# EVALUATION OF LIPID OXIDATION IN HORSE MACKEREL PATTIES COVERED WITH BORAGE-CONTAINING FILM DURING FROZEN STORAGE

Giménez, B., Gómez-Guillén, M.C., Pérez-Mateos, M., Montero, P., Márquez-Ruiz, G.*

Instituto del Frío-ICTAN (CSIC)[1], Ciudad Universitaria, 28040 Madrid, Spain

* Corresponding author. Tel: +34 91 5492300; fax: +34 91 5493627. E-mail: gmarquez@if.csic.es

[1] This centre has implemented and maintains a Quality Management System which fulfils the requirements of the ISO standard 9001:2000

## ABSTRACT

Lipid oxidation of horse mackerel (*Trachurus trachurus*) patties covered with fish gelatin-based films containing a borage seed extract was evaluated, including commonly used analytical indexes (peroxide value, thiobarbituric acid reactive substances, polyene ratio), as well as determination of volatile compounds, quantitation of oxidized triacylglycerols and analysis by Fourier transform infrared (FTIR) spectroscopy, during 240 days of frozen storage and subsequent thawing and 4 days-chilling. Vacuum packaged-patties and control uncovered patties were also tested for comparative purposes. Methods applied to evaluate lipid oxidation in extracted lipids, i.e. peroxide value, quantitation of oxidized triacylglycerols and FTIR, clearly provided a better picture of the oxidation progress and led to similar conclusions. Film had protective effects on lipid oxidation of horse mackerel patties throughout frozen storage and particularly after thawing and chilled storage. Furthermore, as compared to vacuum packaging, film was similarly effective until advanced stages of oxidation were reached

[Escriba aquí]Food Chem. 2011;124(4):1393-1403. DOI:10.1016/j.foodchem.2010.07.097 [Escriba aquí]

and exerted enhanced protection once samples were thawed and exposed to air oxygen under chilling temperature; with the additional advantage of increasing the antioxidant capacity of muscle.

Keywords: Frozen fish patties, gelatin borage-films, lipid oxidation, antioxidant capacity

## INTRODUCTION

Fatty fish contains high levels of ω-3 polyunsaturated fatty acids (PUFAs), such as eicosapentanoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-6). Consumption of ω-3 PUFAs has been related to beneficial effects on human health, e.g. on cardiovascular diseases and arteriosclerosis (Shahidi & Miraliakkbari, 2004). However, because of this high polyunsaturated lipid content as well as the presence of potential activators, such as heme pigments and trace metals (Richards & Hultin, 2002), fatty and medium-fatty species like horse mackerel (*Trachurus trachurus*) are very susceptible to loss of nutritional quality and shortening of shelf life because of lipid oxidation. Recently, an aspect of the utmost interest in this context is the evaluation of the oxidation progress during frozen storage (Medina, González, Iglesias & Hedges, 2009; Pourashouri, Shabanpour, Aubourg, Rohi & Shabani et al., 2009; Mahmoudzadeh et al., 2010, Yerlikaya & Gokoglu, 2010).

The main mechanism for lipid oxidation is autoxidation, an autocatalytic process initiated by formation of radicals in unsaturated lipids followed by oxygen attack (Frankel, 2005). Hydroperoxides are the primary oxidation products formed and further

oxidation, decomposition and polymerization reactions lead to formation of a complex mixture of intermediate and secondary oxidation products which include a multitude of compounds. Non-volatile and volatile compounds, of different molecular weight and polarity, and bearing different oxygenated functions, such as hydroperoxy, hydroxy, aldehyde, epoxy and ketone functions, are formed. Hence great difficulties are normally encountered to evaluate the degree of oxidation (Dobarganes & Márquez-Ruiz, 2003).

Evaluation of fish lipid oxidation is normally based on analytical indexes, peroxide value (PV) and the 2-thiobarbituric acid reactive substances (TBARS) standing out as indicators of primary and secondary oxidation products, respectively (Saeed & Howell, 2002; Aranda, Mendoza & Villegas, 2005; Chaijan, Benjakul, Visessanguan & Faustman, 2006; Quitral, Donoso, Ortiz, Herrera, Araya & Aubourg, 2009; Pourashouri et al., 2009; Yerlikaya & Gokoglu, 2010). However, such methods provide only partial information on the oxidative process. In the case of TBARS, additional drawbacks are that the compound measured, malondialdehyde, is only one of the many possible secondary oxidation products formed and that other compounds, not resulting from the oxidation process, can contribute to TBARS values. Changes in polyunsaturated fatty acids / calculation of polyene ratio is another approach used even though sensitivity is usually low (Márquez-Ruiz, Velasco & Dobarganes, 2000; Mbarki, Sadok & Barkallah, 2009). Recently, the evaluation of volatile compounds has become an additional indicator of lipid oxidation in fish samples (Ross & Smith, 2006; Iglesias & Medina, 2008). With respect to instrumental methods, infrared spectroscopy has lately demonstrated to be a useful tool to monitor oxidative changes in edible oils (Guillén & Cabo, 2000, 2004), due to the high information content of infrared spectra and the possibility to assign specific absorption bands to particular functional groups. However, there are only a few reports dealing with the monitorization of oxidative changes in fish

lipids by this method (Guillén, Ruiz & Cabo, 2004; Chaijan et al., 2006). As a different approach to evaluate oxidation in fish samples, quantitation of total nonvolatile oxidation compounds and their distribution in oxidized triacylglycerol monomers, dimers and polymers has proved to be a useful measurement of early and advanced oxidation stages (Márquez-Ruiz & Dobarganes, 2005; 2006). Even though this methodology has not been applied to fish muscle lipids so far, excellent results have been obtained in studies on microencapsulated fish oils (Márquez-Ruiz et al., 2000; Velasco, Marmesat, Dobarganes & Márquez-Ruiz, 2006; Velasco, Holgado, Dobarganes & Márquez-Ruiz, 2009).

The use of antioxidants is an effective way to prevent lipid oxidation in food products. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been widely used to prevent lipid oxidation, but nowadays the use of natural antioxidants is of growing interest. In recent years, plant extracts and different polyphenolic compounds have been used to successfully improve the oxidative stability of seafood products (Tang, Sheehan, Buckley, Morrissey & Kerry, 2001; Vareltzis, Koufidis, Gavriilidou, Papavergou, & Vasiliadou, 1997; Pazos, Sánchez & Medina, 2005a; Pazos, González, Gallardo, Torres & Medina, 2005b; Medina et al., 2009). These natural compounds, together with others such as chitosan, lysozyme or vitamin C, have been also added to biodegradable packaging materials in order to confer specific attributes, mainly antimicrobial and antioxidant activities, obtaining an active packaging biomaterial (Gómez-Estaca, Montero, Giménez & Gómez-Guillén, 2007; López-Caballero, Gómez-Guillén, Pérez-Mateos & Montero, 2005; Bower, Avena-Bustillos, Olsen, McHugh & Bechtel, 2006; Gómez-Guillén, Ihl, Bifani, Silva & Montero, 2007). In a previous work (Gómez-Estaca, Giménez, Montero & Gómez-Guillén, 2009), the incorporation of a borage extract obtained from seeds remarkably

105 improved the antioxidant activity of fish gelatin films with only minor modifications of
106 the physico-chemical properties of the films.
107 The objective of this study was to evaluate lipid oxidation in horse mackerel patties
108 covered with fish gelatin-based films added with borage extract obtained from seeds
109 during frozen storage for 240 days by different methods, including analytical indexes
110 commonly used (PV and TBARS, determination of polyene ratio), determination of
111 volatile compounds, quantitation of oxidized triacylglycerols and evaluation by FTIR.
112 During frozen storage, samples tested included those covered with films as well as
113 vacuum packaged samples and control samples (neither vacuum packaged nor covered).
114 Additionally, after frozen storage for 240 days, samples were thawed and stored at
115 chilled temperature, thus simulating conditions previous to consumption.
116
117 **MATERIALS AND METHODS**
118
119 **Materials**
120 Commercial fish skin gelatin (Bloom 265/285), mainly obtained from warm water
121 species, was acquired from Rousselot SAS (Courbevoie, France). Borage (*Borago*
122 *officinalis*, Movera variety) seeds were obtained from Semillas Fitó S.A. (Barcelona,
123 Spain). All chemicals were of analytical grade and acquired from Panreac Química
124 (Barcelona, Spain) and Sigma Chemical Co. (Madrid, Spain).
125
126 **Preparation of the borage extract**
127 Borage seeds were ground in an electric grinder for 5 min. The ground meal was mixed
128 with water:ethanol 50:50 (v:v) in a proportion of 20 g/100 mL and the extraction was
129 performed by continuous stirring in a water bath at 75 ºC for 60 min, according to the

optimized method described by Wettasinghe and Shahidi (1999). The extract thus obtained was filtered through Whatman no. 1 filter paper.

**Preparation of the edible films**

The gelatin (final concentration in the film forming solution of 4 g/100 mL) was firstly dissolved in distilled water to a ratio of 4 g/50 mL and a mixture of sorbitol (0.15 g/g gelatin) and glycerol (0.15 g/g gelatin) was added as plasticizer. After adequate mixing of the plasticizers, the borage extract was incorporated at a 1:1 ratio (dissolved gelatin:borage extract) and mixed again (40 ºC, 15 min) to obtain a good blend. No protein precipitation was observed as result of the addition of either ethanol or the borage extract. The films were made by casting an amount of 40 ml on 12 cm x 12 cm-square Petri plates, drying afterwards in a forced-air oven at 45 ºC for 15 h to obtain an uniform thickness (~100 μm; $p \geq 0.05$) in all cases.

**Preparation of fish patties**

Twenty kg of ice-stored Atlantic horse mackerel (*Trachurus trachurus*) were supplied by a local market. After heading, gutting and washing, minced muscle was prepared using a Baader model 694 de-boning machine (Lübeck, Germany) equipped with a drum with 3 mm holes. Sodium salt acquired in a local market was added to a concentration of 1 g/100 g muscle and patties of 50-60 g were then prepared manually. Patties were divided into three batches packed in plastic bags (supplied by Criovac® BB4L): vacuum sealed patties (V), patties covered with borage-added fish gelatin film (F) and control patties (C; neither covered nor vacuum sealed). Patties were frozen in a freezer (Frigoscandia, model AGA0-6373, Helsingborg, Sweden) at -40 ºC for 2 hours and then stored at -20 ºC. Periodically, samples were taken out for analyses. After 240 days of frozen storage, patties were taken

155 out of the bags and kept at chilled temperature (4 ºC) for 4 days in order to simulate
156 conditions previous to consumption under air exposure.
157
158 **Lipid extraction**
159 Lipids were extracted according to the method of Bligh and Dyer (1959) and the lipid
160 content was determined gravimetrically in duplicate.
161
162 **Determination of lipid classes**
163 Separation of neutral and polar lipids in initial samples was carried out using silica gel
164 (230-400 mesh; Sigma-Aldrich, St. Louis, MO) columns and following the procedure
165 described by Bandarra, Batista, Nunes and Empis (2001) with slight modifications. A
166 hexane slurry of silica gel (20 g) was poured into a glass column (16 mm i.d.) plugged
167 with glass wool. One gram of extracted lipids were dissolved in 10 mL hexane:diethyl
168 ether (1:1 v/v) and added to the column. Neutral lipids were eluted with 150 mL
169 hexane:diethyl ether (1:1 v/v) while polar lipids (mostly phospholipids) were eluted in a
170 second fraction with 150 mL methanol followed by 150 mL chloroform:methanol:water
171 (3:5:2 v/v/v). The neutral lipid fraction was further separated in a second silica column
172 using 150 mL hexane:diethyl ether (90:10 v/v) to elute triacylglycerols and 150 mL
173 diethyl ether to elute a minor fraction containing diacylglycerols, monoacylglycerols,
174 free fatty acids and cholesterol (Márquez-Ruiz & Dobarganes, 2005).
175
176 **Fatty acid composition**
177 Fatty acid composition was determined in initial samples by gas-liquid chromatography
178 following derivatization of extracted lipids to fatty acid methyl esters, according to
179 IUPAC methods (IUPAC 1992a,b). Briefly, fatty acid methyl esters were prepared by
180 base-catalyzed methanolysis of the extracted lipids using 2N KOH in methanol. Then,

fatty acid methyl esters were analyzed on a HP-6850 Series chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a flame ionization detector. Fatty acids were separated using a HP Innowax polyethylene glycol capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, USA). The injector and detector temperatures were 250ºC. Oven temperature was set initially at 180ºC and held for 2 min, then raised to 230ºC at a rate of 3ºC/min and held for 20 min. The split ratio was 1:40 and hydrogen was the carrier gas at a flow rate of 1 mL/min. Sample volume injected was 1 μL (50 mg/mL).

**Determination of peroxide value (PV)**

The peroxide value index was assayed according to UNE standard 55-023-73 (1973), and the results were expressed in milliequivalents of $O_2$ per kg of lipids. Determinations were performed in duplicate.

**Determination of thiobarbituric acid reactive substances (TBARS)**

Samples were analysed by the method of Vyncke (1970), which consists in the homogenization of the fish muscle with 7.5% trichloroacetic acid, filtration and reaction with 0.02 M thiobarbituric acid, the absorbance being read at 532 nm. A standard curve was prepared using 1,1,3,3-tetraethoxypropane as per Botsoglou, Fletouris, Papageorgiu, Vassilopoulos, Mantis & Trakatellis (1994). The results were expressed as mg malondialdehyde per kg of patties. Determinations were performed at least in triplicate.

**Polyene ratio**

205 Polyene ratio was calculated on the basis of fatty acid composition of samples
206 throughout storage. Polyene ratio = ([20:5]+[22:6])100/[16:0]. Determinations were
207 performed at least in duplicate.
208
209 **Volatile analysis**
210 Six grams of chopped fish patties corresponding to each sampling point were kept
211 frozen at -80 °C in a 20 mL vial for volatile analysis. All vials were thawed at room
212 temperature and an internal standard (2-octanone in acetone) was added. Sample was
213 inserted automatically through the dynamic headspace injector (TurboMatrix HS-40,
214 Perkin-Elmer, Waltham, MA, USA) and was thermally desorbed with the trap
215 headspace. Injector temperature was set at 80°C for 30 min. Chromatographic
216 separations were performed on an HP-5MS capillary column (30 m x 0.32 mm i.d., 0.25
217 µm film thickness, Agilent Technologies, Palo Alto, CA, USA). The following
218 temperature program was used: 44 ºC for 10 min, 44 ºC to 100 ºC at 10 ºC/min, hold at
219 100 ºC for 5 min, 100 ºC to 190 ºC at 5 ºC/min, 190 ºC to 250 ºC at 50 ºC/min, and
220 finally hold at 250 ºC for 3 min. Mass spectrometry analysis (Agilent 6890-5973MSD,
221 Agilent Technologies, Palo Alto, CA, USA) was operated in the electron ionisation
222 mode and cuadrupole mass filter. The mass range was 30 to 550 amu, ionization energy
223 was 70eV, and transfer line was 230ºC. Identification of the components in each sample
224 was based on their comparison with those of the library of Mass Spectra (NIST05)
225 using the HP ChemStation Software (Agilent Technologies, Palo Alto, CA, USA). The
226 amounts of each compound were expressed as peak areas relative to the peak area of the
227 internal standard. Analyses were carried out in duplicate.
228

**Determination of oxidized triacylglycerol monomers, dimers and polymers.** Quantitative determination of total non-volatile oxidation compounds and their distribution in oxidized triacylglycerol monomers, dimers and polymers was carried out by solid-phase extraction (SPE) and subsequent analysis by high-performance size-exclusion chromatography (HPSEC), according to Márquez-Ruiz, Jorge, Martín-Polvillo and Dobraganes (1996). A volume of 2 mL of a hexane solution containing 50 mg of extracted lipids and 1 mg of monostearin, used as internal standard, was separated into two fractions by SPE. A first fraction, comprising the unoxidized triacylglycerols, was eluted with 15 mL of hexane:diethyl ether (90:10, v/v). The second fraction was eluted with 25 mL of diethyl ether and included all non-volatile oxidation compounds, the internal standard, diacylglycerols, free fatty acids and polar unsaponifiable matter. Efficiency of the separation was checked by TLC using hexane/diethyl ether/acetic acid (80:20:1, v/v/v) for development of plates and exposure to iodine vapor to reveal the spots. The second fraction was analyzed in an HPSEC chromatographic system consisting on a 7725i Rheodyne injector with 10 μL sample loop (Waters, Milford, MA, USA), a Waters 510 pump (Waters, Milford, MA, USA) and a Waters 2414 refractive index detector (Waters, Milford, MA, USA). The separation was performed on two 100 and 500 Å PLgel columns (25 cm x 0.77 cm i.d.) packed with porous, highly cross-linked styrene-divinylbenzene copolymers (film thickness 5 μm) (Agilent Technologies, Palo Alto, CA, USA) connected in series, with tetrahydrofuran (1 mL/min) as the mobile phase. A Agilent 35900E Interface and HP ChemStation software (Agilent Technologies, Palo Alto, CA, USA) were used for data acquisition. The peaks resolved by HPSEC corresponding to oxidation compounds were oxidized triacylglycerol monomers, dimers ans polymers. Determinations were performed in duplicate.

254

255 **FTIR-Attenuated Total Reflectance (ATR) spectroscopy**

256 Infrared spectra between 4000 and 650 $cm^{-1}$ were recorded using a Perkin Elmer
257 Spectrum 400 Infrared Spectrometer (Perkin Elmer Inc, Waltham, MA, USA) equipped
258 with an ATR prism crystal accessory. The spectral resolution was 4 $cm^{-1}$. Measurements
259 were performed at room temperature using approximately 25 mg of the extracted lipids,
260 which were placed on the surface of the ATR crystal, and pressed with a flat-tip plunger
261 until spectra with suitable peaks were obtained. All experiments were performed at least
262 in duplicate. Background was subtracted using the Spectrum software version 6.3.2
263 (Perkin Elmer Inc.).

264

265 **Antioxidant capacity measurement (FRAP assay)**

266 The ferric reducing/antioxidant power (FRAP) assay was used as a measure of the
267 antioxidant capacity of the muscle, following the method of Benzie and Strain (1996).
268 The method is based on increased absorbance at 595 nm due to formation of the
269 tripyridyltriazine (TPTZ)-Fe(II) complex in the presence of tissue reducing agents.
270 Absorbance values were read at 595 nm after 30 min. Results were expressed as μmol
271 $FeSO_4 \cdot 7H_20$ equivalents/g of muscle, based on a standard curve for ferrous iron
272 prepared in advance. All determinations were performed at least in triplicate.

273

274 **RESULTS AND DISCUSSION**

275

276 The lipid content of the minced muscle was about 1.4 %. Similar values were found in
277 mackerel from Islas Baleares (Spain) in March 2002 (Gómez-Guillén, Montero, Solas &
278 Pérez-Mateos, 2005). It is within the usual range for horse mackerel, taking into account
279 that lipid levels vary seasonally (1.4-7.5 % in Bandarra et al., 2001). Lipids were mostly

280 comprised of triacylglycerols (89.9%), phospholipids accounted for only 3.8% and the
281 remainder (essentially diacylglycerols, free fatty acids and cholesterol) summed up to
282 6.3%. Fatty acid composition of total lipids was as follows: C14:0, 2.30%; C16:0,
283 19.51%; C16:1, 3.79%; C18:0, 8.53%; C18:1, 17.44%; C18:2, 2.03%; C18:3, 0.49%;
284 C18:4, 0.60%; C20:0, 0.28%; C20:1, 0.77%; C20:4, 0.34%; C20:5, 5.04%; C22:5,
285 1.98% and C22:6, 22.02%. Values were within similar ranges as those previously
286 reported for horse mackerel lipids (Bandarra et al., 2001; Eymard, Baron & Jacobsen,
287 2009). As expected, high levels of polyunsaturated fatty acids (30.46%) were found,
288 particularly docosahexanoic acid (DHA, C22:6ω3).
289 Crude lipids extracted from horse mackerel were also characterized by FTIR (Figure 1).
290 Generally, the FTIR spectrum exhibited similar regions of functional groups vibrations
291 as reported previously for farmed salmon fillets lipids (Guillén et al., 2004), sardine
292 muscle lipids (Chaijan et al., 2006), as well as for some vegetable oils (Vlachos,
293 Skopelitis, Psaroudaki, Konstantinidou, Chatzilazarou & Tegou, 2006), although slight
294 differences were observed in the frequency values at which some main IR-absorption
295 bands appeared. The weak band associated with the overtone of the glyceride ester
296 carbonyl absorption showed a maximum absorbance near 3473 $cm^{-1}$ (near 3470 $cm^{-1}$ in
297 salmon lipid). The peak at 3012 $cm^{-1}$, related to the C-H stretching vibration of the *cis*-
298 double bond (=CH), appeared at the same wavenumber in salmon lipids (Guillén et al.,
299 2004). Two main peaks at 2922 $cm^{-1}$ and 2853 $cm^{-1}$ (2925 $cm^{-1}$ and 2854 $cm^{-1}$ in salmon
300 lipids) were attributed to the symmetric and asymmetric stretching vibration of the
301 aliphatic $CH_2$ group. The stretching vibration band assignable to the C=O group of
302 triglycerides was found at around 1743 $cm^{-1}$, whereas values of 1746 $cm^{-1}$ and 1741 cm-
303 $^{1}$ were reported in salmon and in sardine lipids, respectively (Guillén et al., 2004;
304 Chaijan et al., 2006). As described for salmon and sardine, a small band was visible at
305 1655 $cm^{-1}$, assignable to C=C stretching vibration of *cis*-olefins. The bands associated

306 with the fingerprint region at frequency values below 1600 $cm^{-1}$ were, generally
307 speaking, similar to those reported for lipids from different sources. However, it should
308 be noted that the peak at 1147 $cm^{-1}$ was reported to appear at higher wavenumbers in
309 salmon lipids (1160 $cm^{-1}$) (Guillén et al., 2004) and also in vegetable oils (1163 $cm^{-1}$)
310 (Vlachos et al., 2006). The deconvolution of this band showed that it was the result of
311 the overlapping of several minor peaks appearing between 1167 and 1143 $cm^{-1}$ (data not
312 shown). According to Guillén and Cabo (1997), the peaks at 1236 and 1147 $cm^{-1}$ would
313 be associated with the stretching vibration of the C-O ester group and with the bending
314 vibration of the $CH_2$ group, both related to the proportion of saturated acyl groups in oil
315 samples.
316
317 Figure 2 shows evolution of **peroxide values** in lipid samples extracted from patties
318 during frozen storage. The last sample corresponds to 4-days chilling following 240
319 days of frozen storage. Initial values were very low (3.6 meq $O_2$/kg on average). During
320 frozen storage, values were generally much greater for C at all sampling points, while
321 similar values were found for V and F up to 120 days, thus showing that the protective
322 effect of the film was comparable to that of vacuum. At 180 days, PV of F increased
323 considerably and, after that, PV tended to decrease in C and F as a consequence of
324 formation of secondary oxidation products. Exceptionally, PV of V, which had
325 remained at low levels during frozen storage, increased after thawing and 4 days-
326 chilling, mainly as a consequence of exposure to air. Peroxide value is a good indicator
327 of lipid oxidation during early stages and is broadly applied in frozen fish (Saeed &
328 Howell, 2002; Aranda et al., 2005; Pourashouri et al., 2009; Park, Hwang, Kim & Kim,
329 2009; Medina et al., 2009). However, as it is clearly reflected in this study, PV does not
330 provide information once formation of secondary products is favoured and hence is
331 usually applied in combination with other indexes.

Figure 3 shows evolution of **TBARS** values during frozen storage and after 4-days chilling following 240 days of frozen storage. Initial values were very low in all samples. V gave always the lowest values while C and F showed similar values except for the first period (15-30 days) when film seemed to exert an antioxidative effect. After this, TBARS values increased in F and C, reaching the highest values at 180 days. Sánchez-Alonso, Jiménez-Escrig, Saura-Calixto and Borderías (2007) reported TBARS values in minced horse mackerel within a similar range (1.5 to 3 mg MDA/kg muscle) during frozen storage at -20°C. Also, Aubourg, Piñeiro and González (2004) found similar increases in TBA values of horse mackerel fillets kept at -20 °C (from an initial value of 0.17 mg MDA/kg fish to 2.37 mg after 12 months of storage). In the present study, a decrease in TBA values occurred at 240 days of frozen storage, probably due to thiobarbituric acid (TBA)-reactive substances cross-linking with proteins producing protein aggregation and toughening of fish meat (Saeed, Fawthrop & Howell, 1999). TBARS values increased significantly in all the frozen-thawed samples after 4 days-chilling. In V, TBARS values reached 7.97 mg/kg after chilled storage, a ten-fold increase was observed as a consequence of the increase in temperature and availability of oxygen after breaking the vacuum. F and C reached values of 11.87 and 12.53 mg/kg, respectively; therefore, there was a two-fold increase in comparison with the highest levels obtained during the frozen storage. Consequently, no protective effect of film was shown at this point according to TBARS values.

Figure 4 shows evolution of **polyene ratio**, which is an indirect measure of oxidation since it reflects the loss of ω-3 PUFAs (C20:5 and C22:6) as a consequence of oxidation. For calculation of such losses, values for the major saturated fatty acid (C16:0), which remains at initial levels, are considered in polyene ratio. No significant

358 changes were observed in polyene ratio, initially 139, for any of the samples during the
359 frozen storage period up to 240 days, when a significant reduction was shown by C.
360 Clearly, polyene ratio was not sensitive enough to detect changes until oxidation
361 reached high levels, as has been previously reported in fish oil samples (Márquez-Ruiz
362 et al., 2000). However, it is a useful measure at advanced stages of oxidation, as it
363 occurred in this study for samples after thawing and 4-days chilling. Both C and V were
364 significantly more oxidized than F. The drastic drop in polyene ratio in V after thawing
365 and 4-days chilling, together with the clear differentiation between either V or C and F,
366 clearly showed that film was highly protective under such conditions.
367
368
369 Table 1 shows the volatile compounds most representative of lipid oxidation in fish
370 muscle according to previous works carried out in horse mackerel (Iglesias & Medina,
371 2008; Medina et al., 2009; Eymard et al., 2009). This table includes results obtained at
372 early oxidation (15 days), advanced oxidation (240 days) and following thawing and 4-
373 days chilled storage (240+4R). No consistent changes were observed in volatile
374 formation along the frozen storage period up to 240 days, when a remarkable increase
375 of heptanal/4-heptenal, 1-octen-3-ol, 2,4-heptadienal and nonanal was found in all the
376 samples. Furthermore, hexanal and octanal noticeably increased in C whereas similar
377 values were found in volatiles between V and F, except in the case of 1-penten-3-ol.
378 The unexpectedly high values observed in F at the beginning of the frozen storage (15
379 days) in 1-pente-3-ol, hexanal and 1-octen-3-ol, together with the high content of 1-
380 penten-3-ol at 240 days, may be in part related to the volatile release from the film
381 and/or the partial retention of volatiles by the film. After thawing and 4 days-chilling,
382 similar values were found in volatiles between C and F, whereas V remained at the
383 lowest values for most volatiles selected. The compound 2-methyl-2-pentenal, although

384 detected throughout the frozen storage, showed an increase in all the samples after four
385 days of chilled storage.
386
387 Table 2 shows quantitation of total **oxidized triacylglycerols** and their distribution in
388 monomers, dimers and polymers throughout storage. Given that lipid samples were
389 mostly composed of triacylglycerols, quantitative determination of oxidized
390 triacylglycerols, thus including virtually all non-volatile oxidation compounds formed,
391 constitutes a good approach to evaluate the progress of oxidation. During early stages of
392 oxidation, only oxidized triacylglycerol monomers increased as they are mostly
393 hydroperoxides (Márquez-Ruiz & Dobarganes, 2005; 2006). Therefore, results found up
394 to 180 days were consistent with those corresponding to PVs. In fact, high correlations
395 between oxidized triacylglycerol monomers and PV during the induction period have
396 been reported elsewhere (Martín-Polvillo et al, 2004). Once oxidation accelerates,
397 marked by formation of dimers, levels of oxTGM kept on increasing in contrast to PV,
398 since oxTGM also include triacylglycerols bearing secondary oxygenated functions,
399 such as hydroxy, epoxy, aldehyde and ketone functions. Before 180 days, V showed the
400 lowest oxTGM values, followed by F and C. Detection of dimers occurred at 120 days
401 for C, and 180 days for F, while only after thawing and 4 days-chilling were detected in
402 V. Formation of dimers and polymers is favoured in highly unsaturated lipids, such as
403 fish oils, even at low temperatures, due to the high instability of polyunsaturated
404 hydroperoxides (Márquez-Ruiz & Dobarganes, 2006). For this reason, PV is not as
405 useful to monitor oxidation in polyunsaturated oils as it is for vegetable oils. Through
406 concomitant quantitation of oxidized triacylglycerol monomers, dimers and polymers a
407 direct and complete determination of primary and secondary non-volatile oxidation
408 products can be achieved, thus allowing to monitor all the oxidation progress and to
409 obtain an objective, quantitative measurement of the level of oxidation.

410

411 At the end of the frozen storage period (240 days) it was clear that V were scarcely
412 oxidized (7.3 mg/g of total oxidized triacylglycerols) while C had a considerably level
413 of oxidation (64.5 mg/g of total oxidized triacylglycerols), including the presence of
414 polymerized triacylglycerols. As to F, oxidation was much higher than in V (48.3 mg/g
415 of total oxidized triacylglycerols) but still lower than that of C (64.5 mg/g of total
416 oxidized triacylglycerols). The influence of thawing and 4-days chilling at air exposure
417 was outstanding for V since their level of oxidation doubled that of F and even
418 polymers, absent in F, were found in considerable amounts (14.5 mg/g). Oxidation also
419 increased substantially (three-fold) in C in contrast to F, which undoubtedly were the
420 least oxidized samples at this point. Values of total oxidized triacylglycerols at
421 advanced oxidation points, namely, 240 days frozen and after 4-days chilling, were
422 consistent with polyene ratios, clearly showing that oxidation level was in the order C >
423 F > V and C > V > F, respectively. Clearly, film showed great protective effects under
424 the most drastic conditions of oxidation (chilling temperature and exposure to air
425 oxygen).

426

427 Figure 5 shows the effect of frozen storage on selected IR regions taken from the
428 spectra of lipids extracted from C. The IR-spectra remained practically unaltered after
429 60 days of frozen storage. However, they exhibited a noticeably increased absorbance in
430 the region between 3600-3100 $cm^{-1}$ after 120 days of frozen storage (Fig. 5A), owing to
431 the absorptions caused by hydroperoxides generated in the oxidation process (Guillén et
432 al., 2004; Chaijan et al., 2006). The absorbance of this band became less intense at day
433 180, and practically returned to initial levels at the end of the storage period (day 240),
434 suggesting the decomposition of hydroperoxide to yield secondary lipid oxidation
435 products. In contrast to the findings reported in salmon lipids by Guillén & Cabo (2004)

436 or in several vegetable oils by Moya Moreno, Mendoza Olivares, Amézquita López,
437 Gimeno Adelantado and Bosch Reig (1999) or Vlachos et al. (2006), a reduction in the
438 degree of lipid unsaturation due to an eventual oxidative process during the storage
439 period could not be evidenced by monitoring the peak at 3012 $cm^{-1}$ (Fig. 5B). However,
440 the bands at 2922 and 2853 $cm^{-1}$ showed a reduced absorbance at days 120 and 180 of
441 frozen storage. Similarly, the shoulder at 2955 $cm^{-1}$ was also slightly reduced at the
442 same sampling time. These findings indicated an appreciable lowering of the
443 concentration of $CH_3$ and $CH_2$ functional groups in this sample. Vlachos et al. (2006)
444 also reported a reduction in the absorbance of these bands as related to oxidative
445 changes in vegetable oils. Changes in the carbonyl absorption of the triglyceride ester
446 linkage at around 1743 $cm^{-1}$ were reported as a main FTIR event denoting lipid
447 oxidation. A decrease in the absorbance at this wavenumber was visible after 15 days of
448 frozen storage, however, the more pronounced drop took place at day 120 of storage
449 (Fig. 5C), showing a clear broadening of the band to lower wavenumbers. According to
450 Guillén et al. (2004), these changes are produced by the appearance of aldehydes and
451 ketones, which are secondary oxidation products derived from the degradation of the
452 hydroperoxides, whose absorbance is near 1728 $cm^{-1}$ causing overlapping with the
453 stretching vibration at 1743 $cm^{-1}$ of the ester carbonyl group of the triglycerides. The
454 broadening in the frequency range of 1700-1726 $cm^{-1}$ largely suggested the formation of
455 new carbonyls from initial aldehyde and ketone compounds (Vlachos et al., 2006). The
456 IR-absorbance between 1743 and 1765 $cm^{-1}$, attributed to the esters of the triglycerides
457 exclusively, was considerably reduced at these sampling days (120 and 180), indicating
458 the lowering of these molecules as lipid oxidation proceeded.
459
460 As described above, the peak at 1655 $cm^{-1}$ was attributed to C=C stretching vibrations
461 of *cis*-olefins. With the oxidative process, one would expect a lowering of this

462 functional group by reduction of the lipid unsaturation degree. This happened during the
463 first 60 days of storage (Fig. 5D). However, the spectral region between 1600 and 1680
464 $cm^{-1}$ showed a noticeably increased absorbance after 120 days of storage, being less
465 intense after 180 and 240 days. According to Hayati, Man, Tan and Aini (2005),
466 spectrum alteration within band 1650-1600 $cm^{-1}$ could be the result of the formation of
467 conjugated double bonds, whose absorption would be overlapped with that of O-H
468 bending, also assignable to this spectral region, both signals coming from
469 hydroperoxides accumulation. These findings are in agreement to the changes described
470 above in the 3100-3700 $cm^{-1}$ region. The frequency of the bands appearing at
471 wavenumbers below 1600 $cm^{-1}$ did not show a definite variation as regard to storage
472 time, however the spectral region between 1280 and 1050 $cm^{-1}$ was slightly altered at
473 days 120 and 180 of frozen storage (Fig 5E). The absorbance of the peaks at 1147 and
474 1116 $cm^{-1}$ showed a clear tendency to decrease at days 120 and 180. Both peaks are
475 related to the proportion of saturated acyl groups. Specifically the latter, associated with
476 the stretching vibration of the C-O group in esters, was reported to be inversely related
477 to the proportion of saturated acyl groups in oil samples (Guillén & Cabo, 1997). The
478 observed lowering in the concentration of C-O, $CH_2$ as well as $CH_3$ functional groups
479 could be related with the production of dimers and polymers of triglycerides in the more
480 advanced stages of lipid oxidation.
481
482 From these results, it seems that the main FTIR changes started to be clearly perceptible
483 at day 120 of frozen storage. However, from day 180 upwards, changes in the main
484 FTIR events were not clearly consistent with accumulation of lipid oxidation
485 compounds (Table 2), especially at day 240, were peak absorbances turned towards
486 higher values. This effect could be attributed to changes in colour and consistency of the
487 extracted fat becoming eye-visible from day 120 onwards, making difficult a proper

488 comparison in the later stages of storage. Therefore, spectra at 120 days were selected to
489 compare samples (Figure 6). At this sampling time, C exhibited a noticeable increase in
490 the absorbance between 3600-3100 $cm^{-1}$ as well as between 1680-1600 $cm^{-1}$ (Fig. 6A
491 and D), as compared to F and V. As described above, both events could be related to
492 accumulation of hydroperoxides. C also showed a reduced intensity of the bands at
493 2922 and 2853 $cm^{-1}$ (Fig 6B) as compared to the other samples, as well as in the
494 spectral region between 1280 and 1050 $cm^{-1}$ (Fig. 6E), being indicative of the lowering
495 in the amount of C-O and $CH_2$ functional groups. This was in agreement with the
496 increase in the concentration of total oxidized triacylglycerols, which was only
497 perceptible at this sampling time in C (Table 2). Similarly, changes in the band at 1743
498 $cm^{-1}$ were much more evident in C, followed by F and V (Fig. 6C). These results
499 confirmed the protective effect of the borage film against lipid oxidation, in a similar
500 way to the vacuum packing system, at least for the first 120 days of frozen storage.
501
502 When comparing the IR-spectra of lipids extracted from the patties which had been
503 frozen stored for 240 days (C240, V240 and F240) with those that were subsequently
504 thawed at this sampling day and chilled stored for 4 days ($C_{4r,}$ $V_{4r}$ and $F_{4r}$), slight
505 differences could be observed, even though, as already noted, the physical properties of
506 the extracted lipids had changed. The absorbance at 3012 $cm^{-1}$, which was higher in
507 V240, revealed some differences in the level of lipid unsaturation among the studied
508 samples (Fig. 7A). Whereas both C and V exhibited a decrease in the height of the 3012
509 $cm^{-1}$ peak after 4 days of refrigerated storage, F remained practically unchanged.
510 Similarly, a slight reduction in the frequency values of this band in $C_{4r}$ and $V_{4r}$ was
511 found, indicating the disappearance of cis olefinic double bonds in these samples, in
512 contrast to the $F_{4r}$. Results were consistent with values found for oxidized
513 triacylglycerols (Table 2). The 1743 $cm^{-1}$ band did not show definite changes

attributable to accumulation of aldehydes and ketones as a result of chilled storage (data not shown), however, the increased absorbance in the region between 1680 and 1652 $cm^{-1}$ in $C_{4r}$ and $V_{4r}$, followed by $F_{4r}$ (Fig. 7B) could be largely related to the production of conjugated double bonds, as well as *trans* C=C groups. No definite changes in absorbance or frequency values in the fingerprint region below 1600 $cm^{-1}$ regarding lipid oxidation during the chilling period were observed (data not shown).

The **antioxidant capacity** of the patties during the frozen storage and subsequent chilled storage was evaluated on the basis of ferric reducing capacity (FRAP) (Figure 8). Both C and V showed a low ferric reducing capacity at the beginning of the frozen storage, which remained practically unchanged during the whole frozen storage. This low antioxidant capacity may be attributed to the endogenous antioxidant system, which includes compounds such as α-tocopherol, ubiquinone or glutathione and is largely consumed with time post mortem (Aubourg et al., 2004; Pazos et al., 2005b).

Applying a complex film to a foodstuff can result in migration of compounds from the film into the food (Chi, Zivanovic & Penfield, 2006). Thus, the reducing ability of F noticeably increased until day 30 of frozen storage most likely as a consequence of the diffusion of phenolic compounds from the borage films to the patties, increasing the antioxidant capacity of the muscle. However, the FRAP values sharply decreased from day 30 to 120 and remained stable until the end of the frozen storage, probably due to the role of phenolics in lipid oxidation, which consequently involves a reduction of the availability of the antioxidant compounds as well as the antioxidant capacity of muscle in spite of the migration from the films. In fact, the decrease of FRAP values from 30 to 120 days in F is consistent with the formation of significant amounts of oxidation products from 120 days onwards (Table 2). When comparing the reducing activity of both C and V frozen for 240 days with those that were thawed and chilled-stored for 4

days, only slight differences could be observed. However, around a two-fold increase of reducing activity was shown in F after thawing and subsequent chilled storage, probably due to an increase of the migration rate of phenolic compounds from the film to the muscle. The physical state of fish muscle may affect both the diffusion and effectiveness of the antioxidant compounds (Medina et al., 2009). At low temperatures the rate of diffusion is lower due to a higher resistance of tissues to mass transfer (Ramesh & Duda, 2001). In frozen systems, the non-frozen fraction may be highly viscous and diffusion may become limited.

In a previous work (Gómez-Estaca et al., 2009) ferric ion reducing ability of gelatin borage-films was evaluated. According to these authors, the incorporation of borage extract to gelatin films gave rise to a high reducing ability, with FRAP values around 800 μmol $FeSO_4$ eq/g film. The borage extract used in our study was obtained from the same borage seeds batch, prepared in the same way and incorporated to the film solution at the same ratio. Therefore, we can deduce that the diffusion of phenolic compounds from the film to the patties is quite low, both in the frozen and chilled storage, given the differences between the FRAP values found in the borage films and the patties.

Considering all results obtained in this study by different analytical approaches, some interesting, general considerations and overall conclusions have been attained. First, difficulties to evaluate lipid oxidation have been clearly reflected in the lack of consistent comparative results between samples at early stages of oxidation or between methods which provide measurements of either primary or secondary oxidation products. This is not strange since methods applied differ greatly in sensitivity and give information of very different compounds formed during the course of oxidation. It is important to note that PV, polyene ratio, oxidized triacylglycerols and IR-spectra were

566 determined in extracted oils while TBARS and volatiles were evaluated directly in the
567 patties, which could have contributed to the different results obtained in some cases.
568 Advantages of determining TBARS and volatiles directly in muscle include avoiding
569 time- and solvent-consuming extraction of lipids while, otherwise, the complexity of the
570 samples evaluated and possible interference sources may add difficulties to
571 determinations of minor compounds. For example, TBARS is based on detection of
572 malondialdehyde, which levels are very low (measured as μmol/g lipid) in comparison
573 with other oxidation products, such as oxidized triacylglycerols (quantitated as mg/g
574 lipid) and, if determined in muscle lipids, other compounds can also react with
575 thiobarbituric acid to give the red chromogen or interfering by forming orange and
576 yellow pigments (Guillén-Sans & Guzmán-Chozas, 1998; Oussalah, Caillet, Salmiéri,
577 Saucier & Lacroix, 2004; Alghazeer, Saeed & Howell, 2008). With respect to volatiles,
578 analysis could be disturbed by the gelatine film or vacuum package, due to compound
579 diffusion from the film to the patties, thus devaluating the results.
580
581 Overall, it was generally observed, and particularly clear in results obtained in extracted
582 lipids, that film had protective effects on lipid oxidation of horse mackerel patties, since
583 F showed lower oxidation levels than C throughout the frozen storage and especially
584 after thawing and chilled storage. Moreover, the protective effect of film against lipid
585 oxidation was similar to that of vacuum packaging except at advanced stages of
586 oxidation during frozen storage, but with the additional advantage of exerting further
587 protection under conditions simulating preparation of patties for consumption and
588 providing extra antioxidant activity to the patties.
589
590 **Acknowledgements**

591 This work was supported by Projects AGL2005-02380/ALI, AGL2007-62922/ALI and
592 AGL2008-00231/ALI (Spanish Ministry of Science and Innovation).

593 **References**

594 Alghazeer, R, Saeed, S., & Howell, N.K. (2008). Aldehyde formation in frozen
595 mackerel (*Scomber scombrus*) in the presence and absence of instant green tea. *Food*
596 *Chemistry*, 108, 801-810.
597 Aranda, M., Mendoza, N., & Villegas, R. (2005). Lipid damage during frozen storage of
598 whole jack mackerel (*Trachurrus symmetricus murphyi*). *Journal of Food Lipids*, 13,
599 155-166.
600 Aubourg, S.P., Piñeiro, C., & González, M.J. (2004). Quality loss related to rancidity
601 development during frozen storage of horse mackerel (*Trachurus trachurus*). *Journal of*
602 *American Oil Chemists' Society*, 81(7), 671-678.
603 Bandarra, N.M., Batista, I., Nunes, M.L., & Empis, J.E. (2001). Seasonal variation in
604 the chemical composition of horse-mackerel (*Trachurus trachurus*). *European Food*
605 *Research and Technology*, 212, 535-539.
606 Benzie, I.F.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a
607 measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-
608 76.
609 Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and
610 purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.
611 Botsoglou, N. A., Fletouris, D. J., Papageorgiu, G. E., Vassilopoulos, V.N., Mantis, A.
612 J., & Trakatellis, A. G. (1994). Rapid, sensitive and specific thiobarbituric acid method
613 for measuring lipid peroxidation in animal tissue, food and feedstuff samples. *Journal*
614 *of Agricultural and Food Chemistry*, 42, 1931–1937.

615 Bower, C.K., Avena-Bustillos, R.J., Olsen, C.W., McHugh, T.H., & Bechtel, P.J.
616 (2006). Characterization of fish-skin gelatin gels and films containing the antimicrobial
617 enzyme lysozyme. *Journal of Food Science,* 71(5), M141-M145.
618 Boyd, L.C., Green, D.P., Giesbrecht, F.B., & King, M.F. (1993). Inhibition of oxidative
619 rancidity in frozen cooked fish flakes by tert-butylhydroquinone and rosemary extract.
620 *Journal of the Science of Food and Agriculture,* 61, 87-93.
621 Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2006). Changes of lipids
622 in sardine (*Sardinella gibbosa*) muscle during iced storage. *Food Chemistry*, 99, 83-81.
623 Chi, S., Zivanovic, S., & Penfield, M. P. (2006). Application of chitosan films enriched
624 with oregano essential oil on Bologna-Active compounds and sensory attributes. *Food*
625 *Science and Technology International*, 12, 111–117.
626 Dobarganes, C., & Márquez-Ruiz, G. (2003). Oxidized fats in foods. *Current Opinion*
627 *in Clinical Nutrition and Metabolic Care*, 6(2), 157-163.
628 Eymard, S., Baron, C.P., & Jacobsen, C. (2009). Oxidation of lipid and protein in horse
629 mackerel (*Trachurus trachurus*) mince and washed minces during processing and
630 storage. *Food Chemistry*, 114, 57-65.
631 Frankel, E.N. (2005). *Lipid oxidation*. Bridgewater, England: The Oily Press.
632 Gómez-Estaca, J., Montero, P., Giménez, B., & Gómez-Guillén, M. C. (2007). Effect of
633 functional edible films and high pressure processing on microbial and oxidative
634 spoilage in cold-smoked sardine (*Sardina pilchardus*). *Food Chemistry*, 105(2), 511-
635 520.
636 Gómez-Estaca, J., Giménez. B., Montero, P., & Gómez-Guillén, M.C. (2009).
637 Incorporation of antioxidant borage extract into edible films based on sole skin gelatin
638 or a commercial fish gelatin. *Journal of Food Engineering*, 92, 78-85.
639 Gómez-Guillén, M.C., Montero, P., Solas, M.T., & Pérez-Mateos, M. (2005). Effect of
640 chitosan and microbial transglutaminase on the gel forming ability of horse mackerel

641 (*Trachurus* spp.) muscle under high pressure. *Food Research International*, 38(1), 103-
642 110.
643 Gómez-Guillén, M. C., Ihl, M., Bifani, V., Silva, A., & Montero, P. (2007). Edible films
644 made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes
645 leaves (*Ugni molinae Turcz*). *Food Hydrocolloids*, 21(7), 1133-1143.
646 Guillen, M. D., & Cabo, N. (1997). Infrared spectroscopy in the study of edible oils and
647 fats. *Journal of the science of food and agriculture*, 75(1), 1-11.
648 Guillén, M. D. & Cabo, N. (2000). Some of the most significant changes in the Fourier
649 transform infrared spectra of edible oils under oxidative conditions. *Journal of the*
650 *Science of Food and Agriculture*, 80, 2028-2036.
651 Guillén, M.D. & Cabo, N. (2004). Study of the effects of smoke flavourings on the
652 oxidative stability of the lipids of pork adipose tissue by means of Fourier transform
653 infrared spectroscopy. *Meat Science*, 66, 647-657.
654 Guillén, M.D., Ruiz, A., & Cabo, N. (2004). Study of the oxidative degradation of
655 farmed salmon lipids by jeans of Fourier transform infrared spectroscopy. Influence of
656 salting. *Journal of the Science of Food and Agriculture*, 84, 1528-1534.
657 Guillén-Sans, R., & Guzmán-Chozas, M. (1998). The thiobarbituric acid (TBA)
658 reaction in foods: a review. *Critical Review in Food Science and Nutrition*, 38, 315-330.
659 Hayati, I. N., Man, Y. B., Tan, C. P., & Aini, I. N. (2005). Monitoring peroxide value in
660 oxidized emulsions by Fourier transform infrared spectroscopy. *European Journal of*
661 *Lipid Science and Technology*, 107(12), 886-895.
662 Iglesias, J., & Medina, I. (2008). Solid-phase microextraction method for the
663 determination of volatile compounds associated to oxidation of fish muscle. *Journal of*
664 *Chromatography A*, 1192, 9-16.
665 IUPAC (1992a) Method 2.301. Preparation of the fatty acid methyl esters. Standard
666 methods for the analysis of oils, fats and derivatives, 7th edn. Pergamon, Oxford.

667 IUPAC (1992b) Method 2.302. Gas-liquid chromatography of fatty acid methyl esters.
668 Standard methods for the analysis of oils, fats and derivatives, 7th edn. Pergamon,
669 Oxford.
670 López-Caballero, M. E., Gómez-Guillén, M. C., Pérez-Mateos, M., & Montero, P.
671 (2005). A chitosan-gelatin blend as a coating for fish patties. *Food Hydrocolloids*,
672 19(2), 303-311.
673 Mahmoudzadeh, M., Motallebi, A., Hosseini, H., Khaksar, R., Ahmadi, H., Jenab, E., et
674 al. (2010). Quality changes of fish burgers prepared from deep flounder
675 (*Pseudorhombus elvatus* Ogilby, 1912) with and without coating during frozen storage
676 (-18 ºC). *International Journal of Food Science and Technology*, 45, 374-379.
677 Márquez-Ruiz, G., & Dobarganes, M.C. (2005). Analysis of non-volatile lipid oxidation
678 compounds by high-performance size-exclusion chromatography. In A. Kamal-Eldin &
679 J. Pokorny (Eds.), *Analysis of Lipid Oxidation* (pp. 40-69). Champaign, IL, USA:
680 American Oil Chemists Society.
681 Márquez-Ruiz, G., Jorge, N., Martín-Polvillo, M., & Dobarganes, M.C. (1996) Rapid,
682 quantitative determination of polar compounds in fats and oils by solid-phase extraction
683 and exclusion chromatography using monoestearin as internal standard. *Journal of*
684 *Chromatography A*, 749, 55-60.
685 Márquez-Ruiz, G., & Dobarganes, M.C. (2006). HPSEC in lipid analysis. In M.M.
686 Mossoba, J.K.çG. Kramer, J.T. Brenna, R.E and McDonald (Eds.), *Lipid Analysis and*
687 *Lipidomics: New Techniques and Applications* (pp. 205-238). Champaign, IL, USA:
688 American Oil Chemists Society.
689 Márquez-Ruiz, G, Velasco, J., & Dobarganes, C. (2000). Evaluation of oxidation in
690 dried microencapsulated fish oils by a combination of adsorption and size exclusion
691 chromatography. *European Food Research and Technology*, 211, 13-18.

692 Martín-Polvillo, M., Márquez-Ruiz, G., & Dobarganes, M.C. (2004). Oxidative stability
693 of sunflower oils differing in unsaturation degree during long term storage at room
694 temperature. *Journal of American Oil Chemists' Society*, 81, 577-583.
695 Mbarki, R., Sadok, S., & Barkallah, I. (2009). Quality changes of the Mediterranean
696 horse mackerel (*Trachurus mediterraneus*) during chilled storage: The effect of low-
697 dose gamma irradiation. *Radiation Physics and Chemistry*, 78, 288-292.
698 Medina, I., González, M.J., Iglesias, J., & Hedges, N.D. (2009). Effect of
699 hydroxycinnamic acids on lipid oxidation and protein changes as well as water holding
700 capacity in frozen minced horse mackerel white muscle. *Food Chemistry*, 114, 881-888.
701 Moya Moreno, M.C.M., Mendoza Olivares, D., Amézquita López, F.J., Gimeno
702 Adelantado, J.V., & Bosch Reig, F. (1999). Analytical evaluation of polyunsaturated
703 fatty acids degradation during thermal oxidation of edible oils by Fourier transform
704 infrared spectroscopy. *Talanta,* 50(2), 269-275.
705 Oussalah, M., Caillet, S., Salmiéri, S., Saucier, L., & Lacroix, M. (2004). Antimicrobial
706 and antioxidant effects of milk protein-based film containing essential oils for the
707 preservation of whole beef muscle. *Journal of Agricultural and Food Chemistry*, 52,
708 5598-5605.
709 Park, J.N., Hwang, K.T., Kim, S.B., & Kim, S.Z. (2009). Partial replacement of NaCl
710 by KCl in salted mackerel (*Scomber japonicus*) fillet products: effect on sensory
711 acceptance and lipid oxidation. *International Journal of Food Science and Technology*,
712 44, 1572-1578.
713 Pazos, M., Sánchez, L., & Medina, I. (2005a). α-tocopherol oxidation in fish muscle
714 during chilling and frozen storage. *Journal of Agriculture and Food Chemistry, 53*,
715 4000-4005.

716 Pazos, M., González, M.J., Gallardo, J.M., Torres, J.L., & Medina, I. (2005b).
717 Preservation of the endogenous antioxidant system of fish muscle by grape polyphenols
718 during frozen storage. *European Food Research and Technology*, 220, 514-519.
719 Pourashouri, P., Shabanpour, B., Aubourg, S.P., Rohi, J.D., & Shabani, A. (2009). An
720 investigation of rancidity inhibition during frozen storage of Wels catfish (*Silurus*
721 *glanis*) fillets by previous ascorbic and citric acid treatment. *International Journal of*
722 *Food Science and Technology*, 44, 1503-1509.
723 Quitral, V., Donoso, M.L., Ortiz, J., Herrera, M.V., Araya, H., & Aubourg, S.P. (2009).
724 Chemical changes during the chilled storage of Chilean jack mackerel (*Trachurus*
725 *murphyi*): Effect of a plant-extract icing system. *LWT - Food Science and Technology*,
726 42, 1450-1454.
727 Ramesh, N., & Duda, J.L. (2001). Predicting migration of trace amounts of styrene in
728 poly(styrene) below the glass transition temperature. *Food and Chemical Toxicology*,
729 39(4), 355-360.
730 Richards, M.P., & Hultin, H.O. (2002). Contribution of blood and blood components to
731 lipid oxidation in fish muscle. *Journal of Agricultural and Food Chemistry*, 50, 555-
732 564.
733 Ross, C.F., & Smith, D.M. (2006). Use of volatiles as indicators of lipid oxidation in
734 muscle foods. *Comprehensive Reviews in Food Science and Food Safety*, 5, 18-25.
735 Saeed, S., & Howell, N.K. (2002). Effect of lipid oxidation and frozen storage on
736 muscle proteins of Atlantic mackerel (*Scomber scombrus*). *Journal of the Science of*
737 *Food and Agriculture*, 82, 579-586.
738 Saeed, S., Fawthrop, S.A., & Howell, N.K. (1999). Electron spin resonance (ESR) study
739 on free radical transfer in fish lipid–protein interaction. *Journal of the Science of Food*
740 *and Agriculture*, 79, 1809–1816.

741 Sánchez-Alonso, I., Jiménez-Escrig, A., Saura-Calixto, F., & Borderías, A.J. (2007).
742 Effect of grape antioxidant dietary fibre on the prevention of lipid oxidation in minced
743 fish: Evaluation by different methodologies. *Food Chemistry*, 101, 372-378.
744 Sánchez-Escalante A., Djenane D., Torrescano G., Beltrán J.A., & Roncales P. (2003).
745 Antioxidant action of borage, rosemary, oregano, and ascorbic acid in beef patties
746 packaged in modified atmosphere. *Journal of Food Science*, 68(1), 339–344.
747 Shahidi, F., & Miraliakkbari, H. (2004). Omega-3 (n-3) fatty acids in health and
748 disease: Part 1 – Cardiovascular disease and cancer. *Journal of Medicinal Food*, 7, 387-
749 401.
750 Tang, S.Z., Sheehan, D., Buckley, D.J., Morrissey, P.A., & Kerry, J.P. (2001). Anti-
751 oxidant activity of added tea catechins on lipid oxidation of raw minced red meat,
752 poultry and fish muscle. *International Journal of Food Science and Technology*, *36*,
753 685-392.
754 Tharanathan, R. N. (2003). Biodegradable films and composite coatings: Past, present
755 and future. *Trends in Food Science and Technology*, 14(3), 71-78.
756 Vareltzis, K., Koufidis, D., Gavriilidou, E., Papavergou, E., & Vasiliadou, S. (1997)
757 Effectiveness of a natural rosemary (*Rosmarinus officinalis*) extract on the stability of
758 filleted and minced fish during froze storage. *Food Research and Technology*, 205, 93-
759 96.
760 Velasco, J., Marmesat, S., Dobarganes, C., & Márquez-Ruiz, G. (2006). Heterogeneous
761 aspects of lipid oxidation in dried microencapsulated oils. *Journal of Agricultural and*
762 *Food Chemistry*, 54, 1722-1729.
763 Velasco, J., Holgado, F., Dobarganes, C., & Márquez-Ruiz, G. (2009). Influence of
764 relative humidity on oxidation of the free and encapsulated oil fractions in freeze-dried
765 microencapsulated oils. *Food Research International*, 42, 1492-1500.

766 Vlachos, N., Skopelitis, Y., Psaroudaki, M., Konstantinidou, V., Chatzilazarou, A., &
767 Tegou, E. (2006). Applications of Fourier transform-infrared spectroscopy to edible
768 oils. *Analytica Chimica Acta*, 573, 459-465.
769 Vyncke, W. (1970). Direct determination of the thiobarbituric acid value in
770 trichloroacetic acid extract of fish as a measure of oxidative rancidity. *Fette Seifen*
771 *Anstrichmittel*, 72, 1084–1087.
772 Wettasinghe, M., & Shahidi, F. (1999). Antioxidant and free radical-scavenging
773 properties of ethanolic extracts of defatted borage (*Borago officinalis* L.) seeds. *Food*
774 *Chemistry*, 67, 399–414.
775 Yerlikaya, P., & Gokoglu, N. (2010). Inhibition effects of green tea and grape seed
776 extracts on lipid oxidation in bonito fillets during frozen storage. *International Journal*
777 *of Food Science and Technology*, 45, 252-257.
778

**Legends to Figures**

**Figure 1.-** Representative Fourier transform infrared spectrum of lipids extracted from starting horse mackerel patties.

**Figure 2.-** Evolution of Peroxide Value (PV) in lipids extracted from horse mackerel patties during frozen storage for 240 days, and after thawing and 4 days-chilled storage. Error bars show the standard deviation of two determinations.

**Figure 3.-** Evolution of Thiobarbituric acid index values in horse mackerel patties during frozen storage for 240 days, and after thawing and 4 days-chilled storage. Error bars show the standard deviation of at least three determinations.

**Figure 4.-** Evolution of Polyene Ratio in lipids extracted from horse mackerel patties during frozen storage for 240 days, and after thawing and 4 days-chilled storage. Error bars show the standard deviation of at least two determinations.

**Figure 5.-** Selected regions of Fourier transform infrared spectra of lipids extracted from control horse mackerel patties during frozen storage.

**Figure 6.-** Selected regions of Fourier transform infrared spectra of lipids extracted from horse mackerel patties (C, V, F) after 120 days-frozen storage.

**Figure 7.-** Selected regions of Fourier transform infrared spectra of lipids extracted from horse mackerel patties after 240 days-frozen storage (C240, V240, F240) and following thawing and 4 days-chilled storage (C4r, V4r, F4r).

**Figure 8.-** Ferric reducing ability of horse mackerel patties during frozen storage for 240 days, and after thawing and 4 days-chilled storage. Error bars show the standard deviation of at least three determinations.