JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Partition Behavior of Virgin Olive Oil Phenolic Compounds in Oil−Brine Mixtures during Thermal Processing for Fish Canning

RAFFAELE SACCHI,*,† ANTONELLO PADUANO,† FRANCESCA FIORE,† DOROTEA DELLA MEDAGLIA,† MARIA LUISA AMBROSINO,† AND ISABEL MEDINA‡

Department of Food Science, University of Naples Federico II, Portici (Naples), Italy, and Instituto de Investigaciones Marinas, CSIC, Vigo, Spain

The chemical modifications and partitioning toward the brine phase (5% salt) of major phenol compounds of extra virgin olive oil (EVOO) were studied in a model system formed by sealed cans filled with oil−brine mixtures (5:1, v/v) simulating canned-in-oil food systems. Filled cans were processed in an industrial plant using two sterilization conditions commonly used during fish canning. The partitioning of phenolic compounds toward brine induced by thermal processing was studied by reversed-phase high-performance liquid chromatographic analysis of the phenol fraction extracted from oils and brine. Hydroxytyrosol (**1**), tyrosol (**2**), and the complex phenolic compounds containing **1** and **2** (i.e., the dialdehydic form of decarboxymethyl oleuropein aglycon **3**, the dialdehydic form of decarboxymethyl ligstroside aglycon **4**, and the oleuropein aglycon **6**) decreased in the oily phase after sterilization with a marked partitioning toward the brine phase. The increase of the total amount of **1** and **2** after processing, as well as the presence of elenolic acid **7** released in brine, revealed the hydrolysis of the ester bond of hydrolyzable phenolic compounds **3**, **4**, and **6** during thermal processing. Both phenomena (partitioning toward the water phase and hydrolysis) contribute to explain the loss of phenolic compounds exhibited by EVOO used as filling medium in canned foods, as well as the protection of *n*−3 polyunsaturated fatty acids in canned-in-EVOO fish products.

KEYWORDS: Virgin olive oil; phenolic compounds; oil−water partition; antioxidants; sterilization; tyrosol; hydroxytyrosol; thermal processing; fish canning

INTRODUCTION

Lipid oxidation occurring in canned food during thermal processing involves chemical and interfacial phenomena between lipid and water phases (*1*, *2*). The rate of oxidation and the mechanisms involved can show important differences depending on the filling medium employed and the polarity of antioxidants present in the medium. In the case of canned fish, lipid oxidation has been found to accelerate in brine packing medium, with a decrease in the quality of the canned fish, these effects being in part inhibited when oils were used as the filling medium (*3*).

Among different oils, extra virgin olive oil (EVOO) used as filling medium of canned fish showed the highest protection on the thermal oxidation of *n*−3 polyunsaturated fatty acids induced during sterilization (*3*). This effect can be attributed to EVOO natural phenolic compounds (absent in refined oils commonly used in the canning industry), which have a well-recognized antioxidant activity in bulk oils (*4*−*6*), micellar systems (*7*), and model systems formed by minced fish muscle heated in oil or in brine (*8*).

Phenolic antioxidants, however, do not have the same behavior in different media. Polar antioxidants are more active in bulk lipids, whereas hydrophobic components are more active in oil-in-water emulsions, according to the so-called "polar paradox" (*2*, *9*). Phenolic compounds, such as butylated hydroxytoluene or natural polyphenols, have been found to be more effective in fish heated in brine than in oil, probably due to the greater affinity of these antioxidants for the more polar interface that exists in the system between muscle lipids and filling medium (*8*).

However, the behavior of EVOO phenolic compounds in an oil−water food system subjected to high-temperature processing is poorly understood. As the phenolic fraction of EVOO is a complex mixture of natural compounds with a wide range of chemical structures (*10*) and polarity (*7*), understanding its physical behavior and chemical modifications in an oil−water food system subjected to high-temperature processing may be of great interest for the food industry. Indeed, the partitioning and chemical changes occurring during heating may influence the antioxidant effectiveness of these compounds in several foods such as canned tuna, tomato sauces, or mayonnaise made by using EVOO as oily ingredient.

* Address correspondence to this author at the Dipartimento di Scienza degli Alimenti, Università di Napoli Federico II, Facoltà di Agraria, I-80055 Portici (Napoli) Italy (telephone +39 081 7752370; fax +39 081 7753734; e-mail sacchi@unina.it).
† University of Naples Federico II.
‡ Instituto de Investigaciones Marinas.

10.1021/jf011526l CCC: $22.00
Published on Web 04/11/2002

This work aims to study the partitioning and chemical behavior of EVOO phenolic compounds and the mechanism involved in their loss from the filling oily phase of sterilized canned-in-oil multiphase foods (*3*). Thus, bulk oil and oil−brine model systems (cans filled with bulk EVOO and oil−brine mixtures) were subjected to two different sterilizing conditions. After thermal processing, the partitioning toward the water phase and the chemical changes of each phenol compound were studied by reversed-phase high-pressure liquid chromatography (HPLC) analysis of phenols in brine and oil before and after processing.

EXPERIMENTAL PROCEDURES

EVOO Samples. Several extra virgin olive oils were obtained from olives (*Olea europaea sativa* L. var. Biancolilla) harvested and extracted at Valle dell'Angelo (Salerno, Italy) in November 1999 in a continuous extraction plant equipped with hammer crushers, a Sinolea (Rapanelli, Foligno, Italy) percolation extractor, a kneader (35 °C, 45 min), a centrifugal decanter, and a separator (Rapanelli). Oils were submitted to chemical analyses and, from a batch selected on the basis of composition, 20 L of oil was collected and stored in a filled container at 16 ± 2 °C in the dark until analyses and thermal processing tests were carried out.

Thermal Processing. Sterilization was performed in an industrial plant for tuna canning (Daunia Food S.p.A., Foggia, Italy). RO-100 cans (6.52 cm in diameter, 3 cm high) were filled with bulk EVOO and a mixture (1:5, v/v) consisting of brine (5% w/v salt concentration) and EVOO. The cans were vacuum-sealed and sterilized under the following conditions: S1 (118 °C for 50 min) and S2 (111.5 °C for 80 min), corresponding to F_0 values (critical lethality, minutes needed at 250 °F or 121 °C to reduce a population of *Clostridium botulinum* of 10^{12}) of 7 and 14 min, respectively. Four cans per treatment were processed. Cans were stored at room temperature until analyses.

EVOO Chemical Analyses. Analyses of free acidity, peroxide value, specific extinction K_{232} (conjugated dienes), and K_{270} (trienes and carbonyl compounds) were performed in duplicate according to the EC official methods for olive oils (*11*). The values exhibited for the EVOO batch before thermal processing were as follows: free acidity of 0.58 g of oleic acid/100 g of oil; peroxide value of 6.05 Mequiv of O_2/1000 g of oil; K_{232} of 2.035; and K_{270} of 0.128. For the determination of fatty acid composition oils were subjected to cold transmethylation in KOH/methanol according to the method of Christie (*12*). Analyses of fatty acid methyl esters (FAMEs) of the EVOO were carried out by gas chromatography using a GC17A chromatograph (Shimadzu, Italy, Milan) equipped with a flame ionization detector and a Quadrex Corp. (New Haven, CT) fused-silica capillary column (60 m long and 0.25 mm i.d.) coated with cyanopropyl methyl silicone (0.25 mm film thickness). The individual FAMEs were identified by comparison with the retention times of pure standards (Larodan, Malmoe, Sweden). The fatty acid composition (weight, percent) of the EVOO used in this study was as follows: palmitic acid (C16:0), 12.39%; palmitoleic acid (C16:1*n*−9), 0.78%; heptadecanoic acid (C17:0), 0.05%; heptadecenoic acid (17:1*n*−10), 0.08%; stearic acid (C18:0), 2.28%; oleic acid (C18:1*n*−9), 74.56%; vaccenic acid (C18:1*n*−7), 2.03%; linoleic acid (C18:2*n*−6), 6.01%; arachidic acid (C20:0), 0.33%; linolenic acid (C18:3*n*−3), 0.58%; eicosenoic acid (C20:1*n*−9), 0.22%; behenic acid (C22:0), 0.09%; and lignoceric acid (C24:0), 0.04%.

Tocopherols were determined by reversed-phase HPLC analysis according to the method reported by Tonolo and Marzo (*13*) on a Shimadzu (Milan, Italy) liquid chromatograph (model LC-10AD) equipped with a diode array detector (model SPD M10A VP). The chromatographic separation was achieved on a 5 mm ODS-3 Prodigy (250 mm × 4.6 mm i.d.) reversed-phase column (Phenomenex, Macclesfield, U.K.). Quantitation was made using *d*-α-tocopheryl acetate (Fluka, Buchs, Switzerland) as an internal standard, and data were expressed as α-tocopherol (response factor calculated using a standard solution of α-tocopherol and *d*-α-tocopheryl acetate). The values exhibited by the EVOO batch before thermal processing were as follows: α-tocopherol, 138 mg/kg of oil; *β*- + *γ*-tocopherol, 12 mg/kg of oil; *δ*-tocopherol, 8 mg/kg of oil.

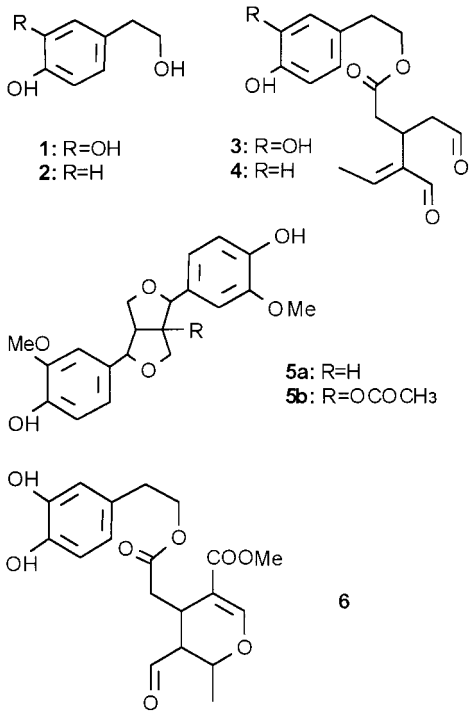

Figure 1. Chemical structures of the main phenolic compounds of virgin olive oil followed by HPLC: hydroxytyrosol (**1**), tyrosol (**2**), dialdehydic form of decarboxymethyl oleuropein aglycon (**3**), dialdehydic form of decarboxymethyl ligstroside aglycon (**4**), pinoresinol (**5a**), 1-acetoxypinoresinol (**5b**), and oleuropein aglycon (**6**).

Extraction of Phenolic Compounds from Oil and Brine. The polar fraction of unprocessed and processed virgin olive oil was obtained by slightly modifying the method described by Vasquez Roncero et al. (*14*). Ten grams of oil dissolved in 10 mL of hexane was extracted with methanol/water (3:2, v/v, 3 × 30 mL). Each extract was washed once with hexane (10 mL) and centrifuged. The three extracts were combined, and the solvent was evaporated to dryness in a flash evaporator (40 °C). The residue was then dissolved in methanol (2 mL), and this solution was used for the HPLC analysis (20 mL injection). Sterilized mixtures of oil−brine were separated by centrifugation (2500 rpm for 3 min). Phenol compounds were extracted from the oily phase and analyzed according to the procedure described above. Phenols were obtained from the aqueous phase collected by centrifugation by drying the brine under vacuum at 40 °C (2 mL) and dissolving the residue in methanol (2 mL) to eliminate salt.

HPLC Separation of Phenolic Compounds. The presence and amount of phenol compounds in unprocessed and processed oils, oily and aqueous phases, were studied by reversed-phase HPLC on the same column and equipment used for tocopherol analysis using a binary gradient elution (*7*). Solvent A was water/trifluoroacetic acid (97:3), and solvent B was acetonitrile/methanol (80:20). A step gradient from 5 to 98% B (45 min) was applied at a flow rate of 1 mL min^{-1}. Peak quantification was carried out at 279 nm. Elenolic acid and its esters were monitored at 239 nm. The main phenol compounds of EVOO (see **Figure 1** for chemical structures) were identified by comparison with relative retention times of pure compounds, when available, or by comparing the relative elution order and UV spectra with those reported in the literature (*15*−*18*). Identification of complex phenols was confirmed by LC-MS analysis, carried out on an API-100 single-quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments) equipped with an atmospheric pressure chemical ionization (APCI) ion source as reported in detail elsewhere (*19*). Quantification of phenol compounds was achieved using tyrosol as an external standard according to the procedure described by Tsimidou et al. (*20*). Data were expressed as milligrams of tyrosol per kilogram of oil for both simple and hydrolyzable phenol compounds.

Partitioning Behavior. The partition coefficient between oil and water (brine) for each EVOO phenol compound was calculated

according to the method of Huang et al. (*21*): partition coefficient (P_c) = $V_w/V_o(W_t/W_w - 1)$, where V_w = volume of brine in the sterilized cans, V_o = volume of oil in sterilized cans, W_t = total amount of the phenol compound in the unprocessed virgin olive oil; and W_w = amount of the phenol compound in brine after partitioning. W_w was calculated by determining the difference between the initial amount of the phenol compound in the oil before partitioning (W_t) and that in oil after thermally induced partitioning (W_o). The amount of each phenolic compound was measured by HPLC as described above.

Statistical Analysis. All determinations were made in duplicate in four different cans subjected to the same treatment. Data were analyzed using the SPSS software package (SPSS Inc., Chicago, IL) and applying the one-way ANOVA. Significance was declared for $P < 0.05$.

RESULTS AND DISCUSSION

The effect of thermal processing on EVOO phenolics was studied by comparing the composition of unprocessed and heated bulk oils subjected to two different times and temperature conditions. The partition of phenolic compounds **1**−**6** (**Figure 1**) toward the water phase in an oil−brine system was determined by studying the composition of the oily and water phases before and after processing.

Figure 2 shows the HPLC profiles of the phenolic compounds extracted from the starting unprocessed EVOO (a), the sterilized bulk oil (b), the oily phase from the sterilized oil−brine system (c), and the brine phase after thermal processing (d). The phenolic profile found in processed bulk oil (**Figure 2b**) slightly varied from that of the starting oil (**Figure 2a**), showing the formation of an unidentified compound eluted at a retention time of 22 min, after the peak of the dialdehydic form of decarboxymethyl oleuropein aglycon (**3**). The HPLC chromatograms obtained for the oily and brine phases after centrifugation of processed mixtures (**Figure 2c**,**d**) were clearly different from that for processed bulk oil (**Figure 2a**). The oily phases seem to have almost lost the dialdehydic form of decarboxymethyl oleuropein aglycon (**3**). The HPLC chromatogram of the brine (**Figure 2d**) showed the presence of the dialdehydic form of decarboxymethyl oleuropein aglycon (**3**), hydroxytyrosol (**1**), and tyrosol (**2**).

Changes in Phenol Composition in the Oily Phase. Table 1 shows the quantity of phenolic compounds found in oil before and after can sterilization made using the conditions S1 and S2. Two thermal treatments caused a significant reduction (∼25−30%) of the hydrolyzable phenolic compounds **3**, **4**, and **6**, of the peak corresponding to pinoresinol and 1-acetoxypinoresinol (**5**), and of hydroxytyrosol (**1**) in bulk virgin olive oil, without affecting the content of tyrosol (**2**). The decrease in complex phenols and hydroxytyrosol was found to be independent of temperatures and processing period.

If compared to the effect induced by thermal processing in bulk oils, the presence of water in the sterilized oil−brine mixtures induced other changes on the overall phenol composition of the oily phase. All simple and complex phenolics showed a significant decrease that was higher for hydroxytyrosol-derived compounds **1**, **3**, and **6** than tyrosol-derived compounds (**2** and **4**) and pinoresinol/1-acetoxypinoresinol (**5**) (**Figure 2c**; **Table 1**). This effect was particularly significant for the dialdehydic form of decarboxymethyl oleuropein aglycon **3** (70% reduced), the EVOO phenolic compound with the highest hydrophilicity at low temperature (*7*).

These changes in the phenolic composition of processed oil−brine mixtures agree with previous results obtained for EVOOs employed as a filling medium of canned fish (*3*); the decrease of tyrosol and the complete loss of hydroxytyrosol and its derivatives, found in filling EVOO after fish canning (*3*), may be explained by the partitioning toward the aqueous phase and/or their oxidation or hydrolysis associated with thermal processing.

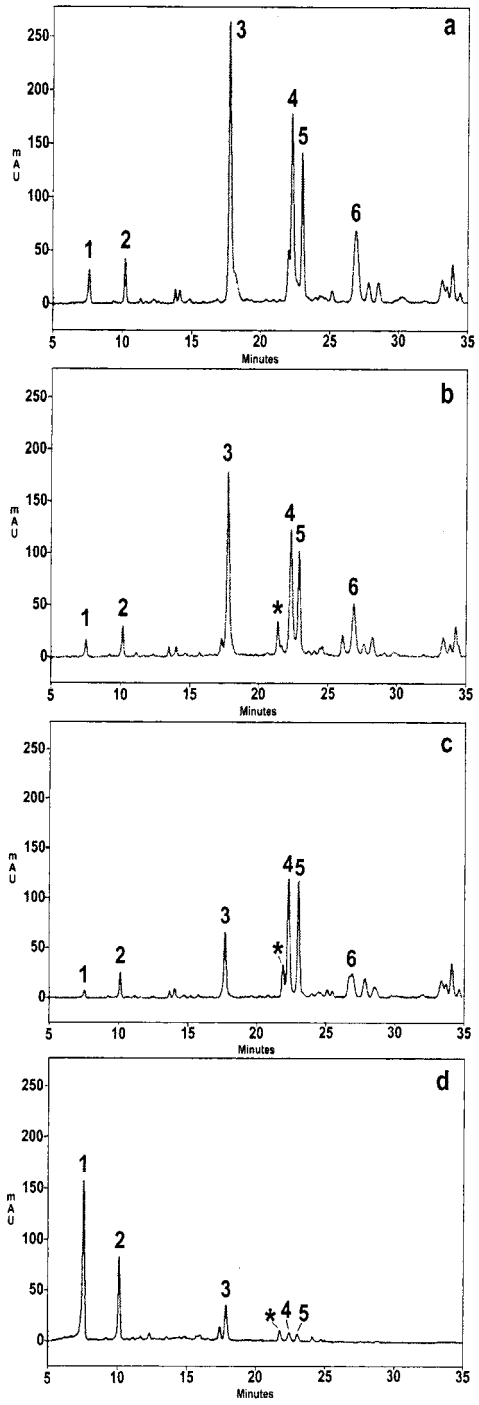

Figure 2. HPLC chromatograms (UV traces at 279 nm) of phenol compounds extracted from extra virgin olive oil before processing (a), after heating of bulk oil in sealed filled cans (b), and after heating of the brine-in-oil model system (c, oil phase; d, brine). Samples were processed under conditions S1 (118 °C for 50 min). Peaks are labeled according to **Figure 1**. Peaks labeled with asterisks are unidentified compounds formed after thermal processing.

Partitioning of Oil Phenol Compounds toward Brine. The partition coefficients (P_c) for each phenol compound after two thermal treatments are shown in **Table 2**. The P_c values were calculated by referring the final amount found in the oily phase to that in the unprocessed oil. Although chemical reactions can also occur parallel to partitioning, we assumed as a starting

Table 1. Phenolic Composition of Virgin Olive Oil and Modifications Found after Thermal Processing in Bulk Oil and in the Brine–Oil Mixtures at Two Different Sterilization Conditions (S1, 118 °C for 50 min; S2, 111.5 °C for 80 min)[a,b]

HPLC peak[c]	starting oil	heated bulk oil		heated oil–brine mixtures			
				oil phase		brine phase	
		S1	S2	S1	S2	S1	S2
1	6.4 ± 0.4^{a}	4.4 ± 0.7^{b}	4.5 ± 0.3^{b}	1.4 ± 0.2^{c}	2.3 ± 0.9^{c}	34.2 ± 4.1	37.6 ± 4.9
2	7.7 ± 0.3^{a}	6.9 ± 0.9^{ab}	6.9 ± 0.3^{ab}	4.8 ± 0.1^{c}	5.4 ± 1.1^{bc}	15.2 ± 2.9	14.3 ± 0.2
3	65.8 ± 5.7^{a}	46.5 ± 2.7^{b}	47.8 ± 5.7^{b}	15.2 ± 2.2^{c}	15.2 ± 2.8^{c}	11.0 ± 1.2	16.6 ± 2.5
4	46.1 ± 3.4^{a}	35.6 ± 0.4^{b}	35.3 ± 2.6^{b}	28.0 ± 3.9^{c}	27.3 ± 0.8^{c}	3.0 ± 0.1	3.3 ± 0.9
5	32.6 ± 1.2^{a}	26.5 ± 0.0^{b}	26.5 ± 0.8^{b}	25.7 ± 1.8^{b}	23.7 ± 2.4^{b}	1.9 ± 0.3	2.0 ± 0.4
6	24.6 ± 0.7^{a}	20.1 ± 0.4^{b}	19.0 ± 1.8^{b}	7.4 ± 0.9^{c}	7.1 ± 2.7^{c}	nd	nd

[a] Data expressed as mg of tyrosol/kg of oil ± standard deviation ($n = 4$). [b] Different superscript letters on the same line indicate a significant difference ($P < 0.05$). [c] Peaks: 1, tyrosol; 2, hydroxytyrosol; 3, dialdehydic form of decarboxymethyl oleuropein aglycon; 4, dialdehydic form of decarboxymethyl ligstroside aglycon; 5, pinoresinol and 1-acetoxypinoresinol; 6, oleuropein aglycon. See **Figure 1** for molecular structures and **Figure 2** for HPLC analysis.

Table 2. Partition Coefficients (P_c, Average ± Standard Deviation, $n = 4$) Calculated from the Residual Amount in Oil of Each Phenol Compound and Percentage Loss of Each Compound in Oil after Two Thermal Treatments[a]

phenolic compound[b]	P_c		loss from oil (%)	
	S1	S2	S1	S2
hydroxytyrosol (**1**)	0.11 ± 0.03	0.11 ± 0.07	77.7	64.4
tyrosol (**2**)	0.83 ± 0.61	0.92 ± 0.73	37.6	29.5
dialdehydic form of decarboxymethyl oleuropein aglycon (**3**)	0.07 ± 0.03	0.07 ± 0.01	77.0	76.9
dialdehydic form of decarboxymethyl ligstroside aglycon (**4**)	0.40 ± 0.08	0.33 ± 0.05	39.4	40.8
pinoresinol and 1-acetoxypinoresinol (**5**)	1.10 ± 0.09	0.79 ± 0.07	21.2	27.3
oleuropein aglycon (**6**)	0.08 ± 0.08	0.07 ± 0.02	70.0	71.0

[a] Sterilization conditions (S1, 118 °C for 50 min; S2, 111.5 °C for 80 min). [b] See **Figure 1** for molecular structures and **Figure 2** for HPLC analysis.

hypothesis of our study that hydrolytic or oxidative phenomena localized at the oil−water interface follow the partition toward the water phase of oil phenol compounds. Therefore, even if this parameter cannot be considered rigorously from a physical point of view, it may represent the real behavior of phenols in the model system studied.

The partition coefficients thus calculated for hydroxytyrosol (**1**) and the hydrolyzable compounds containing hydroxytyrosol (**3** and **6**) were very low ($P_c < 0.1$). In contrast, tyrosol (**2**), the dialdehydic form of the decarboxymethyl ligstroside aglycon (**4**), and pinoresinol/1-acetoxypinoresinol (**5**) showed the highest partition coefficient ($P_c > 0.4$).

After both sterilisation conditions, hydroxytyrosol and its derivatives (compounds **3** and **6**) showed a decrease in the oily phase ranging between 64.4 and 77.7%, whereas compound **4** containing tyrosol lost 29.5−40.8%. Pinoresinol/1-acetoxypinoresinol (**5**) shows the highest thermal stability and hydrophobicity among the main phenols of EVOO. Indeed, only 21−27% of pinoresinol/1-acetoxypinoresinol is lost from the oily phase after sterilization (**Table 2**).

Phenolic Composition of the Brine Phase. To assess the relative contribution of partitioning toward the water phase and decomposition, the phenolic compounds of brine were carefully examined and compared with their recovery in the oily phase after thermal processing. Considerable amounts of hydroxytyrosol (**1**) and the dialdehydic form decarboxymethyl oleuropein aglycon (**3**) were observed in the brine phases resulting from both treatments (**Table 1**). The highest concentration was observed for free hydroxytyrosol (**1**), which showed in brine amounts ranging from 27 to 38 mg/kg of oil. Higher processing temperatures and longer periods of treatments also resulted in greater increases in hydroxytyrosol-derived compounds **1** and **3** in the aqueous phase.

However, the absolute increase of hydroxytyrosol and tyrosol in the water−oil system after processing suggests that hydrolysis

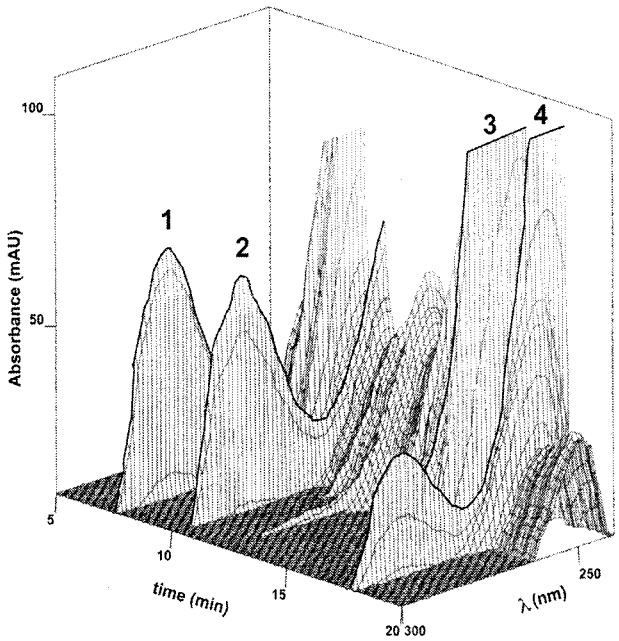

Figure 3. Three-dimensional plot of the HPLC diode array analysis of the phenol fraction extracted from the brine phase collected from the brine-in-oil model system after heating (processing condition S1, 118 °C for 50 min). Peak identification: 1, hydroxytyrosol ($\lambda_{max} = 279$ nm); 2, tyrosol ($\lambda_{max} = 279$ nm); 3, elenolic acid derivative ($\lambda_{max} = 239$ nm); 4, dialdehydic form of decarboxymethyl oleuropein aglycon ($\lambda_{max} = 279$ and 239 nm).

of tyrosol- and hydroxytyrosol esters (**3**, **4**, and **6**) may occur. Hydrolysis of the ester bond between phenyl alcohols (tyrosol and hydroxytyrosol) and elenolic acid was confirmed by the analysis of the brine phase carried out by HPLC with diode array detection, as shown in **Figure 3**. Following both the absorbance of the phenolic moiety ($\lambda = 279$ nm) of simple and hydrolyzable phenolic compounds and that of elenolic acid (λ

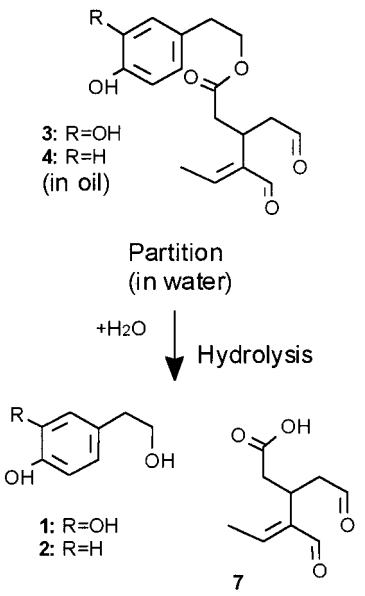

Figure 4. Formation of hydroxytyrosol (**1**), tyrosol (**2**), and dialdehydic form of decarboxymethyl elenolic acid (**7**) in brine from the dialdehydic form of decarboxymethyl oleuropein (**3**) and ligstroside (**4**) aglycons during the sterilization of virgin olive oil–brine mixtures.

= 239 nm) in the three-dimensional HPLC plot obtained from the brine phase separated by centrifugation from a oil−brine sterilized cans, hydrolysis is confirmed. The presence of free hydroxytyrosol (peak 1) and tyrosol (peak 2) was evidenced by their absorbance at 279 nm as well as that of the compound eluted at 15.6 min (peak 3), the UV spectrum of which exhibited a maximum at 239 nm and no absorbance at 279 nm. The UV spectrum of this compound corresponds to those of elenolic acid (*15*, *18*), confirming the hydrolysis of the phenolic compounds **3** and **4** partitioned toward the aqueous phase (**Figure 4**).

The comparison between the absolute amounts of hydroxytyrosol- and tyrosol-derived phenolic compounds **1**−**6** lost from the oil after processing and those found in the corresponding volume of brine (**Table 1**) confirms this mechanism. Indeed, the loss of the hydrolyzable forms of oleuropein (**3** and **6**) and ligstroside (**4**) aglycons from virgin olive oil after processing corresponds closely to the increase in their hydrolysis products **1** and **2** in the corresponding brine volume.

These findings contribute to the understanding of the mechanisms involved in the loss of EVOO phenolic compounds in the oily phase after tuna canning (*3*). The marked partitioning toward the water phase verified in this study for compounds **1** and **3** having the highest antioxidant activity in micellar systems (*7*) may also influence their antioxidant efficiency (*2*, *8*, *21*−*23*) in other multiphase food systems subjected to thermal processing or cooking. Further studies are in progress to check this hypothesis, as well as to evaluate, in cooked-in-EVOO Mediterranean foods, the intake of EVOO phenolic compounds having a well-recognized in vivo antioxidant (*24*−*27*) and hypotensive effect (*28*).

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; EVOO, extra virgin olive oil; FAME, fatty acid methyl ester.

ACKNOWLEDGMENT

G. Pipolo (Frantoio Monacelli, Valle dell'Angelo, Salerno, Italy) supplied EVOO. Sterilization tests were made at Daunia Food (Foggia, Italy) with the assistance of A. Lovoi and L. Palmieri (Istituto Sperimentale per le Conserve Alimentari, MiPAF, Angri, Salerno, Italy).

LITERATURE CITED

(1) Schwartz, K.; Stöckmann, H.; Frankel, E. N.; German, B. J. Partition behaviour as a parameter of efficiency for antioxidants. In *Oil Process and Biochemistry of Lipids*; First European Meeting of the American Oil Chemists' Society, University of Burgundy, Dijon, France; AOCS: Champaing, IL, 1996.

(2) Frankel, E. N. *Lipid Oxidation*; The Oily Press: Dundee, Scotland, 1998.

(3) Medina, I.; Sacchi, R.; Biondi, L.; Aubourg, S. P.; Paolillo, L. Effect of packing media on the oxidation of canned tuna lipids. Antioxidant effectiveness of extra virgin olive oil. *J. Agric. Food Chem.* **1998**, *46*, 1150−1157.

(4) Satue, M. T.; Huang, S.-W.; Frankel, E. N. Effect of Natural Antioxidants in Virgin Olive Oil on Oxidative Stability of Refined, Bleached and Deodorized Olive Oil. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1131−1137.

(5) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenol compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.

(6) Servili, M.; Baldioli, M.; Miniati, E.; Montedoro, G. F. Antioxidant activity of new phenolic compounds extracted from virgin olive oil and their interaction with α-tocopherol and β-carotene. *Riv. Ital. Sostanze Grasse* **1996**, *73*, 55−59.

(7) Fogliano, V.; Monti, S. M.; Ritieni, A.; Gallo, M.; Della Medaglia, D.; Ambrosino, M. L.; Sacchi, R. Antioxidant activity of virgin olive oil phenolic compounds in a micellar system. *J. Sci. Food Agric.* **1999**, *37*, 1458−1465.

(8) Medina, I.; Satué-Gracia, M. T.; German, J. B.; Frankel, E. N. Comparison of natural polyphenols antioxidant from extra virgin olive oil with synthetic antioxidants in tuna lipids during thermal oxidation. *J. Agric. Food Chem.* **1999**, *47*, 4873−4879.

(9) Porter, L. P. Paradoxical behavior of antioxidants in food and biological systems. *Toxicol. Ind. Health* **1993**, *9*, 1−2.

(10) Tsimidou, M. Polyphenols and Quality of Virgin Olive Oil in Retrospect. *Ital. J. Food Sci.* **1998**, *10* (2), 99−116.

(11) European Community Regulation 2568/91. *Off. J. Eur. Communities* **1991**, *L248* (Sept 5).

(12) Christie, W. W. *Lipid Analysis*; The Oily Press: Dundee, Scotland, 1982.

(13) Tonolo, G.; Marzo, S. Determinazione della vitamina E aggiunta e dei tocoferoli naturali negli oli di semi dietetici via HPLC. *Riv. Ital. Sostanze Grasse* **1989**, *66*, 3−6.

(14) Vasquez Roncero, A. Les Polyphenols de l'Huile d'Olive et leur Influence sur les Characteristiques de l'Huile. *Rev. Fr. Corps Gras* **1978**, *25*, 25−26.

(15) Montedoro, G. M.; Servili, M.; Baldioli, R.; Selvaggini, E.; Miniati, A.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil. Spectroscopic characterization of the secoiridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228−2234.

(16) Limiroli, R.; Consonni, R.; Ottolina, G.; Marsilio, V.; Bianchi, G.; Zetta, L. ^{1}H and ^{13}C characterization of new oleuropein aglycones. *J. Chem. Soc. Perkin Trans. 1* **1995**, *47*, 1519−1523.

(17) Brenes, M.; Hidalgo, J. H.; Garcia, A.; Rios, J. J.; Garcia, P.; Zamora, R.; Garrido, A. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. *J. Am. Oil Chem. Soc.* **2000**, *77*, 715−720.

(18) Mateos, R.; Espartero, J. L.; Trujillo, M.; Ríos, J. J.; León-Camacho, M.; Alcudia, F.; Cert, A. Determination of Phenols, Flavones and Lignans in Virgin Olive Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection. *J. Agric. Food Chem.* **2001**, *49*, 2185−2192.

(19) Monti, S.; Ritieni, A.; Sacchi, R.; Skog, K.; Borgen, E.; Fogliano, V. Characterisation of Phenolic Compounds in Virgin Olive Oil and Their Effect on The Formation of Carcinogenic/Mutagenic Heterocyclic Amines in a Model System. *J. Agric. Food Chem.* **2001**, *49*, 3969−3975.
(20) Tsimidou, M.; Papadopoulos, G.; Boskou, D. Determination of phenolic compounds in virgin olive oil by reversed-phase HPLC with emphasis on UV detection. *Food Chem.* **1992**, *44*, 53−60.
(21) Huang, S. W.; Hopia, K.; Schwarz, E. N.; Frankel, E. N.; German, J. B. Antioxidant Activity of α-Tocopherol and Trolox in Different Lipid substrates: Bulk Oil *vs* Oil-in-Water Emulsions. *J. Agric. Food Chem.* **1996**, *44*, 444−452.
(22) Frankel, E. N.; Huang, S.-W.; Kanner, J.; German, J. B. Interfacial phenomena in the evaluation of antioxidants: bulk oil *vs* emulsions. *J. Agric. Food Chem.* **1994**, *42*, 1054−1059.
(23) Stöckmann, H.; Schwarts, K.; Huynh-Ba, T. The influence of various emulsifiers on the partitioning and antioxidant activity of hydroxybenzoic acids and their derivatives in oil-in-water emulsions. *J. Am. Oil Chem. Soc.* **2000**, *77*, 535−542.
(24) Visioli, F.; Galli, C. Natural antioxidants and prevention of coronary heart desease: the potential role of olive oil and its minor constituents. *Nutr. Metab. Cardiovas. Dis.* **1995**, *5*, 306−314.
(25) Visioli, F.; Galli, C. Olive oil phenols and their potential effects on human health. *J. Agric. Food Chem.* **1998**, *46*, 4292−4296.
(26) Manna, C.; Galletti, P.; Cucciolla, V.; Moltedo, O.; Leone, A.; Zappia, V. The protective effects of the olive polyphenol (3,4-dihydroxyphenyl) ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in caco-2 cells. *J. Nutr.* **1997**, *127*, 286−292.
(27) Ramirez-Tortosa, M. C.; Urbano, G.; Lopez-Jurado, M.; Nestares, T.; Gomez, M. C.; Mir, A.; Ros, E.; Mataix, J.; Gil, A. Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. *J. Nutr.* **1999**, *129*, 2177−2183.
(28) Hansen, K.; Adsersen, A.; Christensen, S. B.; Jensen, S. R.; Nyman, U.; Smitt, U. W. Isolation of an angiotensin converting enzyme (ACE) inhibitor from *Olea europaea* and *Olea lancea*. *Phytomedicine* **1996**, *2*, 319−325.

Received for review November 15, 2001. Revised manuscript received February 15, 2002. Accepted February 25, 2002. This work was supported in part by the FSE (research fellow to A. P.) 1994/99 "Ricerca, Sviluppo Tecnologico ed Alta Formazione nelle Regioni dell'Obiettivo 1" (Provision 1.1).

JF011526L