Comparison of Natural Polyphenol Antioxidants from Extra Virgin Olive Oil with Synthetic Antioxidants in Tuna Lipids during Thermal Oxidation

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Polyphenols extracted from extra virgin olive oil (EVOO) were tested for their ability to inhibit lipid oxidation of canned tuna. Hydroperoxide formation during oxidation was monitored by measurement of peroxide value and decomposition of hydroperoxides by static headspace gas chromatographic analysis of volatiles. In tuna oxidized at 40 and 100 °C, 400 ppm of the EVOO polyphenols was an effective antioxidant as compared with 100 ppm of a 1:1 mixture of the synthetic antioxidants butylated hydroxytoluene and butylated hydroxyanisole. However, at concentrations <100 ppm, the EVOO phenolic compounds promoted hydroperoxide formation and decomposition. The EVOO polyphenols were effective antioxidants when added to heated tuna muscle in the presence of either brine or refined olive oil. The oxidation rate in tuna muscle packed in brine was higher than that of tuna packed in refined olive oil. The EVOO polyphenols had higher antioxidant activity in the brine samples than in the refined olive oil. The higher antioxidant activity of EVOO polyphenols in tuna packed in brine may be explained by their greater affinity toward the more polar interface between water and the fish oil system.

Keywords: *Tuna fish lipids; thermal oxidation; antioxidants; polyphenols; packing media (brine, refined olive oil)*

INTRODUCTION

Lipid oxidation has long been recognized as an important cause of food quality deterioration during the processing and storage of fatty fish (Cheftel and Cheftel, 1976; Hsieh and Kinsella, 1989). Because thermal treatment of foods can damage lipids, processes that involve either low or high temperatures, such as smoking or canning, can contribute to the degradation of fish quality by autoxidation of the polyunsaturated fatty acids (PUFA) that are highly concentrated in fish muscle (Sànchez Muniz et al., 1992; Medina et al., 1995). To prolong the shelf life of fatty fish species during food processing and to conserve nutritional value, inhibition of $n-3$ PUFA oxidation is critical (Hale and Brown, 1983; Caroll and Braden, 1986).

Synthetic antioxidants have been used to decrease lipid oxidation during the processing and storage of seafood (Boyd et al., 1993). However, the use of chemical additives has raised questions regarding food safety and toxicity (Chang et al., 1977). Studies on lipid oxidation in fish tissues demonstrated that some polyphenols have potent antioxidant activity compared to butylated hydroxytoluene (BHT) (Ramanathan et al., 1992). Among packing oils used in the fish canning industry, extra virgin olive oil (EVOO) contains natural polyphenols that inhibit fish lipid oxidation during the canning of tuna (Medina et al., 1998a). The natural polyphenols from EVOO may act as free radical acceptors as well as metal chelators (Afanas'ev et al., 1989; Xin et al., 1990). Although fish lipid oxidation was accelerated in brine and refined olive oil packing media and decreased the quality of canned fish, these effects were inhibited when EVOO was used as the packing medium (Medina et al., 1998a). However, the strong fruity flavor of EVOO and its higher cost may limit its use in fish processing. Because oils such as olive oil and seed oils are subject to loss of natural antioxidants during refining (Solinas et al., 1982), polyphenols separated from EVOO may be protective when added during the processing or storage of marine products.

This work presents a study of the antioxidant effectiveness of polyphenols extracted from EVOO when added to tuna muscle subjected to thermal autoxidation after canning. The EVOO polyphenols were added as antioxidants in brine and refined olive oil, which are commonly employed as packing media in fish processing. Assessment of oxidative stability was based on measurements of peroxide value and formation of volatiles by static headspace gas chromatography (Frankel, 1993a).

MATERIALS AND METHODS

Materials. Refined olive oil and EVOO purchased locally were stored frozen in dark bottles under nitrogen. White muscle from six cans of tuna canned in water (~200 g each) was minced, wrapped in filter paper, and thoroughly mixed to obtain homogeneous samples. The antioxidant activity of EVOO phenolics was compared with that of a 1:1 mixture of BHT and butylated hydroxyanisole (BHA) (Sigma, St. Louis, MO). Phenolic compounds used as standards in HPLC analysis and the Folin–Ciocalteu reagent were purchased from Sigma. All chemicals and solvents used were of either analytical or HPLC grade (Fisher Scientific, Fair Lawn, NJ).

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Phenol Extraction and Quantitation. The phenolic compounds from EVOO were extracted with ethanol and water (Vázquez Roncero, 1978). The extract was evaporated to dryness under nitrogen, and the residue was dissolved in 5 mL of ethanol prior to analysis of phenols by reversed-phase HPLC. Total phenol content was determined according to the Folin−Ciocalteu method (Singleton and Rossi, 1965) and expressed as gallic acid equivalents (GAE).

Phenol Composition by HPLC. Total phenol extracts were analyzed by reverse-phase HPLC (Della Medaglia et al., 1996). Peaks were identified by comparing their relative retention times with those of standards. Major peaks corresponding to complex phenols (hydrolyzable phenols) were assigned to the structures identified by Montedoro et al. (1993) on the basis of their relative elution and ^{1}H NMR spectroscopy (Della Medaglia et al., 1996).

Tocopherols. Tocopherols were analyzed in EVOO and refined olive oil by reversed-phase HPLC (Manzi et al., 1996).

Effect of Antioxidants on Tuna Muscle Oxidation. One-milliliter aliquots of aqueous antioxidant solutions were added directly to minced tuna muscle samples (~20 g) at final concentrations of 50−400 ppm of total phenols and 100 ppm of 1:1 BHT/BHA. One-gram portions of tuna muscle were placed into 6-mL headspace vials. Three different tuna cans were used for replicating the experiments.

Packing Media. Two different packing media were added to a second set of tuna muscle samples obtained from three new cans of tuna packed in water. Antioxidants dissolved in 1 mL of brine (20 g of NaCl/L of water) or in 1 mL of refined olive oil were added to 1 g of minced tuna muscle at 400 ppm of EVOO polyphenols and 100 ppm of 1:1 BHT/BHA. These 1-g portions were also placed into 6-mL headspace vials. All experiments were replicated three times.

Volatile Analysis. Six-milliliter headspace vials with 1-g portions of tuna muscle (containing 30−35% of water) were sealed with Teflon-lined caps and heated at 40 °C during 4 days and at 100 °C during 150 min as described previously (Medina et al., 1998b). Following heating of the tuna muscle samples, propanal, 2-ethylfuran, and pentanal were measured by static headspace gas chromatography (Medina et al., 1998b). Each analysis was performed three times under the same conditions.

Lipid Extraction. After analysis of volatiles, the vials were opened and the lipids were extracted from the tuna muscle (Bligh and Dyer, 1959). Lipid content was determined gravimetrically in duplicate and expressed as percentage wet weight (Herbes and Allen, 1983). Lipids extracted from the starting minced tuna muscle were analyzed to determine the initial fatty acid composition.

Fatty Acid Analysis of Oils and Tuna Lipids. Duplicate aliquots of oils, EVOO and refined olive oil, and lipids extracted from the starting tuna muscle were converted to methyl esters (Lepage and Roy, 1986) and analyzed by gas chromatography (Christie, 1982).

Measurement of Peroxides. Peroxide value of tuna muscle corresponding to each vial was determined according to the ferric thiocyanate method (Chapman and McKay, 1949) and was expressed as millimoles of oxygen per kilogram of lipid. Analyses were performed in triplicate.

Statistical Analysis. The data were compared by one-way analysis of variance (Sokal and Rohlf, 1981), and comparisons of the means were made by a least-squares difference method (Statsoft, 1994).

RESULTS

Composition of EVOO, Refined Olive Oil, and Tuna Muscle. EVOO had a fatty acid composition similar to that of the refined olive oil. Both oils had a high content of oleic acid (73−75%) and linoleic acid (8%), whereas tuna muscle was rich in 22:6*n*3 (Table 1). Tuna muscle showed low initial peroxide values (0.6 ± 0.1 mmol/kg of oil). The tocopherol and phenol content and the peroxide values of EVOO and refined olive oil are presented in Table 2. EVOO was richer in phenolic compounds (860 mg of GAE/kg of oil) than the refined olive oil (8 mg of GAE/kg of oil). The concentrations of tocopherols in the two oils were not very different and ranged between 99 and 139 ppm. EVOO phenolics were composed of single phenols, such as tyrosol and hydroxytyrosol, and complex phenols including oleuropein aglycon and elenolic derivatives of tyrosol and hydroxytyrosol, which were the major components (Figure 1 and Table 3). The antioxidant properties of hydroxytyrosol have been recently discussed (Baldioli et al., 1996; Visioli and Galli, 1998). Complex phenols are the main phenolic compounds in EVOO (Montedoro et al., 1993), and their antioxidant activity has been studied in virgin

Table 1. Fatty Acid Composition of Tuna Muscle, EVOO, and Refined Olive Oil[a,b]

fatty acid	tuna muscle	EVOO	refined olive oil
14:0	2.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
16:0	19.3 ± 0.3	8.8 ± 1.3	11.0 ± 1.8
16:1*n*7	2.1 ± 0.3	0.5 ± 0.1	0.7 ± 0.2
18.0	7.7 ± 0.6	3.4 ± 1.2	2.8 ± 0.3
18:1*n*9	18.7 ± 0.9	75.4 ± 4.7	73.4 ± 10.9
18:1*n*7	2.6 ± 0.3	1.8 ± 0.5	2.1 ± 0.4
18:2*n*6	1.5 ± 0.1	8.3 ± 0.9	8.2 ± 1.2
18:2*n*3	0.4 ± 0.1	0.8 ± 0.1	1.0 ± 0.2
20:1*n*9	4.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
20:4*n*6	1.8 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
20:4*n*3	0.6± 0.2	0.0 ± 0.0	0.0 ± 0.0
20:5*n*3	7.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
22:1*n*11	1.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
22:4*n*3	0.75 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
22:5*n*3	1.6 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
22:6*n*3	25.0 ± 1.6	0.0 ± 0.0	0.0 ± 0.0
24:1*n*9	1.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0

[a] Percent of total fatty acids is expressed as mean ± standard deviation for three samples. [b] Minor amounts (<1%) of 15:0, 16:1*n*5, 17:0, 17:1, 20:0, and 22:0 are not included.

Table 2. Peroxide Value and Content of Tocopherols and Phenols in EVOO and Refined Olive Oils[a]

oil	peroxide value	tocopherols[b]	total phenols[c]
EVOO	12.2 ± 1.3	139 ± 20	860 ± 36
refined olive oil	0.4 ± 0.1	99 ± 5	8.1 ± 1.3

[a] Data are expressed as mg/kg of oil, mean ± standard deviation. [b] Expressed as α-tocopherol with a detection limit 0.2 ppm; $n = 3$. [c] Expressed as gallic acid equivalents; $n = 3$.

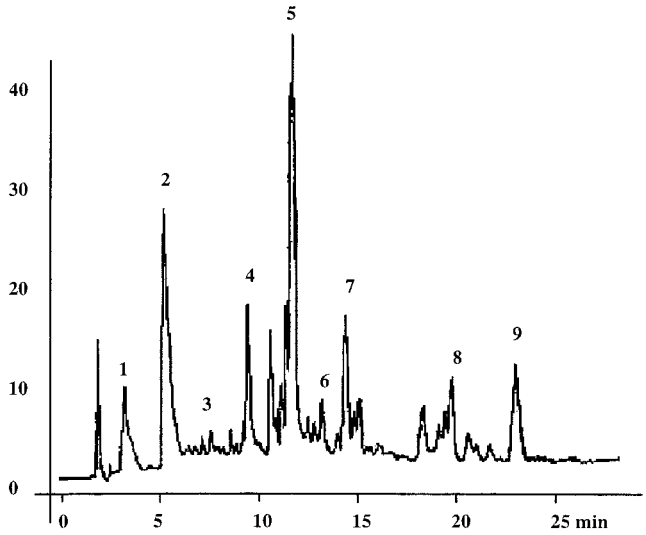

Figure 1. HPLC chromatogram of phenols extracted from EVOO. Peaks are labeled according to peaks designated in Table 3.

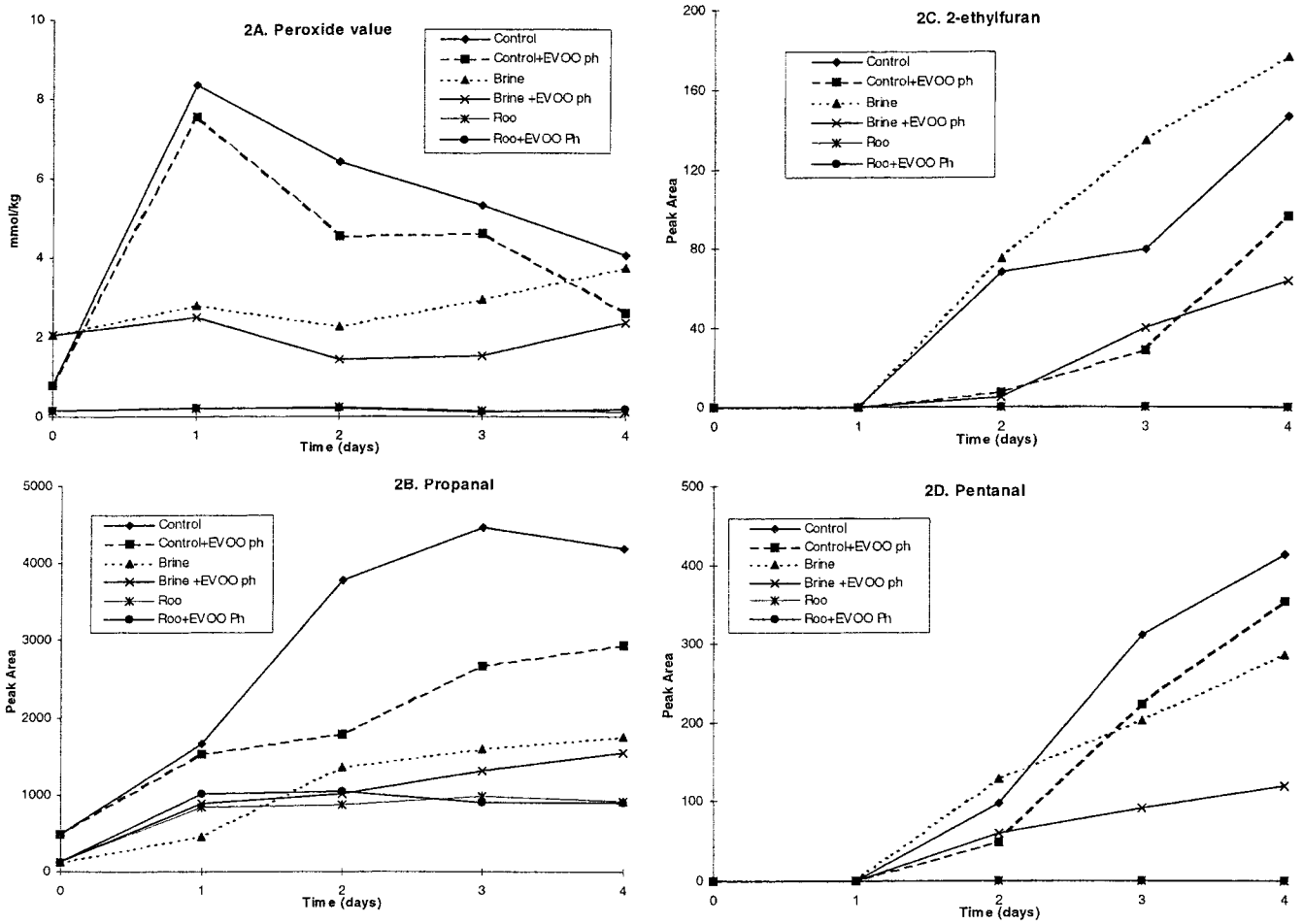

Figure 2. Effect of EVOO phenols on oxidative stability of tuna fish muscle at 40 °C in the presence and absence of packing media: (A) peroxide value; (B) propanal; (C) 2-ethylfuran; (D) pentanal. Abbreviations: control-EVOO ph, tuna sample in the presence of 400 ppm of EVOO phenols; control-brine, tuna muscle packed on brine; brine + EVOO ph, tuna muscle packed in brine and incubated in the presence of 400 ppm of EVOO phenols; control-ROO, fish muscle packed on refined olive oil; ROO + EVOO ph, fish muscle packed in refined olive oil and incubated in the presence of 400 ppm of EVOO phenols.

Table 3. Phenolic Composition of EVOO[a,b]

peak no.	component	phenols (mg/kg of oil)
1	hydroxytyrosol	55.6 ± 0.2
2	tyrosol	85.8 ± 4.3
3	*p*-coumaric acid	18.7 ± 2.2
4	derivative of oleuropein aglycon	85.4 ± 8.4
5	elenolic acid derivative bonded with tyrosol	266.6 ± 20.1
6	elenolic acid derivative bonded with hydroxytyrosol	34.3 ± 2.6
7	elenolic acid derivative bonded with hydroxytyrosol	42.6 ± 6.7
8	unknown	36.4 ± 7.4
9	unknown	42.0 ± 7.6

[a] Quantitative data are expressed as syringic acid, mean ± standard deviation of triplicate analysis. [b] See Figure 1 for HPLC analysis and Montedoro et al. (1993) for molecular structures.

and purified olive oils (Servili et al., 1996; Baldioli et al., 1996).

Effect of Phenol Antioxidants on Tuna Lipid Oxidation at 40 °C in the Absence of Brine or Refined Olive Oil. Lipid oxidation was followed in tuna muscle in the absence of brine or refined olive oil to determine an adequate concentration of EVOO phenolics. As measured by peroxide value, tuna muscle oxidized rapidly at 40 °C without an induction period (Figure 2A). Maximum peroxide values were reached during the first 24 h and then decreased significantly as a result of decomposition.There was no induction or lag period for the formation of propanal from tuna muscle in the absence of brine or refined olive oil during 4 days of oxidation at 40 °C (Figure 2B). Following an induction period of ~1 day, 2-ethylfuran and pentanal were formed in lower amounts than propanal during oxidation (Figure 2C,D). On the basis of hydroperoxide and volatile formation, the rate of oxidation in tuna muscle containing EVOO polyphenols was lower than that in control samples without added antioxidant (Figure 2).

Table 4 shows the calculated inhibition values of hydroperoxide formation, measured as peroxide values, during the oxidation of tuna samples with various concentrations of EVOO phenolics at 40 °C after 4 days: 200−400 ppm of EVOO phenols inhibited significantly oxidation in tuna muscle after 4 days of incubation; however, EVOO polyphenols were prooxidant between 50 and 100 ppm during the first 24 h (−106 and −114% of inhibition, respectively). The BHT/BHA mixture was also effective in inhibiting oxidation. The order of inhibiting hydroperoxide formation after 4 days was 100 ppm of BHT/BHA > 400 ppm of EVOO phenols = 200 ppm of EVOO phenols >100 ppm of EVOO phenols = 50 ppm of EVOO phenols.

EVOO phenolics were usually highly effective in inhibiting volatile formation during oxidation (Table 5).

Table 4. Effect of EVOO Phenolics and BHT/BHA on Hydroperoxide Formation[a,b] Measured as Peroxide Value in Tuna Fish Oxidized at 40 °C in Brine or Refined Olive Oil

packing material	antioxidant	concn (ppm)	day 4
none	control	0	0.0 ± 0.1^{d}
none	EVOO phenolics	400	36.1 ± 3.2^{b}
none	EVOO phenolics	200	46.3 ± 6.8^{b}
none	EVOO phenolics	100	23.8 ± 2.1^{c}
none	EVOO phenolics	50	26.4 ± 0.9^{c}
none	BHA/BHT	100	60.3 ± 4.5^{a}
brine	control	0	0.0 ± 0.1^{c}
brine	EVOO phenolics	400	37.2 ± 0.8^{b}
brine	BHA/BHT	100	53.6 ± 2.6^{a}
refined olive oil	control	0	0.0 ± 0.1^{a}
refined olive oil	EVOO phenolics	400	-4.7 ± 2.4^{a}
refined olive oil	BHA/BHT	100	2.3 ± 1.9^{a}

[a] Percent mean inhibition ± standard deviation of three samples; % inhibition = $[(C - S)/C] \times 100$, where C = hydroperoxide formed in control and S = hydroperoxide formed in sample; negative values represent prooxidant activity. [b] Values in each column for samples packed with the same material and with the same superscript letter were not significantly different ($p < 0.01$).

Concentrations of 200 and 400 ppm of EVOO phenolics inhibited propanal and 2-ethylfuran formation more effectively than did lower concentrations (100−50 ppm), which showed prooxidative effect for propanal formation. Pentanal formation was inhibited to the greatest extent by 400−200 ppm of EVOO phenols. Inhibition by 200−400 ppm of EVOO phenols was lower than that by 100 ppm of 1:1 BHT/BHA for 2-ethylfuran formation and higher for propanal and pentanal formation.

Effect of Phenols on Oxidative Stability of Tuna in Brine or Refined Olive Oil at 40 °C. EVOO phenols (400 ppm) and a 1:1 mixture of BHT/BHA (100 ppm) were added separately to tuna muscle in brine and in refined olive oil and oxidized at 40 °C. The peroxide value of tuna muscle in brine increased slowly during the storage at 40 °C (Figure 2A). Oxidation during 4 days at 40 °C was lower in tuna heated in brine containing 400 ppm of EVOO phenolics than in control samples heated with no antioxidants. Oxidation in tuna with refined olive oil was not observed after 4 days of incubation at 40 °C, and rates of samples with phenolic antioxidants were not significantly different from the control (Figure 2A). On the basis of peroxide value, the initial rate of oxidation in tuna muscle heated in the presence of brine was greater than that in muscle in refined olive oil. Oxidation in tuna with refined olive oil was not observed after 4 days of incubation at 40 °C, and rates of samples with phenolic antioxidants were not significantly different from the control (Figure 2A).

Natural EVOO phenolics at 400 ppm added to samples in brine were effective in inhibiting hydroperoxide formation at 40 °C after 4 days (Table 4). Their antioxidant activity, however, was weaker than that of BHT/BHA. No significant inhibition in hydroperoxide formation was observed in samples heated in refined olive oil in the presence of natural phenolic and synthetic antioxidants (Table 4).

Propanal formation was rapid in tuna samples incubated in brine, showing no induction period (Figure 2B). Oxidation measured by propanal formation during the first day of incubation at 40 °C was greater in samples of tuna muscle in brine heated with EVOO polyphenols than in control samples, but was lower in later periods of oxidation. Propanal was formed rapidly in samples of tuna packed in refined olive oil after the first day of oxidation, but no significant increase was observed after this day. The amounts of propanal produced after the second day of incubation were lower than in samples packed in brine (Figure 2B). The trend of propanal formation was similar to that in brine samples in showing prooxidant activity in the presence of phenolics during the initial period. 2-Ethylfuran and pentanal were produced only in smaller amounts than propanal (∼10 times smaller) after oxidation for 4 days in tuna samples incubated in brine, and they were not significantly produced in refined olive oil (Figure 2C,D). Tuna incubated with EVOO polyphenols in brine showed minor rates of ethylfuran and pentanal formation compared with the control samples.

On the basis of propanal formation, EVOO phenolics showed prooxidant activity initially in tuna in brine (−95.2% of inhibition for the first day), followed by effective inhibition (40.9%) (Table 5). Synthetic antioxidants showed lower inhibition than phenolics (25.7%). No inhibition of propanal formation was observed in tuna incubated in refined olive oil with EVOO polyphenols.

On the basis of 2-ethylfuran formation, phenolic compounds in brine inhibited oxidation (63.8%) after 4 days of oxidation, and the antioxidant activity was significantly greater than the BHT/BHA antioxidant mixture (54.8%) (Table 5). Pentanal formation in tuna samples heated in brine was highly inhibited in samples containing EVOO phenolic antioxidants during the entire incubation period (Table 5). Inhibition of pentanal

Table 5. Effect of EVOO Phenolics and BHT/BHA on Volatile Formation[a−c] by Tuna Muscle Oxidized at 40 °C after 4 Days with and without Added Brine or Refined Olive Oil

packing material	antioxidant	concn (ppm)	propanal	2-ethylfuran	pentanal
none	control	0	0.0 ± 0.0^{c}	0.2 ± 0.1^{e}	0.3 ± 0.1^{b}
none	EVOO phenols	400	29.8 ± 2.2^{b}	34.3 ± 2.9^{ab}	14.4 ± 1.2^{a}
none	EVOO phenols	200	53.7 ± 5.2^{a}	31.7 ± 2.3^{b}	10.6 ± 1.1^{a}
none	EVOO phenols	100	-9.4 ± 2.2^{d}	10.2 ± 1.3^{d}	-41.1 ± 5.7^{c}
none	EVOO phenols	50	-14.3 ± 2.2^{d}	27.7 ± 1.3^{c}	3.6 ± 0.8^{b}
none	BHT/BHA	100	-12.3 ± 3.6^{d}	45.5 ± 4.1^{a}	6.3 ± 4.8^{b}
brine	none	0	0.0 ± 0.1^{c}	0.0 ± 0.1^{c}	0.0 ± 0.0^{b}
brine	EVOO phenols	400	40.9 ± 1.3^{a}	63.8 ± 2.1^{a}	58.0 ± 0.9^{a}
brine	BHT/BHA	100	25.7 ± 3.5^{b}	54.8 ± 3.1^{b}	52.1 ± 4.5^{a}
refined olive oil	control	0	0.0 ± 0.1^{a}		
refined olive oil	EVOO phenols	400	2.4 ± 1.8^{a}		
refined olive oil	BHT/BHA	100	2.3 ± 2.1^{a}		

[a] Percent mean inhibition ± standard deviation of three samples; % inhibition = $[(C - S)/C] \times 100$, where C = volatiles formed in control and S = volatiles formed in sample. [b] Negative values represent prooxidant activity. [c] Values in each column for samples packed with the same material and with the same superscript letter are not significantly different ($p < 0.01$).

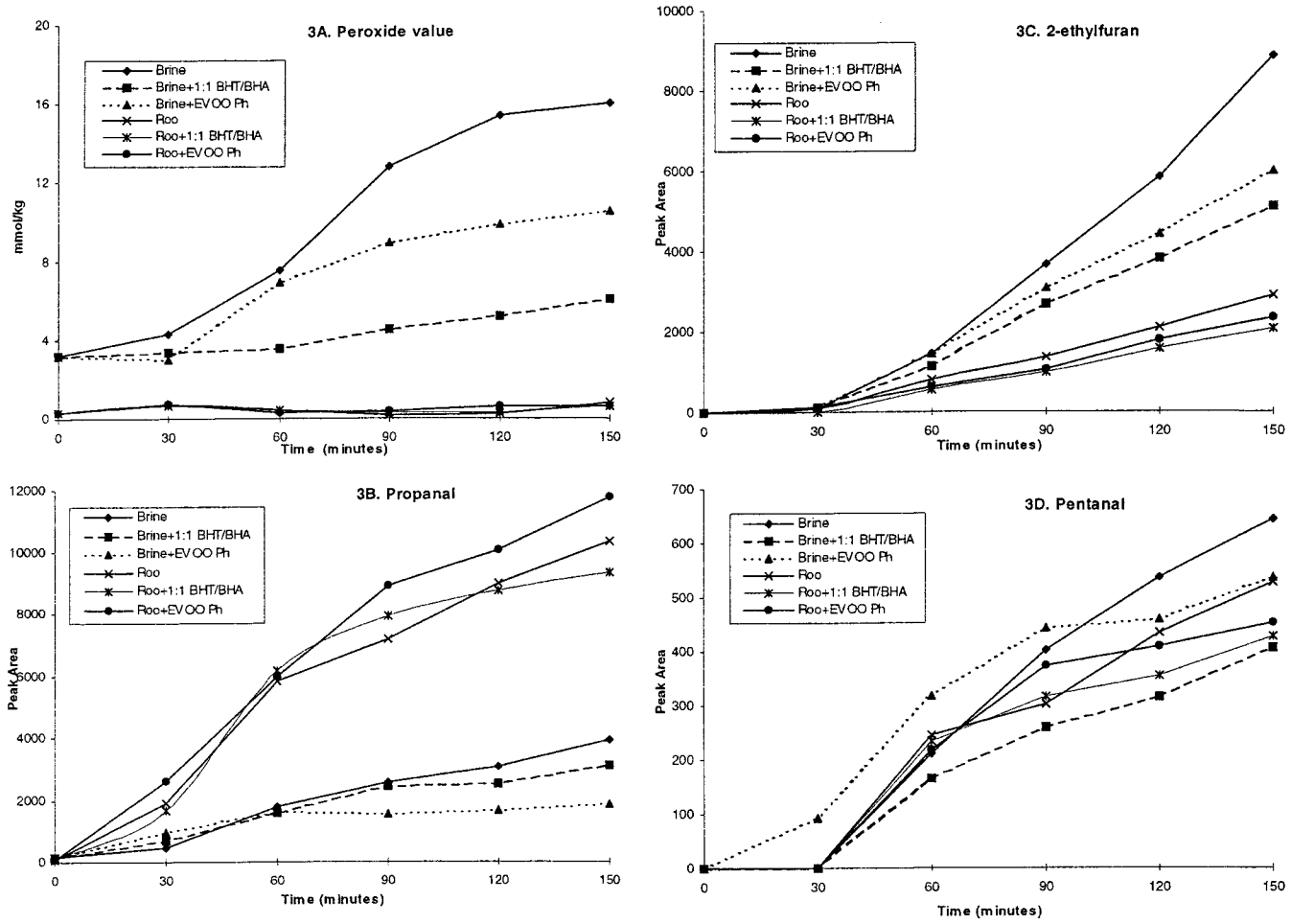

Figure 3. Effect of EVOO phenols and 100 ppm of a 1:1 mixture of BHT/BHA on the oxidative stability of tuna fish muscle at 100 °C: (A) peroxide value; (B) propanal; (C) 2-ethylfuran; (D) pentanal. Abbreviations: brine, fish muscle packed in brine; brine + 1:1 BHT/BHA, fish muscle packed in brine incubated in the presence of 1:1 BHT/BHA; brine + EVOO ph, fish muscle packed in brine incubated in the presence of 400 ppm of EVOO phenols; ROO, fish muscle packed in refined olive oil; ROO + 1:1 BHT/BHA, fish muscle packed in refined olive oil in the presence of 1:1 BHT/BHA; ROO + EVOO ph, fish muscle packed in refined olive oil in the presence of 400 ppm of EVOO phenols.

formation was not significantly different between EVOO phenolic and BHT/BHA antioxidants.

Effect of Phenols on Oxidative Stability of Tuna in Brine or Refined Olive Oil at 100 °C. Tuna heated at 100 °C during 150 min oxidized very rapidly in brine and was more resistant to oxidation in refined olive oil (Figure 3A). These results agree with those obtained with tuna heated at 40 °C. In control samples of tuna heated in the presence of brine, an induction period of ~30 min was observed for the formation of peroxides, followed by an increase during the remaining heating period. EVOO phenolics significantly inhibited hydroperoxide formation during heating of tuna at 100 °C (34.5% inhibition after 150 min). The BHT/BHA mixture showed better antioxidant activity than the EVOO phenolic extract (62.2% inhibition after 150 min). The oxidative stability of the control tuna heated with refined olive oil was not improved with either EVOO phenols or 1:1 BHT/BHA (Figure 3A).

Propanal, 2-ethylfuran, and pentanal formed rapidly in tuna muscle heated with brine or refined olive oil at 100 °C (Figure 3B−D). The rate of propanal formation in tuna heated in the presence of brine was lower than in tuna heated in the presence of refined olive oil. In tuna with added brine, EVOO phenolic extracts inhibited propanal formation more effectively than did the mixture of BHT/BHA (52 and 20% inhibition, respectively, after 150 min). EVOO polyphenol antioxidants were very effective in inhibiting 2-ethylfuran formation during the whole oxidation period and compared to the synthetic antioxidants. After an induction period of 30 min, BHT/BHA was the best inhibitor of pentanal formation in tuna treated with brine (36% of inhibition) after 150 min.

On the basis of volatile formation, tuna muscle heated with refined olive oil oxidized after an induction period of 30 min (Figure 3B−D). EVOO phenolics were highly effective in inhibiting 2-ethylfuran formation during the 150-min heating period (21% inhibition).

DISCUSSION

The present investigation showed that EVOO phenols at ≥200 ppm effectively inhibited both hydroperoxide formation and decomposition in fish muscle heated at 40 °C. Concentrations of EVOO phenols <100 ppm inhibited 2-ethylfuran formation but promoted hydroperoxide formation during the first 24 h. These results agree with a previous report of the significant antioxidant activity of an EVOO extract with a high content of polyphenols for fish during canning (Medina et al., 1998a). EVOO phenols were better antioxidants at 400 ppm than BHT/BHA at 100 ppm as measured by the inhibition of hydroperoxide decomposition. In contrast,

EVOO phenols were less effective antioxidants than the mixture BHT/BHA as measured by the inhibition of hydroperoxide formation. This difference may be attributed to the different molar concentration of the more active polyphenol antioxidants and the synthetics.

When tuna muscle was heated in the presence of brine, phenolic compounds inhibited effectively the formation of hydroperoxides and volatiles during oxidation at 40 °C and hydroperoxide and 2-ethylfuran formation at 100 °C. Small amounts of linoleic and linolenic acid in EVOO remaining in the phenolic extract could account for the pentanal measured in brine tuna samples treated with EVOO phenols at 100 °C and for the propanal observed initially at 40 °C. Therefore, the fatty acids in the refined olive oil may explain the amounts of propanal and pentanal found in samples incubated with this oil at 100 °C.

During canning processing at high temperatures, complex phenols are hydrolyzed to simple compounds (tyrosol and hydroxytyrosol) (Medina et al., 1998a). The antioxidant effect observed previously was attributed to migration of hydrophilic phenols into the tuna muscle−water interface (Medina et al., 1998a). Loss of the hydrogen-donating ability of polyphenols due to hydrolysis may explain the similar effectiveness of EVOO phenols and synthetic antioxidants in tuna samples in brine during oxidation at 100 °C. The activity of phenolic antioxidants is also dependent on temperature and other oxidation conditions (Frankel, 1993b). Phenolic compounds were thus more effective in brine samples oxidized at 40 °C than in the same samples oxidized at 100 °C, a temperature at which phenols were likely decomposed or interacted with other muscle components (Medina et al., 1998a).

On the basis of hydroperoxide and 2-ethylfuran formation, antioxidant efficiency of both kinds of antioxidants, synthetic and natural EVOO phenolics, was greater in tuna heated with brine than in tuna heated with refined olive oil. Hydrophilic antioxidants oriented at the air−oil interface in bulk oil systems are known to be more protective against oxidation than they are in aqueous systems (Frankel et al., 1996). In oil-in-water emulsions, hydroperoxide formation and decomposition are dependent on the effective concentrations of antioxidants in the oil and water phases and the interface (Frankel et al., 1996). The higher antioxidant activity of phenolic compounds in tuna heated in brine may be explained by the greater affinity of these antioxidants toward the more polar interface that exists in this system than in the tuna heated with refined olive oil. In addition, the hydrophobic components are more active in oil-in-water emulsions than in bulk oil (Frankel, 1998).

EVOO phenols may be more efficient than synthetic antioxidants in preventing rancidity in fish. The use of natural antioxidants such as EVOO polyphenols as components of the packing media of marine products subjected to thermal processing may improve their nutritional and sensory quality. Because phenolic compounds are lost during the processing of common oils such as olive oil and seed oils, the use of polyphenols from EVOO or extracted from olive mill waste waters as natural antioxidants may play an important role during the processing or storage of marine products. Studies about the antioxidant activity of the different individual phenols identified in the extract should be continued to elucidate the antioxidant mechanism found in this work.

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