 dilution of sample solution (or water for the control) added, kept at 50 °C for 90 min with lid, cooled rapidly to room temperature and absorbance read at 233 nm, before and after the 90 min incubation. The Trolox (0.25 mg/10 mL) was used as standard substance and the results were expressed as Trolox equivalent (mg Trolox/g leaves (w.b.).

(c) *Ferric-reducing/antioxidant power* (*FRAP*): The FRAP assay was used as a measure of the reducing ability of

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murta extracts following the method of Benzie and Strain (1996). It is based on the increase in absorbance at 595 nm due to the formation of the complex tripiridiltriazine (TPTZ)–Fe(II) in the presence of reducing agents at 37 °C. The aqueous murta extracts were diluted with distiled water (1:10 v/v), and then 30 μL were incubated with 90 μL distiled water and 900 μL FRAP reagent (containing TPTZ and $FeCl_3$). Absorbance values were read after 4 and 30 min. A calibration curve was performed with $FeSO_47H_2O$. Results were expressed as equivalent concentration of $FeSO_47H_2O$ μM at 4 and 30 min.

The FRAP assay was used also as a measure of the reducing ability of the edible films and filmogenic solutions. For this purpose, conditioned films (0.125 g) were dissolved in 5 mL distiled water at 45 °C. When the dissolution of the film was performed in acid medium (after 10 days of storage at room temperature and 58% relative humidity), distiled water was replaced by 0.5 M acetic acid. Dissolved films and filmogenic solutions were measured as described above.

2.3. Film formation

Gelatin was extracted from approximately 1-month-frozen cleaned tuna-fish (*Thunnus tynnus*) skins following basically the procedure reported in Gómez-Guillén and Montero (2001). Gelatin filmogenic solutions were prepared at a protein concentration (Nx5.4) of 2 g/100 mL distiled water, using glycerol (0.25 g/g protein) as plasticizer. Dry gelatin was hydrated at 18–20 °C overnight and dissolved later in water at 45 °C. After complete solubilisation, the remaining water and glycerol were added.

In active films, aqueous extracts from murta (*Ugni molinae* TURCZ) leaves were added in a proportion 1:1 v/v of gelatine solution + glycerol/extract). Two Pumalal ecotypes of murta leaves (Soloyo Grande, *SG*; Soloyo Chico, *SC*) were sampled near Temuco (38°35′39″ South latitude) at the Instituto de Investigación Agropecuaria INIA Carillanca, Chile). Aqueous extracts were obtained by mixing 1.5 g cut up leaves with 20 mL distiled water, heating at 35 °C for 20 min and filtering later with Whatman N° 4 filter paper. Before casting, filmogenic solutions were left at 35 °C for one hour. During this time, solutions containing murta extracts showed some flocculation, attributed to the interaction of the polyphenols of the extracts with the gelatine polypeptides chains (Haslam, 1998). Solutions were then filtered through a 1 μm pore size glass fibre filter. Cleaned filmogenic solutions were applied on plexiglass plates ($11.5 \times 11.5\,cm^2$) in a calculated amount to obtain films of around 100 μm in thickness, and were dehydrated at 42 °C in an oven with air renewal and circulation (Binder FD 240, Tuttlingen, Germany) for 18–20 h.

The resulting films were conditioned at room temperature at 58% relative humidity in dessicators with saturated solutions of NaBr for two days before analysis. Film thickness was measured with a digital micrometre (Mitutoyo MDC-25 M, Kanagawa, Japan) averaging nine different positions.

2.4. Mechanical properties

A puncture test was performed to determine puncture force and puncture deformation of films. Films were fixed in a 5.6 cm diameter cell and perforated to breaking point using a texturometer (Instron 4501, Instron Engineering Corp., Canton, MA, USA) with a round-ended stainless steel plunger ($\varnothing = 3$ mm). Cross-head speed was 60 mm/min and a 100 N load-cell was used. Breaking force was expressed in N, and breaking deformation was expressed in %, according to Sobral et al. (2001). All determinations were carried out at least in quadruplicate.

2.5. Water vapour permeability

Water vapour permeability was determined following the method described by Sobral et al. (2001). Films were fixed onto the opening of cells (permeation area = 5.31 cm^2) containing silica gel and then the cells were placed in dessicators with distiled water. The cells were weighed daily during 7 days at 22 °C. Water vapour permeability was calculated from the equation $WVP = wxt^{-1}\ A^{-1}\ \Delta P^{-1}$, where w is the weight gain (g), x is the film thickness (mm), t is the time of gain (h) and ΔP is the difference of partial vapour pressure of the atmosphere with silica gel and pure water (2642 Pa at 22 °C). Results were expressed as g mm h^{-1} cm^{-2} Pa^{-1}. All tests were made in duplicate.

2.6. Light absorption

The light barrier properties of gelatine films were measured by exposing the films to light absorption at wavelengths ranging from 690 to 200 nm, using a UV-1601 spectrophotometer (Model CPS-240, Shimadzu, Kyoto, Japan). The transparency of the films was calculated by the equation $T = Abs_{600}/x$, where Abs_{600} is the value of absorbance at 600 nm and x is the film thickness (mm). According to this equation, higher values of T would indicate lower degree of transparency.

2.7. Electrophoretic analysis (SDS-PAGE)

Films were dissolved in distiled water at 60 °C and then mixed with a 2-fold concentrated loading buffer (2% SDS, 5% mercaptoethanol and 0.002% bromophenol blue) until reaching a final concentration of 2 mg/mL of protein. Filmogenic solutions were mixed directly with loading buffer at the same protein concentration. Samples were heat-denatured 5 min at 90 °C and analysed by PAGE-SDS according to Laemmli (1970) using 3% stacking gels and 5% resolving gels in a Mini Protean II unit (Bio-Rad Laboratories, Hercules, CA) at 25 mA/gel. The loading

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volume was 15 μL in all lines. Protein bands were stained with Coomassie brilliant Blue R250. Type I collagen from foetal calf skin was used as markers of α-, β- and γ- chain component mobilities.

2.8. Dynamic viscoelastic properties

Dynamic viscoelastic studies of clear filmogenic solutions were performed on a Bohlin CSR-10 rheometer rotary viscometer (Bohlin Instruments Ltd., Gloucestershire, UK) using a cone-plate geometry (cone angle 4°, gap = 0.15 mm). Cooling and heating from 40 to 6 °C and back to 40 °C were performed at a scan-rate of 1 °C/min, frequency 1 Hz, and oscillating applied stress of 3.0 Pa. The elastic modulus (G′; Pa) and viscous modulus (G″; Pa) were represented as a function of temperature. Several determinations were performed for each sample, being the experimental error always below 6%.

2.9. Film microstructure

Cryoscanning electron microscopy (Cryo-SEM) was used to examine representative film surfaces and also the corresponding filmogenic solutions. Samples were mounted with OCT compound (Gurr) and mechanically fixed onto the specimen holder using the Oxford CT1500 Cryosample Preparation Unit (Oxford Instruments, Oxford, England). Samples were frozen in subcooled liquid nitrogen for 2 min and then transferred to the preparation unit. After ice sublimation, the surfaces were gold sputter coated, and subsequently transferred into the cold stage of the SEM chamber. Specimens were observed with a DSM960 Zeiss SEM microscope (Zeiss, Oberkochen, Germany) at −135 °C under a 15 kV acceleration potential.

2.10. Statistical analysis

Statistical tests were performed using the SPSS computer programme (SPSS Statistical Software, Inc., Chicago, Ill.) One-way analysis of variance was carried out. The difference of means between pairs was resolved by means of confidence intervals using a Tukey test. Level of significance was set for $p \leqslant 0.05$.

3. Results and discussion

3.1. Antioxidant properties of murta leaves extracts

The water extracts of murta leaves are rich in derivatives of gallic acid, myricetin and quercetin (Rubilar et al., 2006), which are very polar molecules, with several –OH groups. The identification of some of the phenolic substances showed that SC extract has a higher concentration of some flavonols than SG extract, like the myricetin derivatives: myricetin dirhamnoside, myricetin glucoside or galactoside (Bifani et al., 2006). In correlation to these, one can expect that the antioxidant capacity of the SC extract should be higher than of the SG extract. As can be seen in Table 1, if the radical scavenger activity of the extracts were measured using DPPH, the SC extract showed the highest activity: for 1 μL extract (corresponding to 100 μL of 1:100 dilution), 22.14 mg/g Trolox Equivalent for SC extract versus 10.35 mg/g Trolox Equivalent for SG extract, being the ratio of SC/SG of 2.1.

The antioxidant activity of SG and SC extracts, examined as their protective action toward linoleic acid peroxidation in micelles of sodium dodecyl sulphate in buffer solution, pH 7.4, can be appreciated also in Table 1. The best result was observed with 1 μL extract (corresponding to 100 μL of the 1:100 dilution) of the SC extract (Table 1): 4.76 mg/g Trolox Equivalent for SC and 2.68 mg/g Trolox Equivalent for SG, showing the SC extract 1.8 fold more antioxidant capacity than the SG extract. There can also be appreciated that 300 μL of the extracts were too much for the micelles in the conditions of the experiment and therefore showed lower antioxidant values for both extracts (1.73 mg/g Trolox Equivalent for SG and 2.82 mg/g Trolox Equivalent for SC). Foti et al. (1996) did also not found significant interferences from low concentrations of tested compounds; besides, they found that the method is extremely sensitive and allows the study of the oxidation process at low conversions (less than 10%), at which steady-state kinetic analysis better applies.

There can be seen in Table 2, that the aqueous extracts of both ecotypes show also a high antioxidant capacity when measured through the FRAP method (4 min: 902.8 ± 15.2 μmol $FeSO_4$ in SC and 670.2 ± 9.0 μmol $FeSO_4$ in SG; 30 min: 1475.1 ± 11.1 μmol $FeSO_4$ in SC and 1148.2 ± 35.7 μmol $FeSO_4$ in SG). Therefore, also in this method the SC ecotype showed more antioxidant capacity than the SG ecotype (1.3 fold) and the results present the same relationship than the total polyphenol concentration, expressed as gallic acid content: 283.3 μg mL^{-1} in SC and 224.2 μg mL^{-1} in SG. When comparing with the phenol antioxidant coefficient (PAC) shown by Katalinic, Milos, Kulisic, and Jukic (2006) for 70 infusates of medicinal plants, calculated as the ratio of FRAP (μM/L)/total phenolics (μM of catechin or gallic acid equivalent/L), SC

Table 1
Antioxidant capacity of Soloyo Grande (SG) and Soloyo Chico (SC) Pumalal ecotypes of aqueous murta leaves extracts, diluted 1:100, measured as Trolox Equivalent (mg/g), with DPPH (mean of three determinations) and SDS–linoleic acid (SDS–L) (mean of two determinations) methods

Antioxidant capacity as	Sample volume (1:100) (μL)	Trolox equivalent (mg/g)	
		Soloyo Grande	Soloyo Chico
DPPH	100	10.35 ± 0.40	22.14 ± 1.14
SDS-L	100	2.68	4.76
SDS-L	200	2.15	3.47
SDS-L	300	1.73	2.82

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extract presents a PAC of 3.2 and SG extract of 2.3. Although Katalinic et al. (2006) reported total phenolics as catechin equivalent, and in the present work as gallic acid equivalent, these authors, of the 70 infusates studied, found that *Melissae folium* infusions showed the highest antioxidant capacity and the highest phenol content, with a PAC of 3.3 and discussed the possibility that the molar response of the total phenol content method could be roughly proportional to the number of phenolic hydroxyl groups in a given substrate. The reducing capacity is enhanced when two phenolic hydroxyl groups are oriented ortho or para, which is consistent with the myricetin structure, and the high concentration of this compound found in SC extract (Bifani et al., 2006).

3.2. Addition of murta extracts into filmogenic solutions

It is well known that polyphenols present the ability to form complexes with proteins causing protein precipitation (Haslam, 1998). But there has been shown that tannin–gelatin complexes retain a certain capacity as radical scavengers (Riedl & Hagerman, 2001). The partial flocculation of the protein in the film forming solution is attributed to the polyphenols content of the murta extracts, mainly when SC ecotype was used, because of the higher concentration of the flavonols myricetin and quercetin found, in relation to the SG extract (Bifani et al., 2006). The visible aggregates were eliminated through filtration, but the smaller polyphenol–gelatin complexes most probably remain in solution. After the elimination of the aggregates, the protein concentration remaining in the film forming solutions with murta extract was of 19.42 ± 0.60 mg/mL for the SG ecotype and 17.80 ± 0.23 mg/mL for the SC ecotype. The difference between these protein concentrations obtained with the addition of both water extracts in reference to 20 mg/mL protein of the Control film forming solution was attributed to the removal of polyphenol–protein complexes.

After the drying process, very transparent films were obtained, with a mean thickness of 100 μm. The incorporation of *murta* extracts decreased the transparency of the resulting films, especially in the case of the SC ecotype (Table 3). Also Jongjareonrak et al. (2006) found differences in the transparency of gelatin films, slightly higher for tuna-fish skin than those obtained from bigeye snapper and brownstripe red snapper skin. No significant differences ($p > 0.05$) were found on the thickness of the films made with both ecotypes, neither with the film without extract (Table 3).

3.3. Light absorption

Because of a slightly darker hue of the murta films than the Control film, there could be a modification of their properties, like light barrier. To confirm this, a spectroscopic scanning of the films at wavelenghts between 690 y 200 nm was performed (Fig. 1). At the beginning of the visible spectrum, at wavelengths of about 400 nm, both types of films with antioxidant extracts presented an absorbance level of approximately 6 fold higher than the Control gelatin film. The differences in the ultraviolet spectrum are even higher. This could also mean, besides the results seen in Table 3, that the tuna-fish skin gelatin films, especially those enriched with murta extracts, could be an excellent barrier to prevent UV light-induced lipid oxidation, when applied in food systems. At the wavelength range between 298 and 380 nm the water extract of SC presented absorbance values higher than the SG extract, which was attributed to differences in polyphenolic composition. However, no differences in the spectrums and level of light absorption were found in the films made with both ecotypes. Films from bigeye snapper and brownstripe red snapper skin gelatin have been also reported to exhibit a high absorption to light in the UV range (200–280 nm) (Jongjareonrak et al., 2006). The films obtained with other protein systems offered also a high UV

Table 2
Antioxidant capacity of aqueous extracts of Soloyo Grande (SG) and Soloyo Chico (SC), filmogenic solutions (S) and films (F) with Soloyo Grande (SG) and Soloyo Chico (SC) Pumalal ecotypes, through the FRAP method. (S-C Filmogenic solution control without extract, F-C Film control without extract)

Samples	μmol $FeSO_4$ 7 H_2O	
	4 min	30 min
SG	670.16 ± 8.97	1148.21 ± 35.74
SC	902.79 ± 15.24	1475.09 ± 11.15
S-C	81.31 ± 14.67	200.14 ± 38.03
S-SG	496.20 ± 30.95	908.22 ± 38.81
S-SC	568.27 ± 11.64	908.27 ± 29.52
F-C	299.81 ± 5.96	424.61 ± 8.35
F-SG	394.65 ± 43.13	667.38 ± 70.42
F-SC	542.43 ± 45.70	943.50 ± 44.71

Table 3
Physical properties of edible films with added murta extracts (F-SG : with Soloyo Grande; F-SC: with Soloyo Chico; F-C: Control without extract)

Samples	Transparency	Thickness (mm)	Puncture force (N)	Puncture deformation (%)	WVP (10^{-8} g mm h^{-1} cm^{-2} Pa^{-1})
F-C	0.479	0.098 ± 0.02	5.91 ± 1.49	13.77 ± 0.30	2.16 ± 0.19
F-SG	0.500	0.101 ± 0.01	4.78 ± 1.06	11.39 ± 2.65	2.87 ± 0.40
F-SC	0.804	0.097 ± 0.01	2.75 ± 1.08	3.56 ± 0.03	1.83 ± 0.11

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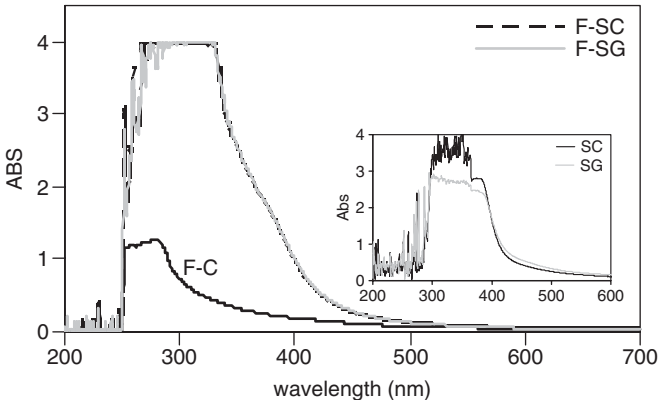

Fig. 1. Changes in absorbance at wavelengths ranging from 200 to 700 nm of films with added murta extracts (F-SG : with Soloyo Grande; F-SC: with Soloyo Chico; F-C: Control without extract). Inner graphic shows absorbance changes of murta leaves water extracts (SG: Soloyo Grande ecotype and SC: Soloyo Chico ecotype).

protection, as were the case of Alaska pollack surimi proteins (Shiku, Hamaguchi, Benjakul, Visessanguan, & Tanaka, 2004) or whey protein (Fang, Tung, Britt, Yada, & Dalgleish, 2002), being considerably higher than in many synthetic polymer films (Shiku et al., 2004).

3.4. Mechanical properties

Puncture force and puncture deformation values are shown in Table 3. In this work, the puncture force in the tuna-fish skin gelatin films is lower than the values reported by Sobral et al. (2001) and Thomazine, Carvalho, and Sobral (2005) in mammalian (pigskin and bovine hide) gelatin films (≈14–16 N), using a comparable amount of plasticizer (25 g of sorbitol or glycerol/100 g gelatine), and also taken into account that the thickness of the mammalian gelatin films was considerably lower (≈around 42 μm). These last authors report a puncture deformation in pigskin gelatin films noticeably lower than in tuna-fish gelatin films (≈3.5%). Films obtained with other proteins and similar concentrations of glycerol as plasticizer have been shown to achieve similar or lower force values than with tuna-fish gelatin, as with films based on gluten (Gontard, Guilbert, & Cuq, 1993), fish myofibrilar protein (Cuq, Aymard, Cuq, & Guilbert, 1995), or muscle proteins from Nile Tilapia (Paschoalick, Garcia, Sobral, & Habitante, 2003). Besides in these films the puncture deformation has also been shown around three times lower than in tuna-fish gelatin films. The addition of the murta extracts at the films produces a lower puncture force and puncture deformation, being significantly different ($p \leqslant 0.05$) only for SC ecotype, and no significantly different ($p > 0.05$) between the Control film and the SG film. A possible explanation lies in the alteration of the plasticizer/gelatin ratio in the *murta* added films, especially in the SC film, as a consequence of the slightly lower protein content. On the other hand, the presence of vegetable tannins in films produced from sunflower protein isolate plasticized with glycerol, has been reported to increase tensile strength in films and decrease elongation at break, due to the capacity to act as a complexing agent of proteins (Orliac, Rouilly, Silvestre, & Rigal, 2002). However, these authors observed that, beyond a certain percentage of tannin addition, tensile strength decreased considerably due to weakening interactions stabilizing the protein network. Therefore, the lower mechanical properties of the film containing the SC extracts, compared to the SG films, could be attributed to both quantitative and qualitative differences in polyphenols content, which is in accordance with all the other results.

3.5. Water vapour permeability

Water vapour permeability of tuna-fish skin gelatin films ranged from 2.87×10^{-8} g mm h^{-1} cm^{-2} Pa^{-1} in the film containing SG to 1.83×10^{-8} g mm h^{-1} cm^{-2} Pa^{-1} in the film with SC (Table 3). Therefore the SC film, even showing lower mechanical properties, presents a reduced permeability to water vapour in relation to the Control film (without extract), and the SG film. Sobral et al. (2001) reported in pigskin gelatin the linearly increase in WVP from 1.8 to 3.2×10^{-8} g mm h^{-1} cm^{-2} Pa^{-1} , between 15 and 65 g sorbitol/100 g gelatin. When using 25 g glycerol/100 g pigskin gelatine, WVP was found to be considerably higher (around 7×10^{-8} g mm h^{-1} cm^{-2} Pa^{-1}) (Thomazine et al., 2005). Glycerol is well recognised to present a higher plasticizing effect than sorbitol, causing an increase in film flexibility but reduced resistance and water vapour permeability (Cuq et al., 1995; Gennadios, Weller, Hanna, & Froning 1996). Thomazine et al. (2005) explained this behaviour in terms of molecular weight and number of molecules of plasticizers in the films. Similar findings have been recently reported working with different concentrations and type of plasticizers in films from skin gelatin of bigeye snapper and brownstripe red snapper (Jongjareonrak et al., 2006).

The results of WVP obtained in the present study with tuna-fish skin gelatin films are noticeably lower than the reported above in pigskin gelatin. A possible explanation lies in differences in aminoacid composition between both gelatins, since fish gelatins are known to be less rich in proline and hydroxyproline (Norland, 1990), thus increasing their hydrophobicity as compared to mammals gelatins. WVP in tuna-fish gelatin films was also lower than in films based on myofibrillar proteins of Atlantic Sardine (Cuq et al., 1995) or muscle proteins from Nile Tilapia (Paschoalick et al., 2003). When compared to other biopolymers, fish gelatin films have been found also to be less permeable to water vapour than films based on cellulose (Psomiadou, Arvanitoyannis, & Yamamoto, 1996) or starch (Arvanitoyannis, Psomiadou, Nakayama, Aiba, & Yamamoto, 1997).

3.6. Viscoelastic properties

On the cleaned film forming solutions with added extracts, the changes observed on the dynamic viscoelasticity upon

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cooling and subsequent heating may serve to show possible interactions between the components of those extracts and the gelatin molecules. Fig. 2 shows the viscoelastic properties of the different filmogenic solutions. Slight differences can be appreciated both on the elastic modulus (G') and viscous modulus (G''), when the temperature decreases from 40 to 6 °C and during the subsequent heating from 6 to 40 °C (Fig. 2). The minimal critical gelling concentration of the filmogenic solution was exceeded, since 2 g gelatin/100 mL was sufficient to induce renaturation of the polypeptide chains into triple helix during cooling down, being the gelation temperature around 13 °C in the three cases assayed. Either the elastic modulus (G') or the gelling/melting temperature of the gelatin is highly dependant on protein concentration. The fact that gel could be obtained with such a low protein concentration, indicates a high gelling capacity of the tuna-fish skin gelatin, as reported previously (Gilsenan & Ross-Murphy, 2000). The addition of both extracts did not modify perceptibly the thermal transition temperatures of the gelatin, because the melting temperature T_m (around 22 °C) was the same for the three samples analysed. At temperatures below T_m a major contribution of the viscous component (G'') in the samples with murta extracts than in the Control were found, showing the interference of both extracts with the polypeptide chains, to form the protein net. Nevertheless, the solution containing the SC extract reached a smaller G' value at 6 °C than with the SG extract, the latter being similar to the gelatin solution without murta extract. After few minutes of maturation at 6 °C, these differences in G' were more pronounced, showing a bigger interference into triple helix of the SC extract in the cold renaturation process of gelatin.

3.7. Electrophoretic analysis

The electrophoretic analysis of the filmogenic solutions showed a molecular weight distribution very similar to the pure tuna-fish gelatin, with predominance of α-chains (≈100 kDa) and their dimmers (β-components) (Fig. 3). The γ-components (≈300 kDa) appeared only at trace levels. In the filmogenic solutions with murta extract, independently of the ecotype used, a protein fraction of lower molecular weight than the α-chains appeared, as well as a trace fraction at the end of the polyacrilamide gel. The presence of these lower molecular weight proteins could be, in part, responsible for the interference of the murta extracts in the formation of the gelatin triple helical structure upon cooling, as observed previously by means of dinamic viscoelastic studies.

When looking at the molecular composition of the dried films, the incorporation of murta extract modifies perceptibly the electrophoretic profile compared to the control film, because γ-trimmers traces disappeared and the presence of β components and α_2 chains are notably reduced. These probably could be due to the contact of the murta polyphenols with certain protein fractions that further interact during the drying of the film, suffering a noticeable aggregation, and thus not present in the electrophoretic gel. In the profile of the film made with the SC ecotype, it can be appreciated a certain amount of degraded low molecular weight protein at the end of the

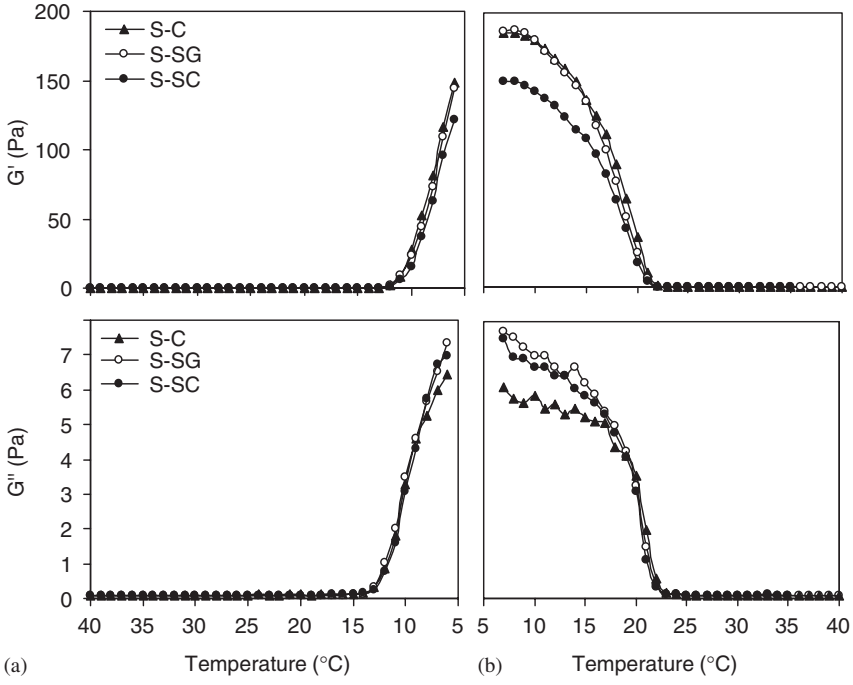

Fig. 2. Viscoelastic properties of film forming solutions (S-SG: with Soloyo Grande; S-SC: with Soloyo Chico; S-C: Control without extract). Changes in the modulus of elasticity G' and modulus of viscosity G'' were monitored during cooling from 40 to 6 °C (a) and subsequent heating from 7 to 40 °C (b).

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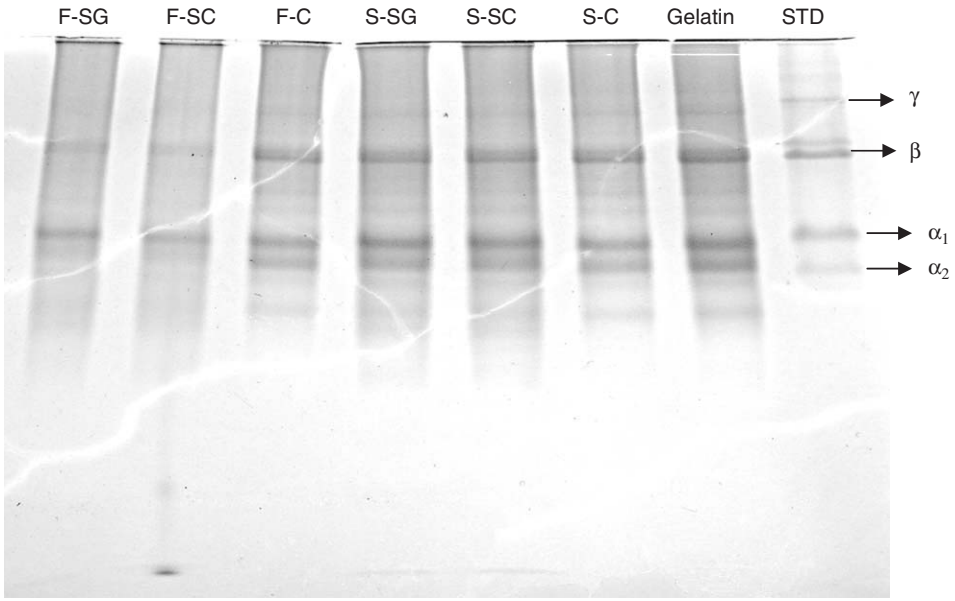

Fig. 3. SDS-PAGE of film forming solutions with added murta extracts (S-SG: with Soloyo Grande; S-SC: with Soloyo Chico; S-C: Control without extract) and resulting edible films (F-SG : with Soloyo Grande; F-SC: with Soloyo Chico; F-C: Control without extract).

electrophoretic gel. This can support the reduced rheological properties of the film formed with this ecotype.

3.8. Scanning electron microscopy

About the electron scanning microscopy at low temperatures (cryo-SEM), in Fig. 4 are shown the microstructures of both the filmogenic solutions and the surfaces of the dried films. On the Control (without extract) filmogenic solution a fibril structure was observed, compartmentalised like beehives into cells, with a fine network inside. On the SG ecotype solution, the arrangement of the cells is similar, although thinner, showing an apparent increase in free volume in relation to the solution without murta extract. With the SC ecotype, the filmogenic solution shows a more aggregated, irregular and diffuse structure.

The microscopic image of the surface of the film containing the SG ecotype is similar to the Control film, with a uniform granular structure, typical of its protein nature. A similar granular and uniform microstructure has been reported in other protein-based films (Fang et al., 2002; Tang, Jiang, & Wen, 2005). The SC film presents a very even and compacted structure, which denotes noticeable greater cohesiveness than both others. The microstructure images found, either in the filmogenic solutions containing SC or in the corresponding dried films, are indications of a greater protein aggregation suffered as a consequence of the qualitative and quantitative differences in the polyphenol content of both extracts, as seen before (Bifani et al., 2006). The more compacted and diffuse surface of the SC film might be responsible for the lower permeability to water vapour of the film, although with inferior mechanical properties.

3.9. Antioxidant capacity

The antioxidant capacity through the FRAP method were measured both for the filmogenic solutions and the dry films (Table 2). The solution without murta extract shows some antioxidant capacity, attributed to the gelatine, because there has been reported that that fish gelatin hydrolysates exhibit high antioxidant and radical-scanvenging capacity, due to their aminoacids content like glycine, proline (Mendis, Rajapakse, & Kim, 2005). After the drying process, the film shows higher values of FRAP than the filmogenic solution. In the solutions containing murta extracts, the antioxidant capacity increased in more than 5 fold in relation to the Control sample, and the SC values even slightly more. In the murta extract films, the antioxidant capacity is also higher than in the Control films and again, the values with SC ecotype were higher. These findings are in direct relation with the higher concentration of polyphenols and the higher antioxidant capacity of the SC extract, as compared to SG extract. After 10 days of storage, the films loose their easy-solubility capacity in water at 45 °C (for the FRAP assay). Therefore, the antioxidant capacity measurements decreased drastically for all the film samples (Table 4). But, with a better solubilisation in acidic media, the FRAP values were similar to that obtained the first day, most probable do to the polyphenol content and antioxidant capacity of the murta extracts added. When looking to the FRAP values of the Control film (gelatin without extract), FRAP values remained low, due to the formation of non-acid-reducible bonds among gelatin polipeptide chains. These is important to take into account when thinking on food applications of the studied films, because it seems to be of critical importance that the application should be done rapidly if

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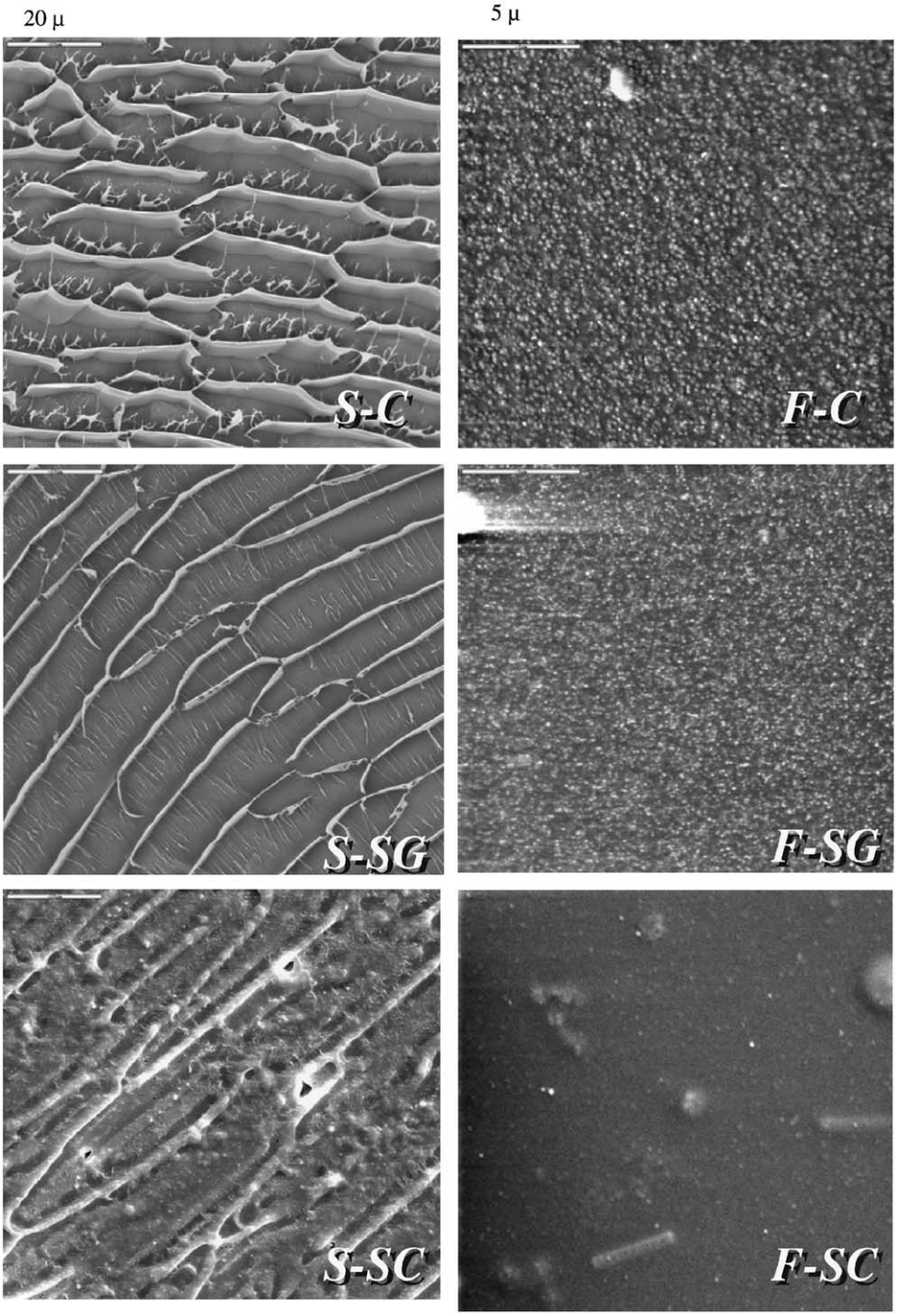

Fig. 4. Scanning electron microscopy at low temperature (Cryo-scanning) of film forming solutions with added murta extracts (S-SG: with Soloyo Grande; S-SC: with Soloyo Chico; S-C: Control without extract) and resulting edible films (F-SG : with Soloyo Grande; F-SC: with Soloyo Chico; F-C: Control without extract).

an adequate diffusion of the polyphenol content into the food surface should be allowed. Further studies would be needed to avoid protein aggregation during film storage.

4. Conclusions

The edible films of tuna-fish gelatin are transparent and show acceptable mechanical properties and barrier properties to water vapour and UV light. It is possible to increase significantly the antioxidant properties of the film, when natural extracts with high polyphenols content are added, producing only minor modifications of the film properties. This would be the case of films with Soloyo Grande ecotypes. When using an extract with a bigger content of polyphenols, like the Soloyo Chico ecoptype, the antioxidant capacity of the film increases, but the mechanical

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Table 4
Antioxidant capacity, measured through the FRAP method, of edible films with added murta extracts (F-SG : with Soloyo Grande; F-SC: with Soloyo Chico; F-C: Control without extract) after 10 days of storage at room temperature and 58% relative humidity

Samples	μmol $FeSO_4$ 7 H_2O			
	Solubilized in water		Solubilized in acetic acid	
	4 min	30 min	4 min	30 min
F-C_{10d}	13.73±8.56	47.22±2.61	14.34±5.52	81.47±18.60
F-SG_{10d}	174.32±2.61	305.92±17.75	433.56±38.78	693.08±56.21
F-SC_{10d}	174.88±5.19	273.63±23.47	595.22±8.29	958.87±41.12

properties decreases, due to a greater interaction between polyphenols and proteins.

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References

Arvanitoyannis, I. S. (2002). Formation and properties of collagen and gelatin films and coatings. In A. Gennadios (Ed.), *Protein-based films and coatings* (pp. 275–304). Boca Ratón, FL: CRC Press.

Arvanitoyannis, I., & Biliaderis, C. G. (1998). Physical properties of polyol-plasticized edible films made from sodium caseinate and soluble starch blends. *Food Chemistry*, *6*(3), 333–342.

Arvanitoyannis, I., Psomiadou, E., Nakayama, A., Aiba, S., & Yamamoto, N. (1997). Edible films made from gelatin, soluble starch and polyols .3. *Food Chemistry*, *60*(4), 593–604.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76.

Bertan, L. C., Tanada-Palmu, P. S., Siani, A. C., & Grosso, C. R. F. (2005). Effect of fatty acids and 'Brazilian elemi' on composite films based on gelatine. *Food Hydrocolloids*, *19*(1), 73–82.

Bifani, V., Ramírez, C., Ihl, M., Rubilar, M., García, A., & Zaritzky, N., (2006). Effects of murta (*Ugni molinae* Turcz) extract on gas and water vapor permeability of carboxymethylcellulose based edible films. *LWT-Food Science and Technology*, in press.

Counet, C., Callemien, D., & Collin, S. (2006). Chocolate and cocoa: new sources of *trans*-resveratrol and *trans*-piceid. *Food Chemistry*, *98*, 649–657.

Cuq, B., Aymard, C., Cuq, J. L., & Guilbert, S. (1995). Edible packaging films based on fish myofibrillar proteins: formation and functional properties. *Journal of Food Science*, *60*, 1369–1374.

Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey proteins. *Journal of Food Science*, *67*(1), 188–193.

Foti, M., Piattelli, M., Baratta, M. T., & Ruberto, G. (1996). Flavonoids, coumarins and cinnamic acids as antioxidants ina micellar system. Structure-activity relationship. *Journal of Agricultural and Food Chemistry*, *44*, 497–501.

Gennadios, A., Weller, C. L., Hanna, M. A., & Froning, G. W. (1996). Mechanical and barrier properties of egg albumen films. *Journal of Food Science*, *61*(3), 585–589.

Gilsenan, P. M., & Ross-Murphy, S. B. (2000). Rheological characterisation of gelatins from mammalian and marine sources. *Food Hydrocolloids*, *14*(3), 191–195.

Gómez-Guillén, M. C., & Montero, P. (2001). *Method for the production of gelatin of marine origin and product thus obtained*. International Patent PCT/S01/00275.

Gontard, N., Guilbert, S., & Cuq, J. L. (1993). Water and glycerol as plasticizers effect mechanical and water vapour barrier properties of an edible wheat gluten film. *Journal of Food Science*, *58*(1), 206–211.

Gudmundsson, M., & Hafsteinsson, H. (1997). Gelatin from cod skins as affected by chemical treatments. *Journal of Food Science*, *62* 37–39, 47.

Haslam, E. (1998). *Practical polyphenolics. From structure to molecular recognition and physiological action*. Cambridge: Cambridge University Press.

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents I licorice root: their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin*, *36*(6), 2090–2097.

Haug, I. J., Draget, K. I., & Smidsrod, A. (2004). Physical and rheological properties of fish gelatine compared to mammalian gelatin. *Food Hydrocolloids*, *18*(2), 203–213.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2006). Effects of plasticizers on the properties of edible films from skin gelatin of bigeye snapper and brownstripe red snapper. *European Food Research and Technology*, *222*(3-4), 229–235.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*, *94*, 550–557.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Ledward, D. A. (1986). Gelation of gelatin. In J. R. Mitchell, & D. A. Ledward (Eds.), *Functional Properties of Food Macromolecules* (pp. 171–201). London: Elsevier Applied Science Publishers.

Leuenberger, B. H. (1991). Investigation of viscosity and gelation properties of different mammalian and fish gelatins. *Food Hydrocolloids*, *5*(4), 353–361.

Mendis, E., Rajapakse, N., & Kim, S. K. (2005). Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. *Journal of Agricultural and Food Chemistry*, *53*(3), 581–587.

Menegalli, F. C., Sobral, P. J. A., Roques, M. A., & Laurent, S. (1999). Characteristics of gelatin biofilms in relation to drying process conditions near melting. *Drying Technology*, *17*(7-8), 1697–1706.

Montenegro, G. (2002). Ugni molinae Turcz. In G. Montenegro, & B. N. Timmermann (Eds.), *Chile nuestra flora útil. Guía de uso apícola, medicinal folclórica, artesanal y ornamental* (2nd ed, pp. 241–242). Santiago: Ediciones Universidad Católica de Chile.

Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatine. *Food Hydrocolloids*, *18*(4), 581–592.

Norland, R. E. (1990). Fish gelatin. In M. N. Voight, & J. K. Botta (Eds.), *Advances in fisheries technology and biotechnology for increased profitability* (pp. 325–333). Lancaster: Technomic Publishing Co.

Orliac, O., Rouilly, A., Silvestre, F., & Rigal, L. (2002). Effects of additives on the mechanical properties, hydrophorbicity and water uptake of thermo-moulded films produced from sunflower protein isolate. *Polymer*, *43*(20), 5417–5425.

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Paschoalick, T. M., Garcia, F. T., Sobral, P. J. A., & Habitante, A. M. (2003). Characterization of some functional properties of edible films based on muscle proteins of Nile Tilapia. *Food Hydrocolloids*, *17*(4), 419–427.

Peschel, W., Sanchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzia, I., Jiménez, D., et al. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chemistry*, *97*(1), 137–150.

Psomiadou, E., Arvanitoyannis, I., & Yamamoto, N. (1996). Edible films made from natural resources; Microcrystalline cellulose (MCC), methylcellulose (MC) and corn starch and polyols .2. *Carbohydrate Polymers*, *31*(4), 193–204.

Riedl, K. M., & Hagerman, A. E. (2001). Tannin-protein complexes as radical scavengers and radical sinks. *Journal of Agricultural and Food Chemistry*, *49*(10), 4917–4923.

Roginsky, V., & Lissi, E. A. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry*, *92*, 235–254.

Rubilar, M., Pinelo, M., Ihl, M., Scheuermann, E., Sineiro, J., & Núñez, M. J. (2006). Murta leaves (*Ugni molinae* Turcz) as a source of antioxidant polyphenols. *Journal of Agricultural and Food Chemistry*, *54*(1), 59–64.

Sánchez-Moreno, C., & Larrauri, J. A. (1998). Main methods used in lipid oxidation determination. *Food Science and Technology International*, *4*(6), 391–399.

Seguel, I., Peñalosa, E., Gaete, N., Montenegro, A., & Torres, A. (2000). Colecta y caracterización molecular de germoplasma de murta (*Ugni molinae* Turcz.) en Chile. *Revista Agro Sur*, *28*(2), 32–41.

Shiku, Y., Hamaguchi, P. Y., Benjakul, S., Visessanguan, W., & Tanaka, M. (2004). Effect of surimi quality on properties of edible films based on Alaska Pollack. *Food Chemistry*, *86*, 493–499.

Simon-Lukasik, K. V., & Ludescher, R. D. (2004). Erythrosin b phosphorescence as a probe of oxygen diffusion in amorphous gelatin films. *Food Hydrocolloids*, *18*(14), 621–630.

Sobral, P. J. A., Menegalli, F. C., Hubinger, M. D., & Roques, M. A. (2001). Mechanical water vapor barrier and thermal properties of gelatin based edible films. *Food Hydrocolloids*, *15*(4-6), 423–432.

Tang, C. H., Jiang, Y., & Wen, Q. B. (2005). Effect of transglutaminase treatment on the properties of cast films of soy protein isolates. *Journal of Biotechnology*, *120*(3), 296–307.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science and Technology*, *14*(3), 71–78.

Thomazine, M., Carvalho, R. A., & Sobral, P. I. A. (2005). Physical properties of gelatin films plasticized by blends of glycerol and sorbitol. *Journal of Food Science*, *70*(3), 172–176.

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