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Hydroxytyrosol Prevents Oxidative Deterioration in Foodstuffs Rich in Fish Lipids

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Hydroxytyrosol, a natural phenolic compound obtained from olive oil byproduct, was characterized as an antioxidant in three different foodstuffs rich in fish lipids: (a) bulk cod liver oil (40% of ω-3 PUFAs), (b) cod liver oil-in-water emulsions (4% of ω-3 PUFAs), and (c) frozen minced horse mackerel (*Trachurus trachurus*) muscle. Hydroxytyrosol was evaluated at different concentration levels (10, 50, and 100 ppm), and its antioxidant capacity was compared against that of a synthetic phenolic, propyl gallate. Results proved the efficiency of hydroxytyrosol to inhibit the formation of lipid oxidation products in all tested food systems, although two different optimal antioxidant concentrations were observed. In bulk oil and oil-in-water emulsions, a higher oxidative stability was achieved by increasing the concentration of hydroxytyrosol, whereas an intermediate concentration (50 ppm) showed more efficiency, delaying lipid oxidation in frozen minced fish muscle. The endogenous depletion of α-tocopherol and ω-3 polyunsaturated fatty acids (ω-3 PUFAs) was also inhibited by supplementing hydroxytyrosol in minced muscle; however, the consumption of the endogenous total glutathione was not efficiently reduced by either hydroxytyrosol or propyl gallate. A concentration of 50 ppm of hydroxytyrosol was best to maintain a longer initial level of α-tocopherol (approximately 300 μg/g of fat), whereas both 50 and 100 ppm of hydroxytyrosol were able to preserve completely ω-3 PUFAs. Hydroxytyrosol and propyl gallate showed comparable antioxidant activities in emulsions and frozen fish muscle, and propyl gallate exhibited better antioxidant efficiency in bulk fish oil.

KEYWORDS: Lipid oxidation; antioxidant; hydroxytyrosol; fish lipids; bulk oil; emulsions; minced muscle

INTRODUCTION

Fish lipids are an important dietary source for ω-3 long-chain polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA, 20:5ω-3), and docosahexaenoic acid (DHA, 22:6ω-3), which have shown potential benefits on human health, particularly in the prevention of cardiovascular diseases (*1*−*3*) and cancer (*4*). Health organizations have made specific guidelines to the general population to increase the intakes of ω-3 PUFAs; for example, the World Health Organization advises that total ω-3 PUFAs should cover 1−2% of the energy, and the American Heart Association and the Scientific Advisory Committee of Nutrition (U.K.) recommend eating fish, particularly fatty fish, at least two times weekly (*5*). In an attempt to achieve these recommended intakes, PUFAs have been recently incorporated into foods typically devoid of these nutrients and with high acceptance for the consumer, such as eggs, bread, baby food, dairy products, juices, cereals, and milk.

However, a high content of fish lipids dramatically compromises shelf life because PUFAs suffer intense oxidative deterioration during processing and storage. Deterioration in flavor, color, texture, and nutritive value and the production of toxic compounds are some of the negative consequences of lipid oxidation (*6, 7*). The application of natural antioxidants is an emerging methodology for controlling lipid oxidation and its deleterious repercussions (*8*−*12*). Previous investigations have demonstrated the efficiency of phenolic extracts from extra virgin olive oil on inhibiting lipid oxidation in tuna muscle subjected to thermal treatments (*13, 14*) and other foods containing ω-3 PUFAs (*15*). The antioxidant capacity of olive oil extracts is attributed to lipophilic phenolic components, mainly tocopherols, and simple phenolics with polar character, such as tyrosol, hydroxytyrosol, and their acetate esters (*16*). Hydrophilic phenolics offer advantages over lipophilic ones as food additives because the former can be easily incorporated into food systems as aqueous solutions. Previous investigations showed high efficiency of hydroxytyrosol in preventing frozen fatty fish fillets from lipid oxidation (*17*) and low antioxidant activity preserving isolated fish membranes under hemoglobin and iron-promoted oxidation (*18*). Moreover, hydroxytyrosol could be profitable obtained from olive oil byproduct (*19, 20*).

The objective of our study was to examine in depth the capacity of hydroxytyrosol (**Figure 1**) to inhibit lipid oxidation in food systems rich in fish lipids. With this purpose, different concentrations of hydroxytyrosol were incorporated into bulk cod liver oil (40% of ω-3 PUFAs), cod liver oil-in-waster emulsions (4% of ω-3 PUFAs), and minced horse mackerel muscle, and the formation of lipid oxidation products was

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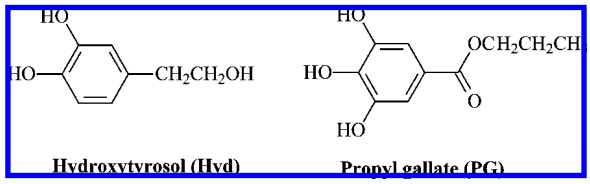

Figure 1. Molecular structures of hydroxytyrosol and propyl gallate.

monitored during controlled storage. The oxidative status in frozen fish muscle was also evaluated by means of studying the depletion kinetics for ω-3 PUFAs and endogenous antioxidants of fish muscle, α-tocopherol, and total glutathione. The antioxidant capacity of hydroxytyrosol was compared with the synthetic phenolic propyl gallate (**Figure 1**) and discussed on the basis of their polar characteristics, electron-donating capacities, and ferrous iron-chelating properties.

MATERIALS AND METHODS

Materials. Fresh horse mackerel (*Trachurus trachurus*) was acquired from a local market, and light muscle was immediately obtained and minced. High-quality cod (*Gadus morhua*) liver oil composed by 40.6% ω-3 PUFAs (3.7% 18:3ω3; 1.3% 20:4ω3; 14.9% 20:5ω3; 2.8% 22:5ω3, and 17.9% 22:6ω3) was purchased from Fluka (New-Ulm, Swizerland). Soybean lecithin containing 40% L-α-phosphatidylcholine (Sigma, St. Louis, MO) was used as surfactant in oil-in-water emulsions. Hydroxytyrosol of 90−95% purity, obtained by hydrothermal treatment of olive oil byproduct (*20*), was kindly provided by the Instituto de la Grasa (CSIC, Sevilla, Spain). Reduced glutathione, 5-sulfosalicylic acid, trichloroacetic acid, and 1,1,3,3-tetraethoxypropane were obtained from Sigma (St. Louis, MO). Propyl gallate was purchased from Merck (Darmstadt, Germany). All chemicals and solvents used were either analytical or HPLC grade (Ridel-Haën, Seelze, Germany). Water was purified through a Millipore-Q plus (Millipore Corp., Bedford, MA).

Polar Character. The partition coefficient between fish oil and water was used to estimate the polar character of hydroxytyrosol and propyl gallate. Briefly, 1 mL of fish oil and 1 mL of water containing phenolics were adequately mixed and centrifuged. The phenolic concentration in the aqueous phase before and after mixing was quantified according to the Folin−Ciocalteu method (*21*). The amount of phenolics in the oil phase was calculated as the difference between the total phenolic amount in water before and after mixing oil in water. The partition coefficients were calculated according to the method of Huang et al. (*22*)

$$\frac{V_W}{V_O} \times \frac{W_O}{W_W}$$

where V_W = volume of water, V_O = volume of fish oil, W_O = amount of phenolic in oily phase, and W_W = amount of phenolic in aqueous phase.

Preparation of Bulk Fish Oil. Cod liver oil samples (5 g) with and without phenolics were prepared in screw-capped 50 mL Erlenmeyer flasks. Hydroxytyrosol and propyl gallate were incorporated in methanol solutions, and methanol was removed under a stream of nitrogen before the addition of cod liver oil. Samples were subsequently sonicated for a total dispersion of the phenolics for 5 min. The oxidative stability of fish oil was monitored during 7 days of storage at 40 °C by measuring the formation of conjugated diene hydroperoxides.

Preparation of Oil-in-Water Emulsions. Cod liver oil emulsions were prepared in water containing 1% lecithin and 10% fish oil, as previously described Huang et al. (*23*). Briefly, cod liver oil was emulsified in water using lecithin as emulsifier and sonicating at high power for 10 min. Phenolics were added in methanol solutions into screw-capped 50 mL Erlenmeyer flasks, and then methanol was removed under a stream of nitrogen before the addition of oil-in-water emulsions (5 g). Samples were subsequently sonicated for 5 min for a total dispersion of phenolics. The oxidative stability of emulsions was monitored during 5 days of storage at 30 °C by measuring the formation of conjugated diene hydroperoxides and fluorescence compounds.

Preparation of Frozen Minced Fish Muscle. Twenty-five different fish of fresh Atlantic horse mackerel (*T. trachurus*) were deboned and eviscerated. White muscle was separated and minced to obtain a muscle homogenate. Hydroxytyrosol and propyl gallate were incorporated into the minced fish muscle as aqueous solution/suspension, and portions of 10 g of minced muscle were placed into screw-capped 50 mL Erlenmeyer flasks and stored at −10 °C for 9 weeks. Lipid oxidation was monitored by the formation of lipid oxidation products: hydroperoxides (peroxide value), conjugated diene hydroperoxides, and thiobarbituric acid reactive substances (TBARS). The consumption kinetics of ω-3 PUFA and the endogenous antioxidants α-tocopherol and total glutathione were also used as indicators of oxidative stress in horse mackerel muscle during frozen storage.

Determination of Total Glutathione. Glutathione was extracted from fish muscle using a modified procedure of Petillo et al. (*24*). Two grams of muscle was homogenized with 10 mL of chilled 5% 5-sulfosalicylic acid. Reduced glutathione (GSH) and oxidized glutathione (GSSG) were quantified together as total glutathione using the method described by Griffith (*25*). Reduced glutathione (Sigma) was used as standard.

Determination of α-Tocopherol. α-Tocopherol was extracted from minced fish muscle by an adaptation of Burton's et al. (*26*) procedure as described by Pazos et al. (*11*). α-Tocopherol was analyzed by HPLC according to the method of Cabrini et al. (*27*).

Lipid Extraction. Lipids were extracted from fish muscle according to the method of Bligh and Dyer (*28*). Lipid content was determined gravimetrically and expressed on a wet weight basis (*29*).

Peroxide Value. Peroxide value was determined in fish muscle by using the ferric thiocyanate method (*30*) and expressed as milliequivalents of oxygen per kilogram of lipid.

Conjugated Diene Hydroperoxides. Bulk fish oil and emulsion samples (10−100 mg each) were dissolved in hexane or ethanol, respectively. The content of conjugated diene hydroperoxides was calculated on the basis of the absorbance at 234 nm in a Beckman DU 640 spectrophotometer (Beckman Instruments Inc., Palo Alto, CA). Absorbance values were converted into millimoles of hydroperoxides per kilogram of oil values using 29000 mL $mmol^{-1}$ cm^{-1} as molar absorption coefficient (*31*). In the case of minced fish muscle, conjugated diene hydroperoxides were determined by dissolving 10−100 mg of the extracted lipids in hexane and measuring the absorbance as above-described.

Thiobarbituric Acid Reactive Substances (TBARS) Analyses. TBARS (mg of malonaldehyde/kg of muscle) were determined according to the method of Vyncke (*32*). 1,1,3,3-Tetraethoxypropane was used as standard.

Measurement of Fluorescent Compounds. Fluorescence compounds, which are generated from the interaction of secondary lipid oxidation products (aldehydes and ketones) with the amino groups of lecithin, were measured after dissolving 100 mg of emulsion in ethanol. Fluorescence was measured at 345/416 and 393/463 nm in a Perkin-Elmer LS 3B spectrophotometer (Perkin-Elmer, Wellesley, MA) and was standardized with a quinine sulfate solution (1 μg/mL in 0.05 M H_2SO_4) at the corresponding wavelengths (*33*). The relative fluorescence (RF) was calculated as follows: RF = F/F_{st}, where F is the sample fluorescence at each excitation/emission maximum and F_{st} is the corresponding fluorescence intensity of the quinine sulfate solution at the corresponding wavelength. The fluorescence shift (δF) was calculated as the ratio between both RF values: $\delta F = RF_{393/463nm}/RF_{345/416nm}$,

Fatty Acid Analysis. Lipids extracted from horse mackerel muscle were converted to methyl esters (*34*) and analyzed by gas chromatography (*35*).

Statistical Analysis. All samples were prepared in triplicate, and experiments were performed at least twice. The data were compared by one-way analysis of variance (ANOVA) (*36*), and the means were compared by a least-squares difference method (*37*). Significance was declared at $p < 0.05$.

RESULTS

Polar Character of Phenolics. The oil/water partition coefficients of hydroxytyrosol and propyl gallate were 0.61 ± 0.02

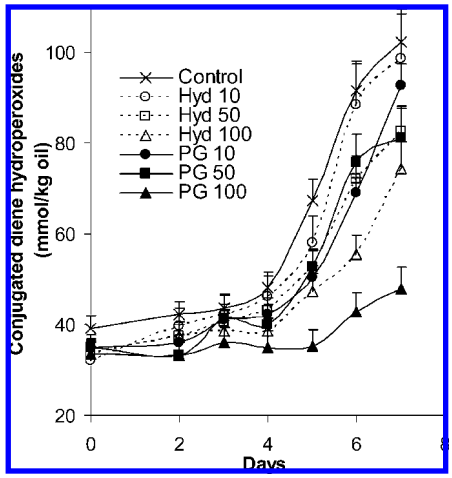

Figure 2. Effect of hydroxytyrosol (Hyd) and propyl gallate (PG) at different concentrations (10, 50, and 100 ppm) on the formation of conjugated diene hydroperoxides in fish oil during oxidation at 40 °C.

and 0.98 ± 0.11, respectively. Therefore, the fractions of hydroxytyrosol and propyl gallate found in the oily phase after the oil/water partitioning were correspondingly 38.0 ± 0.6 and 49.4 ± 2.9%. These partitioning behaviors showed more hydrophilic character for hydroxytyrosol compared to propyl gallate.

Bulk Fish Oil. The antioxidant capacity of hydroxytyrosol (Hyd) and propyl gallate (PG) was tested at different concentrations (10, 50, and 100 ppm) in bulk cod liver subjected to thermal oxidation at 40 °C. Fish oil without phenolic supplementation (control) or supplemented with 10 ppm of hydroxytyrosol showed the fastest lipid oxidation, as observed from the formation of conjugated diene hydroperoxides (**Figure 2**).Therefore, hydroxytyrosol was effective in inhibiting the lipid oxidation rate of bulk oil by supplementation at 50 or 100 ppm, and propyl gallate was effective at phenolic concentrations of 10−100 ppm. With both phenolics, higher oxidation inhibition was reached by supplementation at 100 ppm. The antioxidant efficiency order was found to be PG 100 > Hyd 100 > Hyd 50 ≈ PG 50 ≈ PG 10 > Hyd 10.

Fish Oil-in-Water Emulsions. Control emulsions developed faster generation of conjugated hydroperoxides during the propagation phase of oxidation, compared to those emulsions supplemented with hydroxytyrosol (10, 50, and 100 ppm) (**Figure 3A**). Higher concentrations of hydroxytyrosol (50 and 100 ppm) were also active in delaying induction periods 2−3 days for conjugated diene formation. The decreasing order in inhibiting the formation of conjugated dienes was Hyd 100 > Hyd 50 > Hyd 10 ≈ PG 10. The fluorescent compounds originating from interactions between secondary oxidation products (aldehydes and ketones) and amine groups (the surfactant phosphatidylcholine is a source of amine groups in the sample) were also significantly reduced by hydroxytyrosol; however, such inhibitory activity did not exhibit hydroxytyrosol concentration dependence in the concentration range studied (10−100 ppm) (**Figure 3B**). On the basis of conjugated diene hydroperoxides, the antioxidant activity in oil-in-water emulsions was found to be positively correlated with the hydroxytyrosol concentration, providing hydroxytyrosol at 100 ppm the highest antioxidant efficiency.

Frozen Minced Fish Muscle. Lipid oxidation was effectively delayed in minced horse mackerel muscle (1.2 ± 0.2% of lipid content) during frozen storage at −10 °C by the addition of hydroxytyrosol at 10, 50, or 100 ppm, as indicated by peroxide

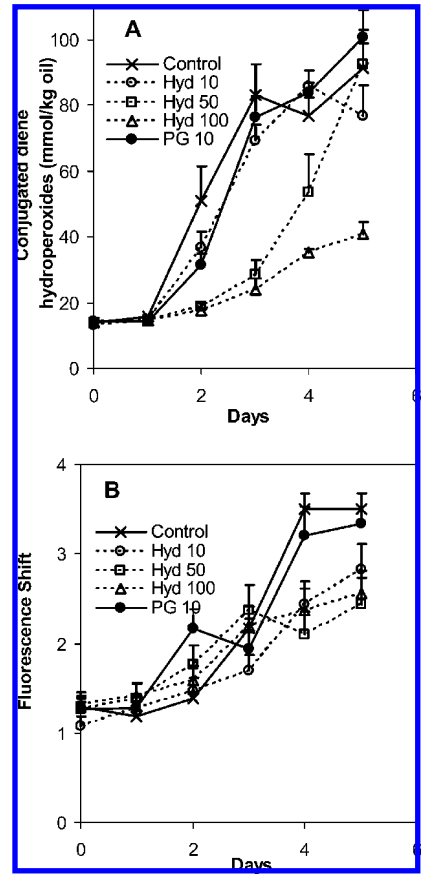

Figure 3. Effect of hydroxytyrosol (Hyd) and propyl gallate (PG) on the formation of conjugated diene hydroperoxides (**A**) and fluorescent compounds (**B**) in fish oil-in-water emulsions during oxidation at 30 °C. Hydroxytyrosol was added at 10, 50, and 100 ppm and propyl gallate at 10 ppm.

value (**Figure 4A**), conjugated hydroperoxides (**Figure 4B**), and TBARS (**Figure 4C**). Fish muscle supplemented with hydroxytyrosol showed induction periods 2−5 weeks longer for peroxide value and conjugated hydroperoxides than for control muscles without phenolics. The supplementation with hydroxytyrosol was also effective in reducing the generation of these oxidation products during the propagation of lipid oxidation. The intermediate concentration of hydroxytyrosol (50 ppm) was found to be the most effective in inhibiting lipid oxidation and was able to delay induction periods of hydroperoxides (peroxide value and conjugated dienes) and TBARS formation for 5 and 3 weeks, respectively. This tendency has not been found in bulk oil and oil-in-water emulsions, in which the strongest antioxidant capacity was achieved with higher phenolic concentration (100 ppm). Hydroxytyrosol showed a capacity similar to that of propyl gallate, both employed at 100 ppm, to delay lipid oxidation in minced fish muscle during frozen storage (**Figure 4**). Therefore, the efficiency order in preserving frozen minced fish muscle against lipid oxidation was Hyd 50 > Hyd 100 ≈ PG 100 > Hyd 10.

The depletion kinetics for the endogenous antioxidants can be also used as oxidative stress indicators in fish tissues (*11, 24*). α-Tocopherol and total glutathione were respectively chosen as representative lipophilic and hydrophilic endogenous antioxidants in fish muscle. In control samples, the initial concentration of α-tocopherol (approximately 300 μg/g of fat) was dramatically reduced after 4 weeks of frozen storage (**Figure 5A**). On the contrary, the addition of hydroxytyrosol preserved intact α-tocopherol for 2−5 weeks. Supplementation with 50

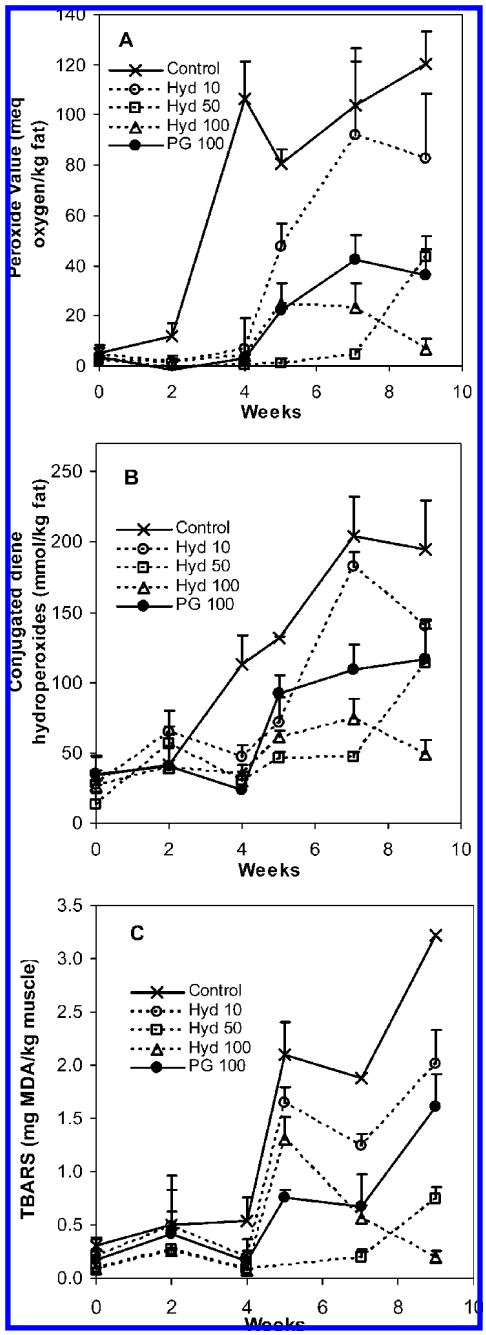

Figure 4. Effect of hydroxytyrosol (Hyd) and propyl gallate (PG) on peroxide value (**A**), conjugated diene hydroperoxides (**B**), and TBARS (**C**) in horse mackerel minced muscle at −10 °C. Hydroxytyrosol was added at 10, 50, and 100 ppm and propyl gallate at 100 ppm.

ppm of hydroxytyrosol was found to be the most effective procedure to maintain longer α-tocopherol, and no differences were observed between adding 10 or 100 ppm to preserve endogenous α-tocopherol. The efficiency of propyl gallate for maintaining α-tocopherol was similar to that achieved for hydroxytyrosol, as can be observed from the corresponding data at 100 ppm. Conversely to this protective effect on α-tocopherol, neither hydroxytyrosol nor propyl gallate was active in inhibiting the consumption of glutathione measured on the basis of total glutathione (**Figure 5B**). Total glutathione concentration suffered a severe diminution within first 6 weeks of frozen storage in fish muscle both with and without phenolic supplementation.

In agreement with the inhibitory effect on lipid oxidation products, hydroxytyrosol was effective in preserving ω-3 PUFAs

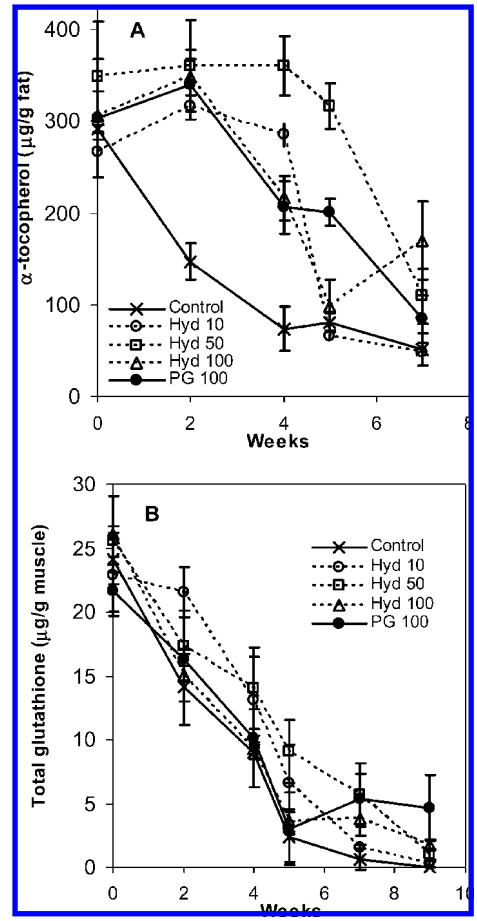

Figure 5. Effect of hydroxytyrosol (Hyd) and propyl gallate (PG) on the depletion kinetics of endogenous α-tocopherol (**A**) and total glutathione (**B**) in minced horse mackerel muscle at −10 °C. Hydroxytyrosol was added at 10, 50, and 100 ppm and propyl gallate at 100 ppm.

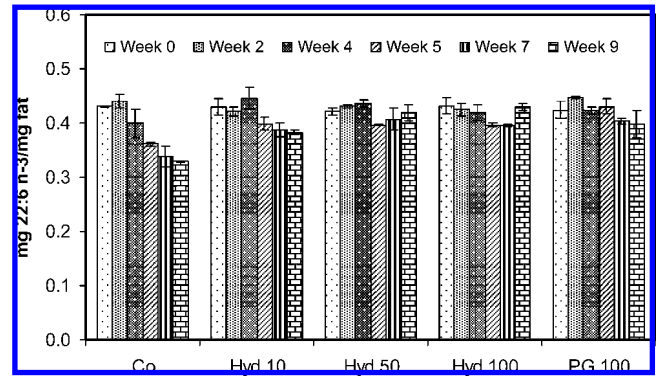

Figure 6. Effect of hydroxytyrosol (Hyd) and propyl gallate (PG) on levels of fatty acid 22:6ω3 in minced horse mackerel muscle during storage at −10 °C. Hydroxytyrosol was added at 10, 50, and 100 ppm and propyl gallate at 100 ppm.

in minced horse mackerel muscle during frozen storage. Docosahexaenoic acid (DHA, 22:6ω-3) was the most abundant ω-3 PUFA in horse mackerel muscle at 0.428 ± 0.010 mg of DHA/mg of fatty acid in fresh muscle. In control samples, the content of DHA was significantly reduced to 0.330 ± 0.001 mg/mg of fatty acid after 9 weeks of frozen storage at −10 °C (**Figure 6**). On the contrary, the supplementation of minced muscle with hydroxytyrosol at 50 or 100 ppm was able to maintain the initial levels of decosahexaenoic acid during the whole experiment (**Figure 6**). Moreover, the addition of propyl gallate at 100 ppm was effective in preserving intact PUFAs

during the whole storage time. Hydroxytyrosol at 10 ppm showed lower activity in maintaining DHA levels, and 0.384 ± 0.006 mg of DHA was found in supplemented minced muscle after 9 weeks of frozen storage (**Figure 6**).

DISCUSSION

The present paper evaluated the suitability of hydroxytyrosol that can be obtained from olive oil byproduct for delaying oxidation events in both primary sources of ω-3 PUFAs, such as bulk fish oil, and potential functional foods based on fish oil-in-water emulsions and minced semifatty fish muscle.

Our investigation clearly demonstrated that hydroxytyrosol can be effective in enhancing the oxidative stability of PUFA-rich foods. Two different tendencies were found regarding the hydroxytyrosol concentration effect on its antioxidant activity. Higher inhibitory properties were achieved in bulk fish oil and oil-in-water emulsions by increasing the hydroxytyrosol concentration from 10 to 100 ppm, whereas a hydroxytyrosol intermediate concentration (50 ppm) was the most advantageous in delaying lipid oxidation in minced fish muscle. A most favorable intermediate concentration has been also found for other phenolics in model systems activated by Fenton-type reactions, whereas higher phenolic concentrations originated the best antioxidant activity when oxidation was catalyzed by peroxyl radicals (*38, 39*). It is known that phenolics can promote metal-catalyzed lipid oxidation given that they lead to the formation of reduced forms of iron [Fe(II)] and copper [Cu(I)], which generate the extremely oxidative hydroxyl radical via Fenton reaction (*40, 41*). Ferrous iron is also 14 times more effective in producing free radicals by decomposition of lipid hydroperoxides than ferric iron (*42*). Regarding the content of those redox-active metals, total iron ranges from 4 to 20 mg/kg of wet muscle in fish flesh, dark-muscle species such as horse mackerel exhibiting almost twice the iron content of white-muscle species (*43*−*45*). Moreover, it is known that the fraction of free ionic iron can increase during fish muscle storage, possibly due to decomposition of heme proteins (*46*). The copper content of fish flesh normally varies from 0.06 to 0.4 mg/kg of wet muscle, and there is no noticeable difference between dark-muscle and white-muscle species (*47, 48*). In contrast, it was reported that fish oil obtained from marinated herring byproducts contained 0.03−0.1 mg/kg for iron and below 0.1 mg/kg for copper (*49*). Therefore, the optimal intermediate concentration observed for hydroxytyrosol in frozen minced muscle could be explained by the significantly high levels of transition metals in horse mackerel muscle and the antioxidant/prooxidant balance of phenolics in those systems mainly activated by transition metals. However, the autoxidation of PUFAs is an important oxidative mechanism in bulk fish oil and emulsion, given that active metals are present in low concentration and their oxidative stability was evaluated at 30−40 °C. Under those conditions, hydroxytyrosol exhibits an essentially antioxidant role by free radical scavenging, and such activity showed a positive relationship with hydroxytyrosol concentration.

Physicochemical properties of phenolic compounds such as electron/hydrogen-donating capacity, chelating activity on iron/copper, capacity to regenerate other antioxidant compounds, and polar character, could modulate the antioxidant behavior of phenolics. Hydroxytyrosol and propyl gallate donate correspondingly 11.9 and 15.4 μmol of electrons/mg of compound to potential free radicals (*17*). These electron-donating capacities are equivalent on a molecular basis to provide 1.7 and 3.2 electrons/molecule of hydroxytyrosol and propyl gallate, which match approximately with the number of hydroxyl groups on their aromatic rings (*18*). Concerning chelating properties, propyl gallate possesses much higher chelating ability on the prooxidant ferrous iron than hydroxytyrosol (*17*). Therefore, the antioxidant efficiency exhibited in bulk fish oil (propyl gallate > hydroxytyrosol) could be explained on the basis of higher electron-donating and ferrous-chelating capacity for propyl gallate. The facts that hydroxytyrosol and propyl gallate differ significantly in chemical properties such as electron-donating and ferrous-chelating activities and partitioning coefficients oil/water are not much different (corresponding a 38.0 and 49.4% of hydroxytyrosol and propyl gallate were found in the oily phase) should restrain the importance of interfacial partitioning of phenolics and the "polar paradox". The polar paradox predicts greater antioxidant efficiency of a hydrophilic phenolic such as hydroxytyrosol in hydrophobic systems such as bulk oil, and conversely (*6*). In oil-in-water emulsions and minced fish muscle, hydroxytyrosol and propyl gallate showed comparable antioxidant behaviors. Therefore, the antioxidant capacity of those phenolics in oil-in-water emulsions and minced muscle cannot be ascribed to chemical properties such as electron-donating and ferrous-chelating capacity.

Hydroxytyrosol was able to inhibit the post-mortem depletion of the endogenous α-tocopherol in minced muscle during frozen storage; however, total glutathione was not significantly preserved by either hydroxytyrosol or propyl gallate. A previous investigation in minced frozen muscle also showed that grape polyphenols preserve better α-tocopherol than total glutathione from consumption (*11*). Moreover, the efficiency order preserving α-tocopherol was similar to that for inhibiting the formation of lipid oxidation products (peroxide value, conjugated hydroperoxides, and TBARS): Hyd 50 > Hyd 100 ≈ PG 100 > Hyd 10. These results point out that the endogenous α-tocopherol reflects the oxidative stress status in fish muscle, which has been previously described in fish muscle (*11, 17, 50*), beef muscle (*51*), and dairy products (*50, 52*). The depletion kinetic for ω-3 PUFAs revealed the efficiency of hydroxytyrosol in preventing the consumption of PUFAs in minced muscle, but that index was not able to distinguish significant differences between the different antioxidant treatments. In previous investigations, the depletion of PUFAs also exhibited lower sensibility to evaluate the oxidative status in frozen minced fish muscle, in comparison with peroxide value and TBARS (*10*).

The results of the present work emphasize the efficiency and versatility of hydroxytyrosol to stabilize foodstuffs rich in functional ω-3 PUFAs. Hydroxytyrosol concentrations ranging from 10 to 100 ppm were able to increase the oxidative stability in bulk fish oil, oil-in-water emulsions, and frozen minced fish muscle, although different optimal concentrations were found in those food systems. Hydroxytyrosol demonstrated an antioxidant capacity similar to that of synthetic propyl gallate in oil-in-water emulsions and frozen fish muscle.

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