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Effect of Packing Media on the Oxidation of Canned Tuna Lipids. Antioxidant Effectiveness of Extra Virgin Olive Oil

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The effectiveness of packing media differing in the content of natural antioxidants has been tested on lipid oxidation occurring during tuna canning. Extra virgin olive oil, having a high content in natural polyphenols, and other filling media lacking phenols (refined olive oil, refined soybean oil, and brine) were selected. The three oils also showed different quantities of tocopherols, the highest amount being detected in refined soybean oil. Different rates of oxidation were observed among the four media after thermal processing and storage of tuna cans; extra virgin olive oil showed a potential antioxidant activity on fish lipids. The verified antioxidant ability may be attributed to the solubilization of hydrophilic phenols into the water−muscle interface. The phenolic composition from extra virgin olive oil studied by reversed-phase HPLC showed a marked change after fish processing, thus suggesting phenol decompositions and strong interactions between oil phenols and fish muscle components. The aqueous environment built by brine made fish lipids more prone to oxidation, presumably due to the accumulation of unsaturated fatty acids at the oil−water interface.

Keywords: *Antioxidants; fish lipid oxidation; canning; packing medium (extra virgin olive oil, refined olive oil, soybean oil, brine); phenols*

INTRODUCTION

Canned tuna is one of the most important fish products in many countries, including Italy and Spain, supporting a significant market demand and playing an important role as components of the Mediterranean diet (Alimarket, 1992). These products are considered of high nutritional quality because of their high proportion of $\omega-3$ polyunsaturated fatty acids (PUFA) (Gallardo et al., 1989; Medina et al., 1995a), which have shown potential benefits to human health, particularly in the prevention of cardiovascular diseases (Carrol and Braden, 1986; Lees and Karel, 1990). Many papers have described the important role that alterations of lipids during food processing have on the quality of the final product (Cheftel and Cheftel, 1976; Pearson et al., 1977). Lipid damage is often focused on the significant number volatiles that can be produced by oxidation of PUFA during thermal treatments of food (Chan, 1987; Hsieh and Kinsella, 1989). Due to the high degree of unsaturated fatty acids present in marine lipids, rancidity as a consequence of lipid degradation is critical in the determination of the shelf life of fatty fish species during storage and processing.

Lipid composition of the processed product can be influenced by raw material composition, process conditions, and packing substrate (Pérez-Camino et al., 1991; Hale and Brown, 1983). Filling medium may produce a different dilution, partial extraction of some components, and different heat transfer in fish muscle as well (Sánchez Muniz et al., 1992; Aubourg et al., 1990). Regarding lypolysis, no differences were found in tuna lipids during canning using oil and brine as filling media (Medina et al., 1995b). However, a different rate of fish lipid oxidation is actually expected depending on the physical state of $\omega-3$ PUFA in the food system, thus involving the packing medium employed in canning (Frankel et al., 1994; Coupland et al., 1996). In this way activities of natural antioxidants in fish oils have been demonstrated to be different in bulk oil or oil-in-water emulsion systems (Frankel et al., 1996).

Oil and brine are two of the most common packing media used in canning manufacturing. Among filling oils employed in the tuna canning industry, virgin olive oil has been demonstrated to contain natural polyphenols having a key role in determining oxidation (Papadopulos and Boskou, 1991; Tsimidou et al., 1992). The antioxidant activity of polyphenols has been attributed to their molecular structural features that enable them to act as free radical acceptors as well as chelators (Xin et al., 1990; Afanas'ev et al., 1989). Studies carried out on control lipid oxidation in fish tissues demonstrated a potent antioxidant activity of some polyphenols comparable to that of butylated hydroxytoluene (BHT) (Ramanathan and Das, 1992). Through the use of synthetic antioxidants, decreased lipid oxidation of storage and processed seafood has been achieved as well (Boyd et al., 1993). However, the use of chemical additives raises questions with regard to food safety and toxicity (Chang et al., 1977). Refined olive oil and other edible seed oils are subjected to a technological treatment involving the loss of natural antioxidants (Solinas and Cichelli, 1982). A different oxidation of fish lipids could be expected depending on the antioxidant content and stability of the packing oils employed.

The aim of this work was to study the effect of different packing media on the oxidation of lipids

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occurring during tuna canning. An extra virgin olive oil (EVOO) having a high content of polyphenols was selected, and its antioxidant activity was compared with that of brine and oils lacking phenols [refined soybean oil (RSO) and refined olive oil (ROO)].

MATERIALS AND METHODS

Packing Oils. ROO and EVOO, six months old, were supplied by Olefici Italiani (Bari, Italy). Fresh RSO was obtained from an Italian commercial market. They were kept frozen in dark glass bottles under nitrogen.

Raw Material, Cooking, and Canning. Three frozen Atlantic tuna (*Thunnus alalunga*) samples (A, 67 cm, 8.2 kg; B, 63.8 cm, 6.7 kg; C, 65.5 cm, 7.2 kg) were obtained from commercial sources. Fish were caught 1 month before, and they were kept frozen in polyethylene bags. After arrival at the laboratory, fish were processed in the pilot plant of the Instituto de Investigaciones Marinas del CSIC (Vigo, Spain). Whole eviscerated and beheaded fish were steamed (102−103 °C) until a final backbone temperature of 65 °C was achieved (90 min); they were then cooled and held at room temperature (14 °C) for ∼5 h. The fish were cleaned, and the red muscle was removed. Portions of 90 g of cooked white muscle were placed in RO-100 cans (6.52 cm diameter, 3 cm height). Brine (20 mL of aqueous sodium chloride; 99.99% purity; maximum level of metals, 0.008%; 20 g L^{-1}) or oil (20 mL of oil with 2 g of sodium chloride) was then added. Three different oils were used: RSO, ROO, and EVOO. The cans were vacuum-sealed, sterilized in a retort at 115 °C (60 min, pressure 1.76 kg/cm^2), and stored at room temperature until required for analysis (2 and 5 months). Cans containing only oils were subjected to the same sterilization conditions (blank oil cans).

Lipid Extraction. Cans were opened and packing media were carefully collected and centrifuged to separate lipid and aqueous phases. Tuna muscle was minced and wrapped in filter paper; lipids were then extracted from 5 g of the resulting minced muscle by homogenization with 30 mL of a mixture of dichloromethane/methanol/water (2:2:1) (Bligh and Dyer, 1959). Lipid extracts were stored at −20 °C in chloroform until analysis; propyl gallate was used as antioxidant. Lipid content was determined as described by Herbes and Allen (1983).

Lipid Oxidation. The thiobarbituric acid index (TBA-i) (milligrams of malonaldehyde per kilogram of muscle) was determined according to the method of Vyncke (1970). Peroxide value (milliequivalents of oxygen per kilogram of lipids) was determined according to the AOCS (1989) method. Conjugated diene and triene indices were also determined according to the AOCS (1989) method. Fluorescence formation was studied in the packing medium as the ratio between fluorescence intensities obtained at 393/463 and 327/415 nm (Aubourg et al., 1995).

Transmethylation. Oils were subjected to acidic transmethylation according to the procedure of Lepage and Roy (1986). One hundred milligrams of oil was dissolved into 1 mL of toluene and 2 mL of acethyl chloride (5% in methanol). They were held overnight at 50 °C. Five milliliters of potassium carbonate (6%) was added, and methyl esters were extracted into 10 mL of hexane.

Fatty Acid Composition. Methyl esters were analyzed by gas chromatography as described by Christie (1982).

Phenol Extraction. The phenolic compounds from EVOO were obtained according to the method described by Vázquez Roncero (1978): 5 g of oil was dissolved into 50 mL of hexane. An extraction in 60 mL of a mixture of methanol/water (3:2) was then carried out. The extract was evaporated to dryness in a flash evaporator (40 °C), and the content was evaluated gravimetrically. The residue was then dissolved in methanol (5 mL), and this solution was used for the reversed-phase HPLC analysis.

From aqueous phases obtained after centrifugation of the packing media, phenols were extracted using a previous acidification (pH 2), a washing step with hexane, and an extraction in ethyl acetate according to the method of Brenes et al. (1995). The organic extract was then evaporated to dryness in a flash evaporator (40 °C) and dissolved in 100 μL of methanol.

Phenols were extracted from canned tuna muscle using the method described by Brenes et al. (1990). Tuna muscle (5 g) was washed with 100 mL of ethanol (80%, 400 ppm of sodium metabisulfite) and then extracted in 100 mL of ethanol/acetone (1:1). The ethanolic phase was concentrated in a flash evaporator (40 °C), and then it was washed with hexane and reextracted in ethyl acetate. Finally, the organic extract was evaporated to dryness in a flash evaporator (40 °C) and dissolved in 100 μL of methanol.

Phenol Separation. Aliquots of total phenol extracts (20 μL) were subjected to reversed-phase HPLC. Separation was performed on a Spherisorb ODS column (4.6 mm i.d. × 25 cm.; Bedfordshire, U.K.), coupled with a Varian 5000 liquid chromatograph (Palo Alto, CA), and a variable-wavelength UV detector set at 280 nm (Varian model 50) fitted with a Hewlett-Packard 3394 integrator (Palo Alto, CA). The chromatographic separation was achieved using a gradient elution. Solvent A was water/trifluoroacetic acid (97:3). Solvent B was acetonitrile/methanol (80:20) (Fluka, Switzerland). The following gradient was used: from 10 to 35% B for 3 min, 100% B in 27 min. The flow rate was 1 mL/min. Identity of peaks was verified by comparing their relative retention times (RRT) and UV spectra obtained through a Beckman 168 diode array detector (Palo Alto, CA) coupled with the HPLC with those of standards (Sigma, St. Louis, MO). Major peaks corresponding to complex phenols (hydrolyzable phenols) were assigned to the molecular structures identified by Montedoro et al. (1993) on the basis of their relative elution order and ^{1}H NMR spectroscopy. Quantitative results of the main phenols were expressed as syringic acid employed as internal standard.

Tocopherols. Tocopherols were analyzed by reversed-phase HPLC (2.5 g of oil in 25 mL of ethyl acetate, injection 20 μL) using the same column and equipment employed for phenol analysis. A modification of the method described by Manzi et al. (1996) was carried out: isocratic elution in metanol/water/acetonitrile (73.2:1.8:25), UV detection at 290 nm, and a washing step in ethyl acetate during 15 min. Di-α-tocopheryl acetate (Fluka, Buchs, Switzerland) was used as internal standard. Quantitative data were expressed as α-tocopherol.

^{1}H NMR Spectroscopy. Complex phenols separated by HPLC were collected after five successive injections into the column and a washing step with potassium carbonate (6%) for subsequent ^{1}H NMR analysis. ^{1}H NMR spectra were recorded on a Bruker AM-400 instrument in methanol-δ solutions according to the method of Della Medaglia et al. (1996). Peak assignment was made by referring to the chemical shift data previously reported in the literature (Montedoro et al., 1993; Limiroli et al., 1995).

Statistical Analysis. For each sampling time, 2 and 5 months, three different cans corresponding to the same tuna sample (A) were opened and analyzed individually. This strategy was also followed with the other tuna individuals (B and C), and all analyses were performed in duplicate. Data obtained were subjected to analysis of variance (ANOVA) one-way method according to the procedure of Sokal and Rohlf (1981). Comparisons of means after ANOVA test were performed using a least squares difference (LSD) method (Statsoft, 1994).

RESULTS AND DISCUSSION

To study the effect of oil composition on the modification taking place on tuna lipids during processing, canning tests on pilot scale were designed using brine and three different oils as packing media. Oils were selected on the basis of their composition: an RSO rich in linoleic (55.5%) and linolenic acids (8.44%), an ROO having a high content of oleic acid (72%), and a high-quality EVOO having a fatty acid composition similar

Table 1. Tocopherol and Phenol Content of Original Oils[a]

	tocopherols[b]			phenolic content[c]
	α	$\beta+\gamma$	δ	
initial EVOO	108.3 ± 3.2[a]	13.3 ± 1.3[a]	5.0 ± 1.3[a]	1280 ± 25.6[a]
initial ROO	82.2 ± 1.2[b]	6.1 ± 0.8[b]	10.2 ± 2.1[b]	0.0 ± 0.0[b]
initial RSO	59.4 ± 0.9[c]	542.1 ± 10.3[c]	211.1 ± 2.6[c]	0.0 ± 0.0[c]

[a] Data are expressed as mg/kg of oil, mean ± standard deviation. Values with different following letters in the same row are significantly different. Significance was declared at $p < 0.05$. [b] Expressed as α-tocopherol; detection limit 0.2 ppm. [c] Gravimetrically determined.

to that of the ROO but rich in phenolic compounds (about 1.2 g/kg of oil; Table 1). The greatest tocopherol amounts were found in RSO with important contents of β-,γ-, and δ-tocopherol. EVOO and ROO showed similar concentrations of α-tocopherol around 100 mg/kg of oil, whereas β-, γ-, and δ-tocopherols were present at low levels (Table 1). Literature data have demonstrated that the relative antioxidant order for the four tocopherol homologues varies widely, being influenced by variables such as the type of oil and the processing temperature (Warner, 1997).

Figure 1A shows the HPLC profile of phenols extracted from initial EVOO used as packing media, showing as minor components tyrosol (Ty) and hydroxytyrosol (OHTy) and high contents of complex phenols constituted of Ty and OHTy esters with elenolic acid derivatives (Table 2). Previous works have attributed the oxidative stability of olive oils to the antioxidant activity of *o*-diphenols (Papadopulos and Boskou, 1991).

Oxidation Monitoring in Tuna Muscle and Packing Media. TBA-i and fluorescence measurements were performed to follow tuna lipid oxidation after canning and storage during 2 and 5 months (Table 3**)**. Data resulting from the ANOVA analysis of TBA-i showed significant differences between the content of aldehydic compounds in the same tuna sample when different packing media were used. In both experiments, cans opened at 2 and 5 months, the formation of aldehydic compounds was significantly higher in RSO and ROO than in EVOO, suggesting a protective role of natural EVOO antioxidants. Since the tocopherol contents was much greater in RSO (Table 1), the antioxidant ability detected must be mainly attributed to EVOO polyphenols.

The highest TBA-i values were found in canned tuna muscle using brine as dipping medium (Table 3), thus indicating a lower protection in the muscle kept in a highly aqueous environment. Oxidation observed is difficult to attribute to pro-oxidants present in the salt since levels of metals in brine used during this study were lower than usually reported to induce lipid oxidation (Angelo, 1992). Recent works have described the influence of the physical state on the rate of oxidation of oils (Frankel et al., 1996; Coupland, 1996). Depending on the partitioning of PUFA in oil-in-water emulsions, the oxidation can increase (Coupland, 1996). Lipids were more susceptible to oxidation in these emulsions, since unsaturated fatty acids are high surfactant actives and tend to accumulate at the oil−water interface (Coupland, 1996). In this way, they can be easily oxidized by the action of aqueous radicals that are more abundant in the water phase. Values of TBA-i detected in brine cans confirm these findings and could be presumably explained by considering the physical state of tuna lipids immersed in high-water-content media.

Fluorescence measurements of compounds resulting from interaction of carbonyls and amino groups have recently demonstrated their application to the determination of the oxidative range of a fish product

Table 2. Phenolic Composition of EVOO during 2 Months of Canning (Milligrams per Kilogram of Oil)[a]

peak	RRT[b]	initial	heated	packing
(1) hydroxytyrosol	0.52	3.55 ± 0.02[a]	2.99 ± 0.21[a]	0.00 ± 0.00[b]
(2) tyrosol	0.73	6.59 ± 0.43[a]	5.28 ± 1.02[a]	0.52 ± 0.02[b]
(3) *p*-coumaric acid	1.13	1.16 ± 0.22[a]	1.90 ± 0.35[a]	0.00 ± 0.00[b]
(4) unknown	1.24	0.00 ± 0.00[a]	0.00 ± 0.00[a]	1.24 ± 0.25[b]
(5) dialdehydic form of oleuropein aglycon	1.50	33.37 ± 1.06[a]	36.39 ± 0.89[a]	0.00 ± 0.00[b]
(6) unknown	1.61	0.00 ± 0.00[a]	0.00 ± 0.00[a]	10.67 ± 0.22[b]
(7) elenolic acid derivative bonded with Ty	1.80	67.08 ± 2.01[a]	77.13 ± 2.05[b]	0.00 ± 0.00[c]
(8) elenolic acid derivative bonded with Ty	1.89	20.59 ± 0.26[a]	19.93 ± 1.40[a]	1.93 ± 0.10[b]
(9) elenolic acid derivative bonded with OHTy	2.05	35.20 ± 0.76[a]	35.21 ± 1.74[a]	0.00 ± 0.00[b]
(10) unknown	2.44	22.48 ± 0.76[a]	23.20 ± 0.93[a]	0.00 ± 0.00[b]

[a] Quantitative data are expressed as syringic acid (mean ± standard deviation of three different tuna samples). Values with different following letters in the same row are significantly different. Significance was declared at $p < 0.05$. [b] Relative retention time. Their variation coefficients were lower than 0.5%.

Table 3. TBA-i and Fluorescence (FL) Values Found in Three Different Tuna Cans Expressed as Milligrams of Malonaldehyde per Kilogram of Muscle and as Ratio between Fluorescence Intensities of the Packing Media Obtained at 393/463 and 327/415 nm, Respectively[a]

	EVOO		ROO		RSO		brine	
	TBA-i	FL	TBA-i	FL	TBA-i	FL	TBA-i	FL
tuna A[b]	0.26 ± 0.01[a]	1.22 ± 0.10[α]	0.46 ± 0.05[b]	1.00 ± 0.06[β]	0.35 ± 0.08[c]	0.71 ± 0.03[γ]	0.79 ± 0.12[d]	9.08 ± 1.03[δ]
tuna B[b]	0.13 ± 0.01[a]	1.47 ± 0.12[α]	0.46 ± 0.03[b]	0.99 ± 0.03[β]	0.76 ± 0.10[c]	0.70 ± 0.13[γ]	0.58 ± 0.04[d]	48.05 ± 2.24[δ]
tuna C[b]	0.18 ± 0.02[a]	1.30 ± 0.09[α]	0.35 ± 0.01[b]	0.97 ± 0.02[β]	0.26 ± 0.05[c]	0.68 ± 0.08[γ]	0.48 ± 0.02[d]	3.53 ± 0.58[δ]
tuna A[c]	0.51 ± 0.06[a]	1.47 ± 0.01[α]	0.55 ± 0.02[ab]	1.07 ± 0.14[β]	0.57 ± 0.09[b]	0.79 ± 0.07[γ]	0.75 ± 0.10[c]	18.84 ± 0.98[δ]
tuna B[c]	0.57 ± 0.03[a]	1.71 ± 0.11[α]	1.22 ± 0.08[b]	1.07 ± 0.05[β]	0.80 ± 0.11[c]	0.77 ± 0.15[γ]	3.55 ± 0.43[d]	21.17 ± 1.03[δ]
tuna C[c]	0.27 ± 0.02[a]	1.50 ± 0.02[α]	0.60 ± 0.13[b]	1.01 ± 0.08[β]	0.42 ± 0.03[c]	0.74 ± 0.02[γ]	4.42 ± 0.28[d]	7.01 ± 0.62[δ]

[a] Values are expressed as mean ± standard deviation. Values with different following Roman letters for TBA-i or different Greek letters for FL, in the same row are significantly different. Significance was declared at $p < 0.05$. [b] Cans opened after 2 months. [c] Cans opened after 5 months.

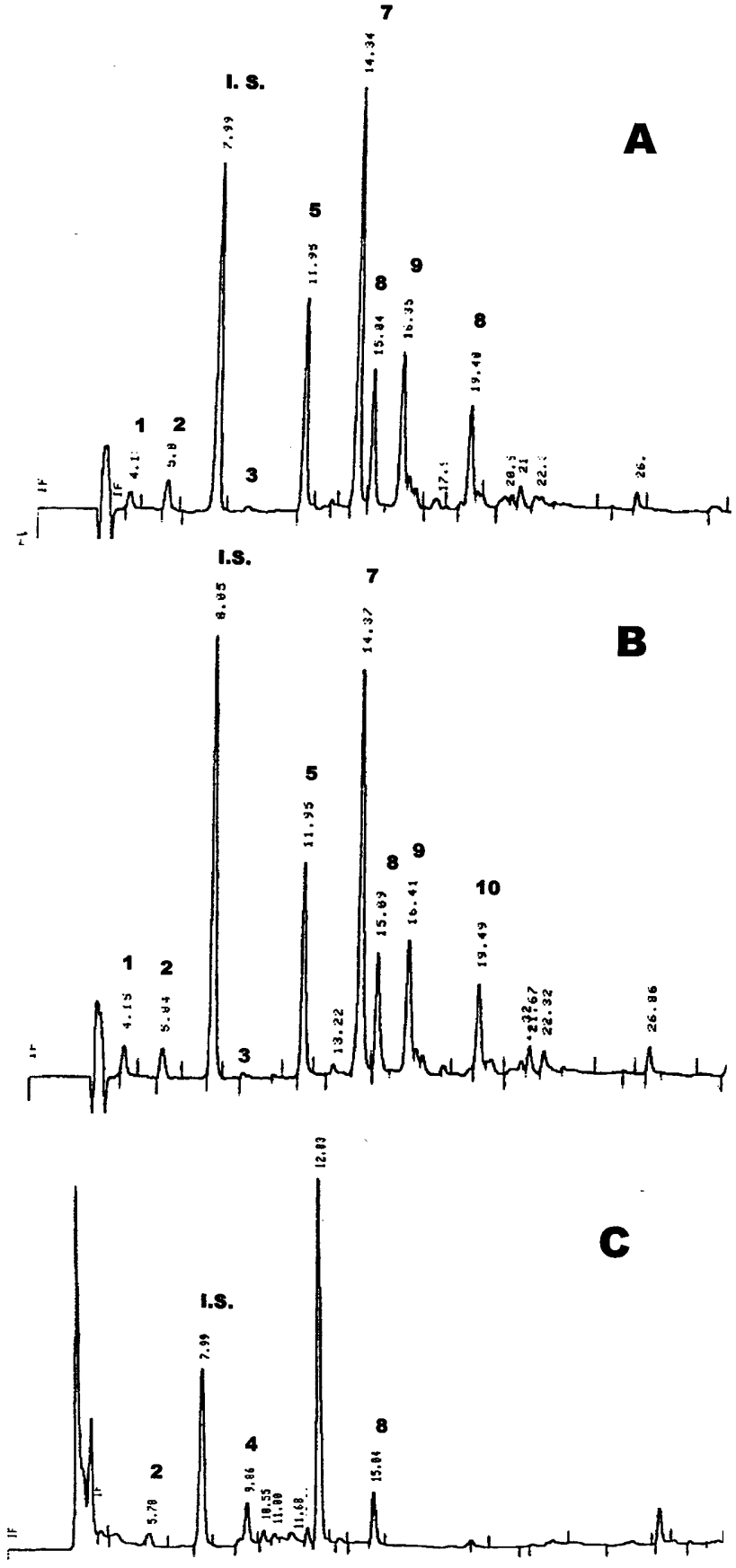

Figure 1. HPLC chromatograms of phenols extracted from EVOO: (A) initial EVOO; (B) blank canned EVOO; (C) packing EVOO. Peaks are labeled as in Table 2. Syringic acid was used as internal standard (I.S.).

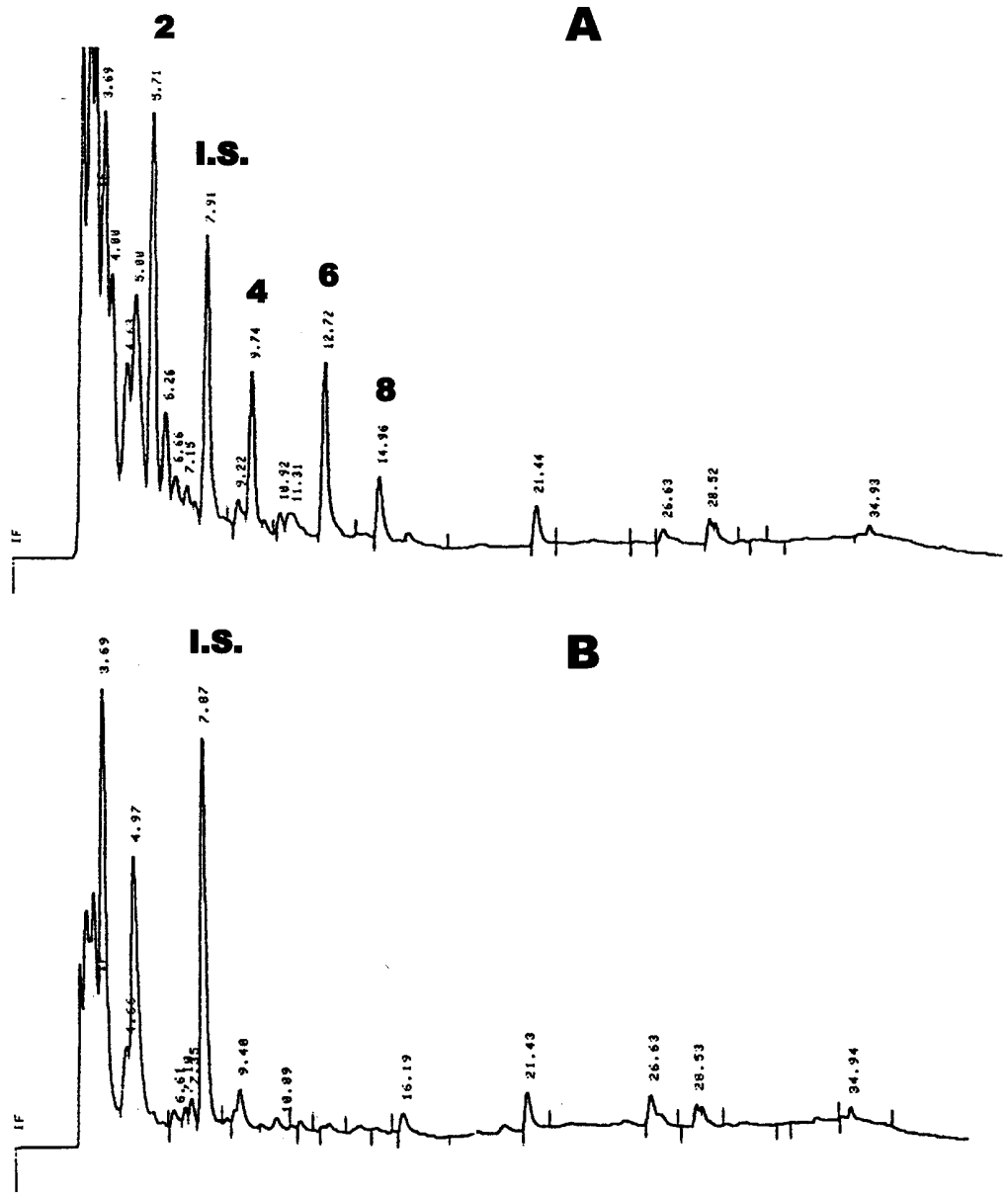

Figure 2. HPLC chromatograms of phenols extracted from the aqueous phase of tuna cans packing media: (A) tuna canned with EVOO; (B) tuna canned with ROO. Peaks are labeled as in Table 2. Syringic acid was used as internal standard (I.S.).

(Aubourg et al., 1995, 1997). Particularly in the case of fish canned products, the fluorescence measurements of the dipping media have shown to have a good correlation with the quality of the starting material (Aubourg and Medina, 1997). However, values obtained in the present work demonstrated a different evolution of these oxidation products depending on the packing medium employed (Table 3). Brine achieved the highest values due to the major rate of oxidation already observed and probably an extraction of fluorescent compounds in this polar medium as well. These results are in agreement with those of TBA-i relating a higher lipid oxidation in these cans; however, the fluorescence responses of oily cans showed a different tendency from their corresponding TBA-i values, being higher for EVOO than for RSO and ROO (Table 3). The presence of phenolic groups in original EVOO likely involved higher fluorescence response, and so did a different evolution during canning. As a result, no significant correlations between fluorescence of the dipping medium and TBA-i values from canned tuna muscle could be obtained. These data suggested that no relevant information regarding fish oxidation is supplied by comparing the fluorescence values in oily packing media with different compositions.

The analysis of packing media offered further elements to understand the mechanism involved in tuna lipids−oil and −brine interactions. Table 4 shows the oxidation measurements of oils extracted from cans after 2 months of storage. Taking into account the values within groups of oily media, there were no changes in peroxide content between initial and blank canned oils. However, with regard to packing media, ROO peroxides suffered a larger decrease than peroxides of RSO and EVOO did, thus suggesting a superior decomposition of primary oxidation products of ROO during canning. The mechanism involved in such decomposition was not easy to explain, and it likely

Table 4. Peroxide and Conjugated Dienes and Trienes of Initial and Processed Oils[a]

	peroxide[b]	conjugated diene[c]	conjugated triene[d]
initial EVOO	9.50 ± 0.75[a]	2.16 ± 0.23[a]	0.24 ± 0.02[a]
blank canned EVOO	11.03 ± 1.12[a]	1.84 ± 0.37[a]	0.20 ± 0.11[a]
packing EVOO	9.91 ± 0.58[a]	1.82 ± 0.13[a]	0.23 ± 0.08[a]
initial ROO	4.50 ± 0.87[b]	2.25 ± 0.12[a]	0.62 ± 0.14[b]
blank canned ROO	5.79 ± 1.02[b]	2.26 ± 0.35[a]	0.57 ± 0.15[b]
packing ROO	1.59 ± 0.38[c]	2.45 ± 0.42[a]	0.56 ± 0.06[b]
initial RSO	1.49 ± 0.26[d]	4.86 ± 0.83[b]	2.16 ± 0.24[c]
blank canned RSO	2.18 ± 0.64[d]	3.50 ± 0.54[b]	1.51 ± 0.45[c]
packing RSO	1.00 ± 0.17[e]	3.63 ± 0.39[b]	1.53 ± 0.36[c]

[a] Data are mean ± standard deviation of three different tuna cans opened after 2 months. Values with different following letters in the same column are significantly different. Significance was declared at $p < 0.05$. [b] Expressed as mequiv of oxygen/kg of lipids. [c] Expressed as specific extinction at 234 nm. [d] Expressed as specific extinction at 270 nm.

concerns a prooxidant activity of some components of fish muscle on oily media, an action that provided a little effect during RSO and EVOO canning. Because of the content of natural polyphenols of EVOO and natural tocopherols of RSO, it can be concluded that there is an inhibition of peroxide decomposition in both media, and since there were no statistical differences within the EVOO group (initial, heated, and packing), protection attributed to polyphenols was higher than that of tocopherols.

No increases in conjugated diene and triene values were detected along the process; carbonylic products from peroxide decomposition of oils seemed to react with tuna muscle compounds. In addition, carbonylic compounds from tuna lipid oxidation were not extracted into the oil medium, confirming the accuracy of TBA-i values calculated from tuna muscle.

Phenolic Profile Analysis. *Lipid Phase of Packing Media.* Analysis of peroxides, conjugated dienes, and TBA-i values made above suggested an important oxidative inhibition by EVOO phenol compounds on fish lipids during canning and storage. To determine the contribution of phenols in this process, their changes were studied by extracting the minor polar compounds from packing EVOO. Parts A, B, and C of Figure 1 show the HPLC profiles obtained from initial EVOO, blank canned EVOO, and packing EVOO, respectively. Blank canned EVOO was prepared to assess the effect of physical treatment, avoiding the interaction between oil and tuna muscle. Table 2 shows the individual phenol composition obtained by HPLC. Results achieved in initial and blank canned EVOO confirmed previous findings in which no significant changes occurred in EVOO phenols during storage and thermal stress (Della Medaglia et al., 1996). However, a significant variation was observed in phenols extracted from packing EVOO after sterilization and 2 months of storage of tuna cans (Figure 1C). Chromatograms showed a strong decrease in all peaks. Tyr and the most hydrophobic Tyr esters, peaks 2 and 8, respectively, were detected at lower levels; other compounds disappeared completely. Two new compounds, peaks 4 and 6, were formed (Table 2). These compounds could originate through a degradation of oil phenols or be lipid-soluble compounds arising from tuna muscle. To verify their origin, phenol extraction was carried out on the packing ROO of tuna cans, but none of these products could be detected; they came from degraded phenols. However, since no significant differences were found between the phenol compositions in initial and blank canned EVOO, the rate of phenol changes detected in packing EVOO was difficult to explain just by taking into account the formation of these new products (Table 2). Other mechanisms must be involved in the rate of phenol losses, phenol migration into the aqueous phase (exudate) present in the packing media, and interaction of phenols with fish muscle components as well (Waters et al., 1994).

Aqueous Phase of Packing Media. An acidic extraction and HPLC analysis of minor polar components was then carried out on the aqueous phase of packing media in EVOO and ROO tuna cans. EVOO chromatograms showed a significant number of peaks; two of them could be identified as Tyr and Tyr esters and the two unknown compounds, peaks 4 and 6 as well (Figure 2A). The HPLC analysis of the aqueous phase from ROO did not show any of these last peaks (Figure 2B); it can be concluded they were entirely related with phenol reactions. The relative content of Tyr in the aqueous phase of EVOO tuna cans was higher than that of Tyr aglycon, so a hydrolysis of the phenol complex to give single phenols seemed to occur during processing or cans storage. Because of the profile obtained from packing EVOO (Figure 1C), the proportion of free Tyr was higher in the aqueous phase than in oil. A migration of hydrophilic EVOO phenols from the oil to the water−muscle interface seemed to take place. OHTy was difficult to detect due to the overlap of the peak with other muscle components.

Tuna Muscle. Chromatograms of phenolic compounds directly extracted from tuna muscle did not show any original phenols. Only peaks 4 and 6 could be identified but in low concentrations. Since most of the polyphenols were not found in oil and aqueous phases of the EVOO packing or tuna muscle either, reactions with other components in the food system seem to be occurring. Interactions between proteins and phenolic compounds used to take place effortlessly in food when the system involved high levels of phenols (Waters et al., 1994). EVOO phenols could interact with polar compounds from tuna muscle, forming new complexes, their antioxidant activity to test. Work is now in progress to check the role of these protein−phenol compounds in lipid oxidation.

The data obtained seem to indicate a potential antioxidant activity of phenolic compounds of extra virgin olive oils on lipid oxidation occurring during thermal process and/or storage of fish products. The antioxidant ability verified during tuna canning may be attributed to migration of hydrophilic EVOO phenols from the oil to the water−muscle interface where lipid oxidation occurs (Coupland et al., 1996). The results demonstrated the influence of an aqueous phase such as brine employed as packing medium in a major rate of oxidation on fish lipids and subsequently in a decrease on the quality achieved when EVOO is employed at the same processing conditions.

The use of natural antioxidants as components of the packing media in these processed products may improve their quality, nutritional and sensorial as well. Sensory and economical aspects such as the strong fruity EVOO flavor and the higher production cost have to be evaluated. Assessment of the possibility of using only the phenol extract is in progress.

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LITERATURE CITED

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