Food Chemistry 112 (2009) 18–25

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts

J. Gómez-Estaca *, L. Bravo, M.C. Gómez-Guillén, A. Alemán, P. Montero

Instituto del Frío (CSIC), José Antonio Novais, 10, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 10 December 2007
Received in revised form 27 March 2008
Accepted 8 May 2008

Keywords:
Food packaging
Antioxidant properties
Gelatin
Oregano extract
Rosemary extract

ABSTRACT

We have investigated the antioxidant properties (FRAP ferric-reducing ability and ABTS radical-scavenging capacity) as well as the light barrier properties of gelatin-based edible films containing oregano or rosemary aqueous extracts. For comparative purposes both bovine-hide and tuna-skin gelatins were studied. The oregano and rosemary extracts were first characterised by both their total phenolic content and antioxidant activity, with the oregano extract showing the higher values. HPLC and confocal laser scanning microscopy revealed qualitative differences between extracts although rosmarinic acid was the most abundant phenolic compound in both. After determining the total quantities of phenolics on films, the polyphenol–protein interaction was found to be more extensive when tuna-skin gelatin was employed. However, this did not clearly affect the antioxidant properties of the films, although it could affect the phenol diffusion from film to food. The light barrier properties were improved by the addition of oregano or rosemary extracts, irrespective of the type of gelatin employed.

1. Introduction

Plastic packaging has come into widespread use, thanks to its good mechanical properties and effectiveness as a barrier to oxygen and water. However, because plastic is not biodegradable, build-up in the environment poses a serious ecological problem. As a consequence, in recent years, research attention has turned to developing biodegradable and/or edible packaging made from natural polymers, often from waste products of agriculture, livestock raising, or fishing. Such polymers may be protein, lipid, or polysaccharide-based (Tharanathan, 2003), and the physical properties of the resulting films can be highly variable, depending on the type of polymer employed.

Gelatin is a protein-based polymer, widely used in the manufacture of edible films. In consequence, the properties of films made from mammalian (chiefly porcine and bovine) gelatins have been widely studied (Arvanitoyannis, 2002; Bertan, Tanada-Palmu, Siani, & Grosso, 2005; Menegalli, Sobral, Roques, & Laurent, 1999; Simon-Lukasik & Ludescher, 2004; Sobral, Menegalli, Hubinger, & Roques, 2001). Recent research has focused increasingly on films made from fish gelatins, largely because of religious objections to eating pork-based products and health concerns about the spread of diseases such as bovine spongiform encephalopathy to humans. Our understanding of fish gelatins and how to extract them has grown in recent years (Gudmundsson & Hafsteinsson, 1997; Gómez-Guillén & Montero, 2001a; Muyonga, Cole, & Duodu, 2004) but there is still little published information on their use in making biodegradable and edible films (Gómez-Guillén, Ihl, Bifani, Silva, & Montero, 2007; Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006a, 2006b; Muyonga et al., 2004).

Film coatings improve storage, mainly as a result of their ability to act as barriers to water, preventing dehydration, and to oxygen and light, reducing lipid oxidation (Gennadios, Hanna, & Kurth, 1997). Furthermore, active substances like plant extracts can be added, conferring antimicrobial and/or antioxidant properties (Kim et al., 2006; Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004; Zivanovic, Chi, & Draughon, 2005). Delayed time-release of antioxidants of this type from films maintains high concentrations on the surface of the food, thereby improving the efficacy of the films (Gómez-Estaca, Montero, Giménez, & Gómez-Guillén, 2007). According to Papadokostaki, Amanratos, and Petropoulos (1997), polymer structure is related to the release of active components. Still, little information is available on the addition of antioxidant extracts to gelatin-based films (Gómez-Guillén et al., 2007). There may be substantial differences according to the type of gelatin employed, since gelatin attributes differ considerably, depending on the source, that is, between fish (cold-water and warm-water species) and mammalian gelatins (Gómez-Guillén et al., 2002). Interactions with the protein matrix may not only affect the release of active components to the food but may also alter the physicochemical properties of the films, thereby altering the films' solubility and their barrier properties (Gómez-Guillén et al., 2007).

* Corresponding author. Tel.: +34 91 5445607; fax: +34 91 5493627.
E-mail address: jgomez@if.csic.es (J. Gómez-Estaca).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.05.034

The object of this experiment was to determine the antioxidant properties of films made from two kinds of gelatins: tuna-skin and bovine-hide gelatin, with added oregano and rosemary extracts.

2. Materials and methods

2.1. Preparation of the antioxidant extracts

Freeze–dried oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) leaves were purchased at local markets. An amount of 5 and 20 g, respectively, was added to 100 ml of distilled water that had been pre-warmed to 45 °C and extracted by continuous stirring in a warm-water bath at 45 °C for 10 min. The aqueous extract thus obtained was filtered through Whatman No. 1 filter paper.

2.2. Determinations performed on the antioxidant extracts

Polyphenols were quantified spectrophotometrically at 750 nm using Folin–Ciocalteau reagent (Montreau, 1972) and caffeic acid as standard. The results are the mean values of three determinations.

Identification of the polyphenols in the plant extracts was carried out by HPLC-DAD analysis using an Agilent 1100 series liquid chromatograph equipped with a diode array detector (Model G1315B; Agilent Technologies, Waldbron, Germany). The liquid chromatographic equipment included a quaternary pump (Model G1311A) and an on-line vacuum degasser (Model G1322 A). Data acquisition and analysis were carried out using the Agilent ChemStation. HPLC separation was performed on a Nucleosil 120 C18 reversed-phase column (250 × 4.6 mm i.d., 5 m particle size; Tecknochroma, Barcelona, Spain) with an ODS RP18 guard column. A binary gradient of 4.5% formic acid in deionised water (solvent A) and acetonitrile (solvent B) was as follows: from 10% to 20% solvent B over 20 min, from 20% to 25% solvent B over 10 min, from 25% to 35% solvent B over 10 min, and then isocratic conditions for a further 10 min. The flow rate was 1 ml/min. Samples (20 μl) were filtered through 0.45 m syringe filters prior to injection. The DAD settings were 280 and 296 nm. Quantification was by comparison with known standards when possible (caffeic, chlorogenic, gallic and protocatechuic acids were all from Sigma Chemical Co., Madrid, Spain). Caffeic acid was used to estimate the concentration of rosmarinic acid. All determinations were performed in duplicate.

The FRAP and ABTS methods were used to measure the antioxidant activity of the plant extracts. FRAP is a measure of reducing ability and was performed according to the method described by Pulido, Bravo, and Saura-Calixto (2000). Dilutions of the extracts were made up to obtain absorbance measurements falling within a readable range. An amount of 30 μl of the diluted aqueous oregano or rosemary extract (10^{-2} and 10^{-1} dilutions, respectively) was incubated with 900 μl of FRAP reagent (containing TPTZ and $FeCl_3$) and 90 μl of distilled water at 37 °C. Absorbance values were read at 595 nm after 4 min and 30 min. Results were expressed as mM of $FeSO_4 \cdot 7H_2O$ equivalents, based on a standard curve for the same compound prepared previously. The ABTS method estimates free radical-scavenging capacity and was performed according to the method described by Re et al. (1999), as slightly modified by Kim and Lee (2004). An amount of 20 μl of diluted (1/20) oregano or rosemary extract was mixed with 980 μl of ABTS reagent. The mixture was then left to stand at 30 °C for 10 min, and absorbance values were read at 734 nm. Results were expressed as mg of ascorbic acid equivalents/ml of extract based on a standard curve for ascorbic acid prepared previously. All determinations were performed at least in duplicate.

Confocal laser scanning microscopy (CLSM; Model TCS-SP2-AOBS, Leica, Barcelona, Spain) was also used to observe possible differences and characterise the extracts, by scanning the autofluorescence of the extracts under a range of fluorescence-inducing laser wavelengths. The fluorescence excitation wavelengths of the extract components were 351 nm (blue), 488 nm (green), and 543 and 633 nm (red). To prepare samples for viewing, a drop of the 1.25 g/100 ml oregano extract or the 20 g/100 ml rosemary extract was placed on a microscope slide. The sample was then dried at 45 °C and covered with a cover slip for viewing under CLSM.

2.3. Edible film formation

The film-forming solutions were made using tuna-skin gelatine, prepared according to the method described by Gómez-Guillén and Montero (2001b), or bovine-hide gelatine, purchased at a local market (Sancho de Borja SL, Saragossa, Spain; Bloom 200/220), at a

Table 1
Total phenols (μg of caffeic acid/ml of extract), reducing ability (FRAP method, expressed as mM of $FeSO_4 \cdot 7H_2O$ equivalents), and free radical-scavenging capacity (ABTS method, expressed as mg of ascorbic acid equivalents/ml of extract) for oregano (5 g/100 ml) and rosemary (20 g/100 ml) extracts

	Oregano	Rosemary
Total phenols	2 080 ± 23	665 ± 11
FRAP 4 min	57.0 ± 0.14	10.1 ± 0.05
FRAP 30 min	92.9 ± 0.2	17.2 ± 0.1
ABTS	1.048 ± 0.016	0.141 ± 0.029

Results are the mean values (total phenols performed in triplicate; FRAP and ABTS in duplicate) ± standard deviation.

Table 2
Phenolics detectable in the aqueous oregano (5 g/100 ml) and rosemary (20 g/100 ml) extracts

Peak	Substance	λ_{max}	Rosemary (μg/ml extract)	Oregano (μg/ml extract)
1	Gallic acid	272		75.8 ± 2.05
2	Protocatechuic acid	260 (294)		74.4 ± 1.50
3	NI 1	288		
4	Chlorogenic acid	326	6.49 ± 0.43	
5	NI 2	265		
6	Caffeic acid	324	5.17 ± 0.38	9.54 ± 0.41
7	NI 3 (flavone)	271, 336		
8	NI 4 (hydroxybenzoyl derivative)	260 (290)		
9	NI 5	278		
10	NI 6 (flavone)	256 (265) 348		
11	NI 7	274		
12	NI 8 (hydroxybenzoyl derivative)	262 (295)		
13	NI 9 (flavone)	267, 338		
14	NI 10	255, 283,347		
15	NI 11	271, 338		
16	NI 12 (flavone)	256 (265) 348		
17	NI 13 (flavone)	256 (265) 348		
18	NI 14	273		
19	Rosmarinic acid	328	24.6 ± 1.32	177 ± 11.6
20	NI 15 (flavone)	268, 342		
21	NI 16 (flavone)	268, 331		
22	NI 17 (flavone)	268, 342		
23	NI 18 (flavone)	268, 342		

Determinations were performed in duplicate and results are the mean values ± standard deviation.
NI, not identified.

concentration of 4 g/100 ml of distilled water. Sorbitol (0.15 g/g of gelatin) and glycerol (0.15 g/g of gelatin) were employed as plasticisers. The control batch was not supplemented with any additive. Oregano or rosemary extract was added to a final concentration of 0.3 g/100 ml or 1.25 g/100 ml (oregano) and 2.5 g/100 ml or 20 g/100 ml (rosemary), using distilled water to make up the corresponding dilutions. All admixtures were warmed and blended by stirring at 40 °C for 15 min, and the films were made by casting an amount of 40 ml on 12 cm × 12 cm-square plates and drying in a forced-air oven at 45 °C for 15 h to obtain a uniform thickness (100 μm; $p \leqslant 0.05$) in all cases. Before performing the determinations, the films were conditioned in desiccators over a saturated solution of NaBr at 22 °C (yielding 58% relative humidity) for 2 days.

2.4. Determinations performed on the films

To avoid protein interference in the Folin–Ciocalteau method, a slightly modified version of the method of phenol quantification, described by Cardinal et al. (2004) was used, and the results have been expressed as g of phenol/g of film, according to a standard curve of the same compound prepared in advance. Films were first dissolved in 0.5 M acetic acid at 40 °C. The resulting solution was shaken vigorously in a 95% ethanol solution for 30 min to extract the phenols. Phenol–protein interactions were then evaluated on the assumption that extractability decreases with the degree of interaction. The mixture was left to stand for 10 min, and the supernatant was employed as the extract for the total phenol determination. All assays were performed at least in triplicate.

The potential antioxidant power of films was measured as total reducing ability by the FRAP method and as free radical-scavenging capacity by the ABTS method. For both determinations, the film was dissolved in 0.5 M acetic acid at 40 °C, and adequate dilutions were made up to yield absorbance measurements within a readable range. FRAP was performed as described for the plant extracts, and the results were expressed as mM of $FeSO_4 \cdot 7H_2O$ equivalents/μg of film. The ABTS method was also performed as described for the plant extracts, and the results have been expressed as mg of ascorbic acid equivalents/g of film. All determinations were performed at least in duplicate.

The light barrier properties of the films were measured according to Gómez-Guillén et al. (2007). Films were exposed to light at wavelengths ranging from 670 to 270 nm, and absorbance was measured using a UV-1601 spectrophotometer (Model CPS-240, Shimadzu, Kyoto, Japan). Results have been expressed as percentage transmittance.

2.5. Statistical analyses

Statistical tests were performed using the SPSS® computer program (SPSS Statistical Software Inc., Chicago, IL). One-way and two-way analysis of variance (ANOVA) were carried out. Differences between pairs of means were assessed on the basis of confidence intervals using the Tukey-b test. The level of significance was $p \leqslant 0.05$.

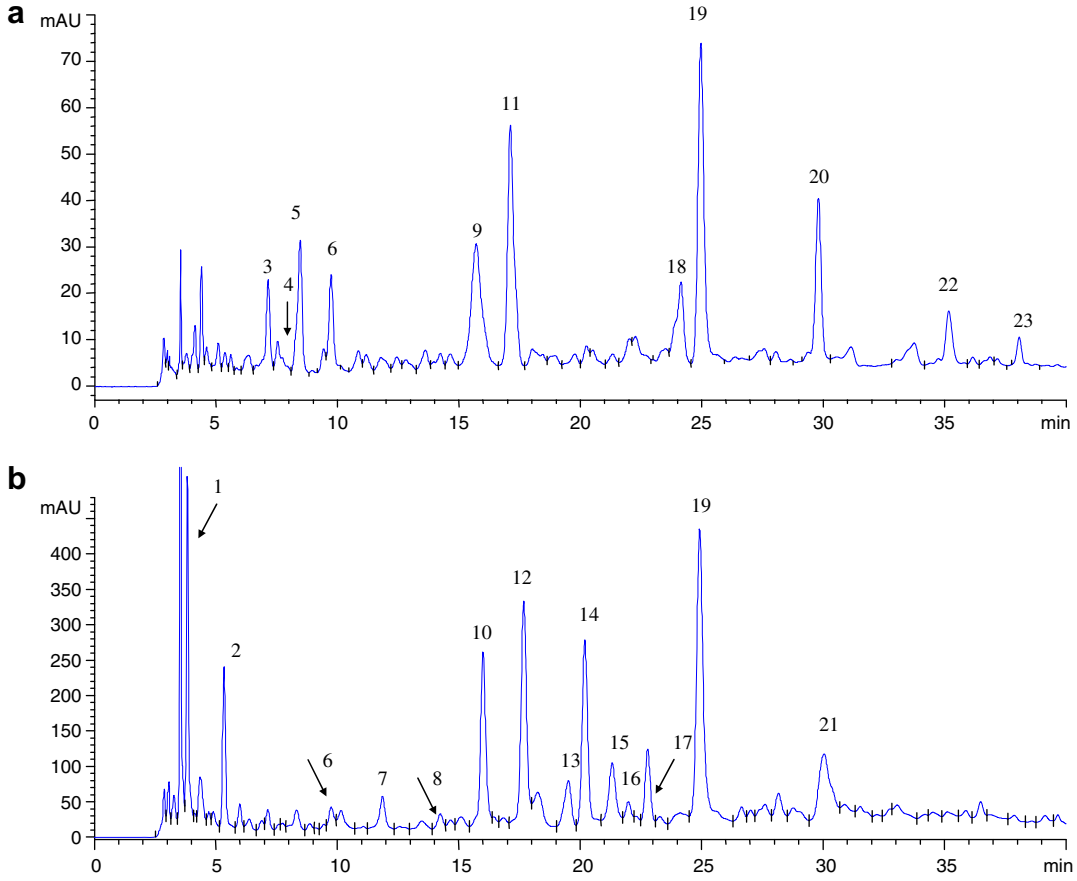

Fig. 1. HPLC-DAD chromatograms for the aqueous extracts of (a) rosemary and (b) oregano. Peak 1, gallic acid; peak 2, protocatechuic acid; peak 4, chlorogenic acid; peak 6, caffeic acid; peak 19, rosmarinic acid; peaks 3, 5, 7–18, 20–23, unidentified substances.

3. Results and discussion

3.1. Antioxidant extracts

Quantitative analysis of the phenols in the aqueous oregano and rosemary extracts (Table 1) yielded higher levels in the oregano extract (~2,000 μg of caffeic acid/ml) than in the rosemary extract (~600 μg of caffeic acid/ml), despite the lesser amount of dried leaf used in the former (5 g/100 ml *vs.* 20 g/100 ml). This might be attributed to the higher phenolic content of oregano, which has been reported to have two to five times as much total phenolics as rosemary, depending on the extraction procedure (Shan, Cai, Sun, & Corke, 2005; Zheng & Wang, 2001). In addition, a higher extraction yield of the polyphenols from oregano than from rosemary cannot be ruled out. Although hot water is not the most efficient solvent for polyphenol extraction, this was selected ahead of other more commonly used solvents (e.g., aqueous methanol, acetone) because the extracts were to be added to edible films, and intense flavours should be avoided. Although elimination of these solvents is possible, this would be expensive and environmentally unfriendly.

Rosmarinic acid was the most abundant polyphenol in both extracts (Table 2, Fig. 1) and was 7.2-fold more concentrated in the oregano (177 μg/ml) than in the rosemary extract (24.6 μg/ml). Other researchers who have worked with both aqueous and alcoholic extracts have also reported higher rosmarinic acid concentrations in oregano as compared to rosemary (Shan et al., 2005; Zheng & Wang, 2001). Caffeic acid was also detectable in small quantities in both samples. The oregano extract also contained appreciable quantities of gallic acid and protocatechuic acid (approximately 75 μg/ml each). In addition, appreciable amounts of a series of other compounds that could not be identified were also observed to be present, though based on their absorption spectra these may have been hydroxybenzoic acid derivatives, caffeic acid derivatives, and various flavonoids (primarily flavone derivatives).

Other researchers have shown rosmarinic acid to be the principal phenolic compound in rosemary and oregano extracts (Almela, Sánchez-Muñoz, Fernández-López, Roca, & Rabe, 2006; del Baño et al., 2003; Kivilompolo & Hyötyläinen, 2007; Kulisic, Dragovic-Uzelac, & Milos, 2006; Shan et al., 2005; Zheng & Wang, 2001), not only in aqueous extracts but also in alcoholic and oleaginous extracts. At the same time, other phenolic acids, such as gallic acid, chlorogenic acid, caffeic acid, vanillic acid, syringic acid, ferrulic acid, and *p*-coumaric acid have been described in both rosemary and oregano extracts. In addition, both these herbs are rich in phenolic substances, chiefly flavone derivatives like apigenin and luteolin (Almela et al., 2006; del Baño et al., 2003; Kulisic et al., 2006; Shan et al., 2005; Zheng & Wang, 2001). Other major components in oregano and rosemary extracts are the diterpenic phenols, carnosic acid, carnosol, rosmanol, epirosmanol, and rosmadial in rosemary (Almela et al., 2006; del Baño et al., 2003; Shan et al., 2005) and carvacrol in oregano (Shan et al., 2005). However, none of these substances was identified in the extracts prepared in this study. It is likely that the mild extraction conditions (45 °C water for 10 min) were not strong enough to extract these highly water-insoluble components.

Figs. 2 and 3 present CLSM images of the extracts. The oregano extract (Fig. 2) exhibited quite intense emission of fluorescence (503–630 nm) when excited in the green region (488 nm) and less intense (650–764 nm) when it is excited at wavelengths in the red

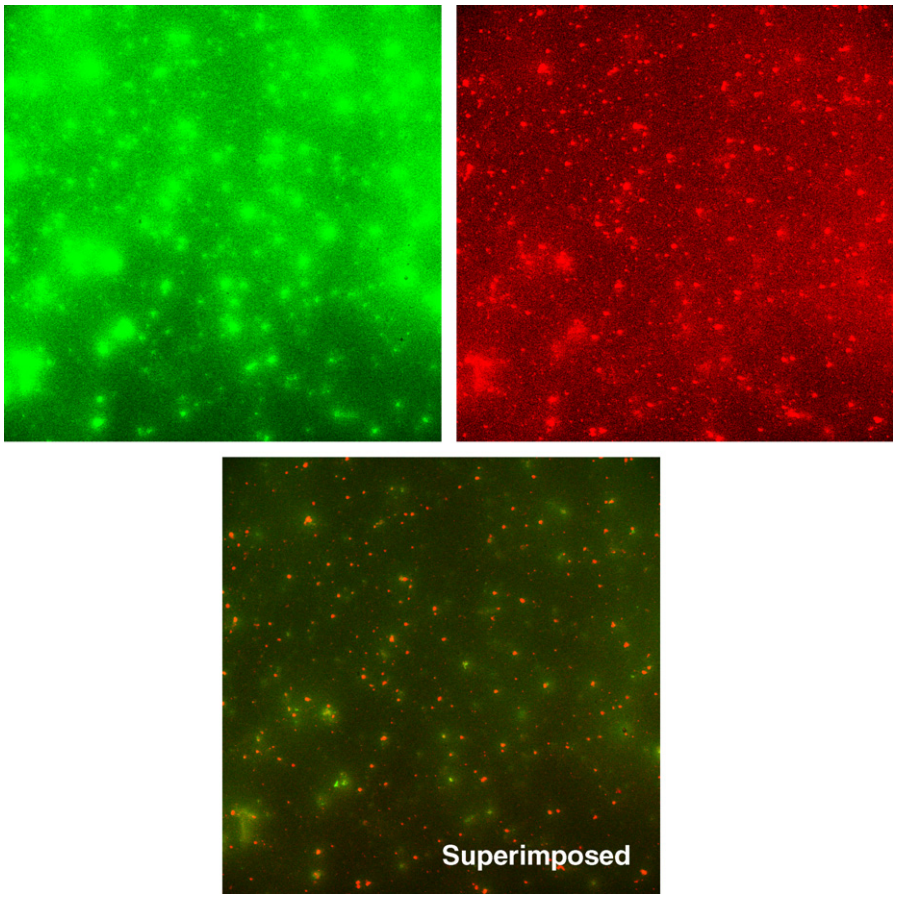

Fig. 2. Confocal micrographs of oregano extract (1.25 g/100 ml).

region (633 nm). In the three emission images the extract exhibits small, very bright spots that were largest in the green region. The merged images show that the bright spots in the green and red regions did not always overlap; hence, they have been deemed to have different structural groups as their source. The rosemary extract (Fig. 3) also exhibited fluorescence in the green region (488 nm excitation, 503–591 nm emission) and in the red region (mainly when it is excited at 630 nm, very little at 543 nm, emitting in the 558–666 and 650–764 nm regions, respectively), though emission was lower in terms of both intensity and quantity, because the spots observed were smaller and less numerous. This is indicative of the quantitative differences between the two extracts already referred to above. Further, unlike the case of the oregano extract, fluorescence was also observed in the blue region (excitation at 351 nm and emission at 355 nm), an indication of qualitative differences as well. The emission of fluorescence was much stronger in the oregano extract whatever the wavelength. The oregano extract had a considerably higher total phenolic content than the rosemary extract (Table 1), despite the lesser amount of dried leaves used. Thus the intensity of the emission seems to be related to the phenolic content of the plant extracts.

There is not much information in the literature on the fluorescence excitation/emission spectra of polyphenols. Flavonoids and anthocyanins have been reported to emit fluorescence at around 450–600 nm. Specifically, a methanolic solution of the flavonol quercetin has been reported to emit fluorescence in the ultraviolet (300 nm), blue (450–500 nm) and yellow (570–580 nm) regions when excited at 266, 266–355, and 480 nm, respectively (Lai, Santangelo, Zorreéis, & Fantoni, 2007), while it emitted at 500 nm with an excitation wavelength of 430 nm when dissolved in phosphate buffered saline (Nifli et al., 2007). The quercetin–aluminium complex has emission and excitation wavelengths of 485 and 422 nm, respectively (Hollman, van Trijp, & Buysman, 1996). As for anthocyanins, pelargonidin chloride also exhibited emission at 380 and 450 nm when excited at 266 and 355–400 nm, respectively, showing a very intense emission band at 600 nm (575–675 nm) under excitation wavelengths between 230–450 nm (Lai et al., 2007). The 3,5-diglucosides of the anthocyanins malvidin and peonidin have also been reported to emit fluorescence under UV light (Santos-Buelga & Williamson, 2003). Considering all the above, the fluorescence observed in the oregano and rosemary extracts, with emissions recorded at both 488 nm and, though quite weakly, at 534 nm in both extracts, the flavonoid content would appear not to be particularly high, in agreement with the chromatographic data. However, although anthocyanin emission peaks are found at 380 nm and 450 nm, and around 600 nm, the emission peaks in the oregano and rosemary extracts are not due to the presence of anthocyanins, since these flavonoids were not detected in the extracts after HPLC-DAD analyses.

The results for antioxidant activity measured as reducing ability (FRAP method) and free radical-scavenging capacity (ABTS method) likewise yielded higher values for the oregano extract (Table 1), respectively, 5.5 and 7 times the values recorded for the rosemary extract. Furthermore, somewhat higher activity was also observable in the oregano extract when antioxidant activity was expressed as μg of caffeic acid (Table 3). According to Shan et al. (2005), the higher antioxidant power was ascribable to higher phenol content, based on the positive linear correlation they observed between the two. The main polyphenols identified in the oregano and rosemary extracts in this study were hydroxycinnamic acid derivatives, like caffeic, rosmarinic, and chlorogenic acids, and hydroxybenzoic acid derivatives, like gallic acid and

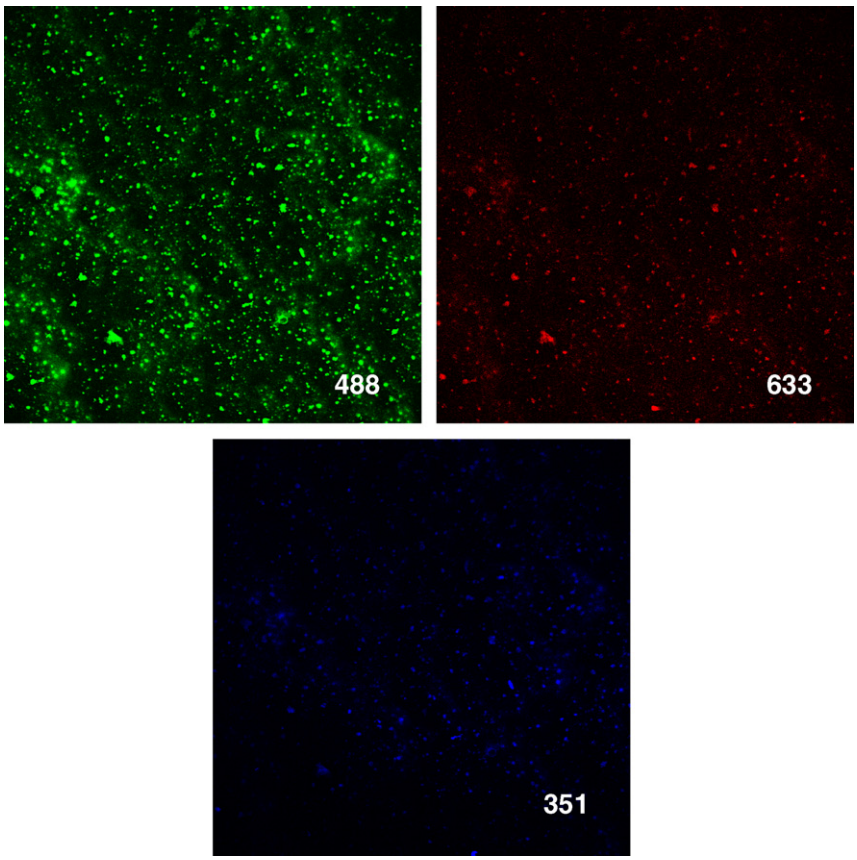

Fig. 3. Confocal micrographs of rosemary extract (20 g/100 ml).

protocatechuic acid. In the form of simple acids, both these classes of phenols have exhibited similar levels of reducing ability, as measured by the FRAP method (Pulido et al., 2000). However, differences in the level of antioxidant activity of the different components have been reported to depend on the chemical form of the compounds present. For instance, combining two or more phenolic acids, as in the case of rosmarinic acid (a dimer of caffeic acid and hydrocaffeic acid), greatly increases the antioxidant power, whereas esterification lowers the antioxidant power, as in the case of chlorogenic acid (caffeic acid esterified with quinic acid) (Chen & Ho, 1997; Cuvelier, Richard, & Berset, 1992). Consequently, the greater antioxidant activity of the oregano extract when expressed either as ml of extract or as μg of caffeic acid was consistent with the findings of higher concentrations of both rosmarinic acid and caffeic acid and of the presence of gallic acid and protocatechuic acid in the extract. Other workers have also reported higher quantities of phenols and higher antioxidant activity in oregano extract as compared to rosemary extract (Shan et al., 2005; Zheng & Wang, 2001), which agrees with the findings observed here.

FRAP readings taken at two different times help assess an antioxidant's activity rate and check whether it is active only at the outset or whether its activity level increases with time. Taking the 30-min FRAP reading as 100%, the 4-min FRAP activity level was ~61% for the oregano extract and ~59% for the rosemary extract. This finding indicates that the antioxidant activity trend was similar in both extracts, that is, over half of the potential reducing ability was achieved at the outset, and the activity level then increased with time.

Table 3
Reducing ability according to the FRAP method and free radical-scavenging capacity according to the ABTS method expressed as a function of total phenolic content

	Oregano	Rosemary
FRAP 4 min	0.027	0.015
FRAP 30 min	0.044	0.025
ABTS	5.03×10^{-4}	2.12×10^{-4}

FRAP and ABTS antioxidant activity per μg of caffeic acid.

Table 4
Total phenols (μg of phenol/g of film) for the different films

	Control	Oregano 0.3 g/100 ml	Oregano 1.25 g/100 ml	Rosemary 2.5 g/100 ml	Rosemary 20 g/100 ml
Bovine-hide	–	1 673 (30) bx	5 819 (209) cx	50 (2) ax	1 598 (364) bx
Tuna-skin	–	178 (11) by	498 (39) dy	40 (13) ax	289 (1) cy

Different letters (a, b, c, d) in the same row indicate significant differences between the different film formulations made using the same type of gelatin. Different letters (x, y) in the same column indicate significant differences for each film formulation according to gelatin type. Determinations were performed in triplicate and results are the mean values (standard deviation).

3.2. Films

As expected, the unsupplemented films (without added antioxidant) contained no phenols (Table 4). Adding the oregano and rosemary extracts raised the phenol contents of the films. The phenol content was higher in the films containing oregano than in the films with rosemary. As the phenol content was adjusted to be similar in the films for both extracts, this finding could be indicative of some differences in the interaction degree as a function of the type of extract, being higher in the rosemary one. Furthermore, the phenol content in the films differed considerably according to the type of gelatin employed, even though the quantity of antioxidant extract added was similar. Thus, the films made using the fish gelatin were observed to have significantly lower ($p \leqslant 0.05$) phenol concentrations than the films made using the mammalian gelatin. The explanation for this lies in the interactions between the gelatin's polypeptide chains and the polyphenols, which seemed to be higher in the fish gelatin. In this sense, fish collagenous material is well known to possess a lower content in imino acids (Pro + Hyp) (Norland, 1990) and so protein–protein interactions may be reduced, compared to mammalian gelatins. This intrinsic characteristic may favour the tuna gelatin polypeptides to interact with polyphenols and therefore decrease the free phenol content in the corresponding films.

Discussing in more detail the data for the rosemary-added films, it can be seen that the phenol content for the tuna-skin gelatin films was sevenfold higher at 20 g/100 ml and 32-fold higher for the bovine-hide gelatin made films, compared with films at 2.5 g/100 ml. This can be due to saturation of the protein–polyphenol interaction, in the case of the bovine-hide gelatin films, as the ability of this kind of gelatin to interact with itself is higher than that of fish species, due to the higher imino acid content.

Table 5 sets out the potential antioxidant activity of the films, expressed as reducing ability (FRAP method) and free radical-scavenging capacity (ABTS method). The control films displayed some antioxidant activity, as measured by both these indices, irrespective of the type of gelatin employed, but this finding was particularly pronounced for the ABTS index. This antioxidant activity is ascribable to the gelatin, particularly to the peptide fraction (Kim et al., 2001; Kim, Kim, Byun, Park, & Ito, 2001). Mendis, Rajapakse, and Kim (2005) reported the peptide sequence His–Gly–Pro–Leu–Gly–Pro–Leu, present in hydrolysates of hoki (*Johnius belengerii*) skin gelatin, to be a powerful scavenger of free radicals. The results obtained in the present study are in agreement with previous findings in films made from tuna-fish gelatin, which also exhibited certain antioxidant capacity (Gómez-Guillén et al., 2007).

Table 5
Reducing ability (mM of $FeSO_4 \cdot 7H_2O$ equivalents/μg of film) and free radical-scavenging capacity (ABTS method, expressed as mg of ascorbic acid equivalents/g of film) of the different films

Formulation	FRAP 4 min		FRAP 30 min		ABTS	
	Bovine-hide	Tuna-skin	Bovine-hide	Tuna-skin	Bovine-hide	Tuna-skin
Control	0.04 (0.01) ax	0.04 (0.009) ax	0.07 (0.01) ax	0.07 (0.01) ax	2.78 (0.01) ax	2.49 (0.01) ay
Oregano (0.3 g/100 ml)	1.31 (0.03) cx	1.45 (0.05) by	2.83 (0.07) cx	2.48 (0.09) by	7.10 (0.11) dx	6.74 (0.30) cx
Oregano (1.25 g/100 ml)	5.02 (0.14) ex	5.48 (0.78) dx	9.05 (0.19) ex	8.17 (1.03) dx	16.10 (0.33) ex	11.04 (1.16) dy
Rosemary (2.5 g/100 ml)	0.55 (0.02) bx	0.51 (0.06) ax	0.96 (0.03) bx	0.81 (0.05) ay	3.67 (0.10) bx	3.73 (0.42) bx
Rosemary (20 g/100 ml)	3.59 (0.23) dx	3.32 (0.29) cy	5.98 (0.01) dx	5.27 (0.13) cy	5.56 (0.09) cx	7.00 (0.24) cy

Different letters (a, b, c, d) in the same row indicate significant differences between the different film formulations made using the same type of gelatin. Different letters (x, y) in the same column indicate significant differences for each film formulation according to gelatin type. Determinations were performed in duplicate and results are the mean values (standard deviation).

The films supplemented with the added antioxidant extracts exhibited higher reducing ability and free radical-scavenging capacity values than the control films (Table 5), the degree of antioxidant power generally being proportional to the amount of extract added. The activity trend for reducing ability followed the same pattern as in the extracts, with more than half the potential being achieved at the outset and then increasing. Differences were again observed depending on the type of extract added; with higher activity levels being observed for the oregano extract supplemented films. With the higher oregano concentration, the radical-scavenging capacity was found to be significantly lower in the tuna gelatin films. This suggests that the supposed higher degree of protein–polyphenol interactions with the fish gelatin may eventually affect the potential antioxidant power of the supplemented film.

The films with added oregano or rosemary extracts, although maintaining their transparency, were darker, and this could affect the light transmission properties of the films. To ascertain whether or not this was the case, the films underwent spectrophotometric scanning at wavelengths ranging from 670 to 270 nm, and the percentage light transmittance was recorded. Fig. 4 shows that the unsupplemented films had transmittance values of around 80–90% at 400 nm. On adding extract, the transmittance values became inversely proportional to the amount of extract added. These findings suggest that, in addition to the potential antioxidant benefits afforded, films supplemented with added oregano or rosemary extracts may also possess good light barrier properties, especially to ultraviolet light, a powerful lipid-oxidising agent. These light barrier attributes are in part ascribable to the polyphenol composition of the extracts added to the films, since the transmittance results for the extracts were indicative of a similar pattern of light absorption (Fig. 4, inset). These results are in agreement with previous findings (Gómez-Guillén et al., 2007), that reported better light barrier properties when antioxidant extract from murta was added to gelatin-based edible films. Other protein-made films from gelatin (Jongjareonrak et al., 2006b), myofibrillar proteins (Shiku, Hamaguchi, Benjakul, Visessanguan, & Tanaka, 2004), and milk whey proteins (Fang, Tung, Britt, Yada, & Dalgleish, 2002) have also displayed good light barrier properties. As a general rule, light transmission by this type of food packaging is much lower than that of synthetic polymers (Shiku et al., 2004).

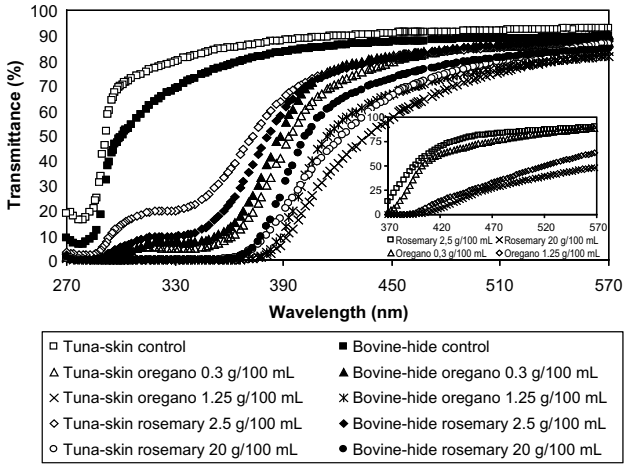

Fig. 4. Changes in percentage of transmittance at wavelengths ranging from 670 to 270 nm for tuna-skin and bovine-hide gelatin films either unsupplemented or supplemented with an added oregano or rosemary antioxidant extract (each extract used at two concentrations). Inset depicts the percentage transmittance of the antioxidant extracts at the different concentrations employed.

4. Conclusions

The antioxidant power of biodegradable and edible films made from bovine-hide and tuna-skin gelatin can be augmented by adding polyphenol-rich oregano or rosemary water extracts. However, while the potential antioxidant activity of the films was not clearly affected by the source of the gelatin employed, the supplemented films did release differing amounts of phenols, with the bovine-hide gelatin films releasing larger amounts, presumably because protein–polyphenol interactions were formed to a higher extent in the fish gelatin films. The light barrier properties of the films were unaffected by gelatin type but were enhanced by supplementation with the plant extracts.

Acknowledgements

This research was supported by the Spanish "Ministerio de Educación y Ciencia" (Project AGL2005-02380/ALI).

References

Almela, L., Sánchez-Muñoz, B., Fernández-López, J. A., Roca, M. J., & Rabe, V. (2006). Liquid chromatographic–mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. *Journal of Chromatography A, 1120*, 221–229.

Arvanitoyannis, I. S. (2002). Formation and properties of collagen and gelatin films and coatings. In A. Gennadios (Ed.), *Protein-based films and coatings* (pp. 274–304). Boca Ratón, Florida: CRC Press.

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 gelatin. *Food Hydrocolloids, 19*, 73–82.

Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J. L., & Leroi, F. (2004). Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. *Food Research International, 37*, 181–193.

Chen, J. H., & Ho, C.-T. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry, 45*, 2374–2378.

Cuvelier, M.-E., Richard, H., & Berset, C. (1992). Comparison of the antioxidative activity of some acid-phenols: Structure–activity relationship. *Bioscience, Biotechnology and Biochemistry, 56*, 324–325.

del Baño, M. J., Lorente, J., Castillo, J., Benavente-García, O., del Río, J. A., Ortuño, A., et al. (2003). Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, ítems, and roots of *Rosmarinus officinalis*. Antioxidant activity. *Journal of Agricultural and Food Chemistry, 51*, 4247–4253.

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*, 188–193.

Gennadios, A., Hanna, M. A., & Kurth, L. B. (1997). Application of edible coating on meats, poultry and seafoods: A review. *Lebensmittel-Wissenschaft und Technologie, 30*, 337–350.

Gómez-Estaca, J., Montero, P., Giménez, B., & Gómez-Guillén, M. C. (2007). Effect of functional edible films and high pressure processing on microbial and oxidative spoilage in cold-smoked sardine (*Sardina pilchardus*). *Food Chemistry, 105*, 511–520.

Gómez-Guillén, M. C., & Montero, P. (2001a). Extraction of gelatin from megrim (*Lepidorhombus boscii*) skins with several organic acids. *Journal of Food Science, 66*, 213–216.

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

Gómez-Guillén, M. C., Ihl, M., Bifani, V., Silva, A., & Montero, P. (2007). Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae Turcz*). *Food Hydrocolloids, 21*, 1133–1143.

Gómez-Guillén, M. C., Turnay, J., Fernández-Díaz, M. D., Ulmo, N., Lizarbe, M. A., & Montero, P. (2002). Structural and physical properties of gelatin extracted from different marine species: A comparative study. *Food Hydrocolloids, 16*, 25–34.

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

Hollman, P. C. H., van Trijp, J. M. P., & Buysman, M. N. C. P. (1996). Fluorescence detection of flavonols in HPLC by postcolumn chelation with aluminium. *Analytical Chemistry, 68*, 3511–3515.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2006a). 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*, 229–235.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2006b). Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. *Food Hydrocolloids, 20*, 492–501.

Kim, S., Kim, Y., Byun, H., Nam, K., Joo, D., & Shahidi, F. (2001). Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska Pollack skin. *Journal of Agricultural and Food Chemistry, 49*, 1984–1989.

Kim, S. K., Kim, Y. T., Byun, H. G., Park, P. J., & Ito, H. (2001). Purification and characterization of antioxidative peptides from bovine skin. *Journal of Biochemistry and Molecular Biology, 34*, 219–224.

Kim, D. O., & Lee, C. Y. (2004). Comprehensive study an vitamin C equivalent antioxidant capacity (VEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Critical Reviews in Food Science and Nutrition, 44*, 253–273.

Kim, K. M., Lee, B.-Y., Kim, Y. T., Choi, S.-G. C., Lee, J., Cho, S. Y., et al. (2006). Development of antimicrobial edible film incorporated with green tea extract. *Food Science and Biotechnology, 15*, 478–481.

Kivilompolo, M., & Hyötyläinen, T. (2007). Comprehensive two-dimensional liquid chromatography in análisis of Lamiaceae herbs: Characterisation and quantification of antioxidant phenolic acids. *Journal of Chromatography A, 1145*, 155–164.

Kulisic, T., Dragovic-Uzelac, V., & Milos, M. (2006). Antioxidant activity of aqueous tea infusions prepared from oregano, thyme and wild thyme. *Food Technology and Biotechnology, 44*, 485–492.

Lai, A., Santangelo, E., Zorreéis, G. P., & Fantoni, R. (2007). Analysis of the main secondary metabolites produced in tomato (*Lycopersicon esculentum*, Mill) epicarp tissue during fruit ripening using fluorescence techniques. *Postharvest Biology and Technology, 43*, 335–3342.

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*, 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*, 1697–1706.

Montreau, F. R. (1972). On the análisis of total phenolic compounds in wines by the Folin–Ciocalteau method. *Connaissance Vigne, 24*, 397–404.

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

Nifli, A. P., Theodoropoulos, P. A., Munier, S., Castagnino, C., Roussakis, E., Katerinopoulos, H. E., et al. (2007). Quercetin exhibits a specific fluorescence in cellular milieu: A valuable tool for the study of its intracellular distribution. *Journal of Agricultural and Food Chemistry, 55*, 2873–2878.

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, PA: Technomic Publishing Co.

Oussalah, M., Caillet, S., Salmiéri, S., Saucier, L., & Lacroix, M. (2004). Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *Journal of Agricultural and Food Chemistry, 52*, 5598–5605.

Papadokostaki, K. G., Amanratos, S. G., & Petropoulos, J. H. (1997). Kinetics of release of particules solutes incorporated in cellulosic polymer matrices as a function of solute solubility and polymer swellability. I. Sparingly soluble solutes. *Journal of Applied Polymer Science, 67*, 277–287.

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 48*, 3396–3402.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Santos-Buelga, C., & Williamson, G. (Eds.). (2003). *Methods in polyphenols analysis*. Cambridge, UK: The Royal Society of Chemistry.

Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry, 53*, 7749–7759.

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*, 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*, 423–432.

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

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 59*, 5162–5170.

Zivanovic, S., Chi, S., & Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. *Journal of Food Science, 70*, M45–M51.