JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Molecular Structure of Phenolic Families as Hydroxycinnamic Acids and Catechins on Their Antioxidant Effectiveness in Minced Fish Muscle

I. MEDINA,*,† J. M. GALLARDO,† M. J. GONZÁLEZ,† S. LOIS,† AND N. HEDGES‡

Instituto de Investigaciones Marinas del CSIC, Eduardo Cabello 6, E-36208 Vigo, Spain, and Unilever, Sharnbrook Beds, MK44 1LQ, United Kingdom

The antioxidant effectiveness of two different families of phenolic compounds, hydroxycinnamic acids and catechins, added as a power (0.001% w/w) to chilled minced horse mackerel muscle was evaluated. Caffeic acid, chlorogenic acid, *o*-coumaric acid, and ferulic acid were selected as hydroxycinnamic acids with similar molecular structures. Commercial catechins with different numbers of hydroxylic groups, including catechin, gallocatechin, catechin gallate, and gallocatechin gallate, were also tested. The effectiveness found was individually discussed for each family as a function of the molecular structure. The capacity of hydroxycinnamic acids for donating electrons seems to play the most significant role for retarding the development of rancidity in fish muscle. Conversely, the properties related to the ability for chelating metals and the distribution between oily and aqueous phases were not correlated with the inhibitory activities. Among hydroxycinnamic acids, the results highlighted the potent antioxidant activity of 10 ppm caffeic acid in inhibiting lipid oxidation in fish muscle. Its antioxidant efficacy was similar to that of propyl gallate. Among catechins, catechin showed the highest antioxidant activity. There was an increment of efficacy in fish muscle using concentrations ranging between 10 and 100 ppm of both caffeic acid and catechin.

KEYWORDS: Oxidation; fatty fish; storage; hydroxycinnamic acids; catechins

INTRODUCTION

Fatty fish species are considered to be of great nutritional importance. This is due mainly to their naturally high content of essential n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (20:5ω-3) and docosahexaenoic acid (22:6ω-3). These acids have been shown to have potential benefits for human health (*1*, *2*). Some studies have also demonstrated strong effects of docosapentaenoic acid (22:5ω3) in, for example, the inhibition of angiogenesis (*3*). Studies of this type highlight the importance of including oily fish species with high proportions of n-3 PUFAs in the diet and not focusing purely on the fat content. Despite these obvious benefits, one major barrier to the use of oily fish exists. Storage and processing of these seafoods are still limited due to the susceptibility of their lipids to develop lipid oxidation. The products of oxidation cause anomalous flavors and structural changes, which in turn lead to rejection of products by the consumer (*4*, *5*). There is a strong interest by fishing factories and food multinationals to develop methods that minimize lipid oxidation and rancidity in fatty fish, which extends their shelf life and preserves quality.

Randicity in fish is associated with high amounts of PUFAs together with the presence of heme pigments and trace amounts of metallic ions (*4*, *6*). The use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences (*7*). Metal chelators such as citric acid and agents that maintain reduced hemoglobin have been widely employed in model systems for fish muscle, and improvements have been demonstrated (*8*). There is a great potential for some natural compounds present in vegetable and fruit extracts. These molecules have been shown to effectively scavenge radicals and inhibit oxidation (*9*). Recent studies have investigated the supplementation of fish products with natural extracts from different fruit and vegetable sources, such as from grape pomace, rosemary, etc. (*10*−*12*). However, the activity of these antioxidants is often difficult to predict in real food products because of the different potential mechanisms involved in the antioxidant function (*7*). Also, the same antioxidant may work by a number of routes. These include the capacity for chelating metals, the reducing power of the antioxidant, or the ease of incorporation of the antioxidants into the sensitive oxidative sites of fish muscle. All of these mechanisms have been suggested to play a significant role in the antioxidant activity of phenolics as stabilizing agents for fish lipids (*12*).

Phenolics are secondary metabolites present typically in plants. Among these, hydroxycinnamic acids are widely distributed and are common to seeds, fruits, tubers, and the herbaceous parts of many vegetable species (*13*). They occur naturally in combination with other compounds, usually in the

* To whom correspondence should be addressed. Tel: +34 986 231930. Fax: +34 986 292762. E-mail: medina@iim.csic.es.
† Instituto de Investigaciones Marinas del CSIC.
‡ Unilever.

10.1021/jf063498i CCC: $37.00
Published on Web 04/24/2007

form of esters. For example, chlorogenic acid is an ester of caffeic acid and quinic acid. Phenolic compounds can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes (*14*). In addition, they may delay the onset of lipid oxidation via the decomposition of hydroperoxides (*15*, *16*).

Other phenolic compounds present in vegetable sources include catechins. In recent years, catechins, which belong to the flavonoid family and are common components of the human diet, have attracted much attention in relation to their physiological role as antimutagenic and antitumorigenic agents (*17*, *18*). Furthermore, they have been recognized as efficient antioxidants. Their proposed mechanism of action is by scavenging oxygen radicals and by chelating metal ions (*19*, *20*). Their antioxidant activity in fish model systems has been compared to that of other natural and synthetic antioxidants. The results suggested that their antioxidant properties were attributed to the ability to chelate iron. The extensive hydroxylation of the flavanoids was also proposed as being important in explaining their efficiency (*21*, *22*). Furthermore, green tea catechins were also shown to inhibit lypooxygenase activity in mackerel muscle (*23*).

This work is aimed to elucidate the effect of the molecular structure on the antioxidant effectiveness in fish muscle of two different families of natural antioxidants, the hydroxycinnamic acids and the catechins. The molecular structure and physicochemical parameters such as the reducing and chelating abilities or the affinity for oily or water phases were determined for a given family and correlated with the efficiency for inhibiting the oxidation of chilled minced horse mackerel muscle. The work attempts to identify the significant molecular features for a correct design of antioxidants in seafood.

MATERIALS AND METHODS

Materials. Different batches of fresh Atlantic horse mackerel (*Trachurus trachurus*), 80−100 fish caught during April and June 2004, were supplied by a local market. The first batch (April 15, 2004) was employed to perform the experiments with hydroxycinnamic acids, and the second one (May 20, 2004) was used for the experiments with catechins. The third (April 29, 2004) and fourth (May 26, 2004) batches were employed for testing the effect of the antioxidant concentration. Caffeic acid, *o*-coumaric acid, chlorogenic acid, ferulic acid, catechin, gallocatechin, catechin-gallate, gallocatechin-gallate, propyl gallate, butylhydroxytoluene (BHT), ferrozine, bovine hemoglobin, $FeCl_2 \cdot 4H_2O$, and $FeCl_3 \cdot 6H_2O$ were supplied by Sigma (St. Louis, MO). 2,4,6-Tri-(2-pyridyl)-*s*-triazine was obtained from Fluka (New-Ulm, Swizerland). All chemicals and solvents used were either analytical or high-performance liquid chromatography grade (Ridel-Haën, Seelze, Germany).

Polarity of Phenolic Compounds. The polarity of phenolics was determined by their partition between aqueous and oily phases according to Huang et al. (*24*) and Pazos et al. (*25*). Briefly, 1 mL of fish oil and 1 mL of water containing antioxidants were well mixed and centrifuged. The antioxidant concentrations in the aqueous phase before and after mixing were quantified, and the amount of antioxidant in the oily phase was calculated as the difference between the total amount of antioxidant in water before mixing and the amount after mixing with oil. Coefficients were calculated as V_w/V_o (W_o/W_w), where V_w = volume of water, V_o = volume of oil, W_o = amount of antioxidant in the oily phase, and W_w = amount of the antioxidant in the aqueous phase.

Reducing Power of the Phenolic Compounds. The FRAP (ferric reducing/antioxidant power) method was used by adaptation (*25*) of the procedure of Benzie and Strain (*26*). The number of donated electrons was calculated from the slopes of the lineal adjustments between the phenolic concentration and the FRAP activity.

Chelating Capacity of the Phenolic Compounds. The capacity of the phenols for chelating ferrous iron was determined according to the procedure of Kolayli et al. (*27*) and the adaptation by Pazos et al. (*12*). The chelating activity was expressed as the percentage of ferrous iron (20 μM) chelated by 20 μM phenolic compound.

Chilled Minced Horse Mackerel. Batches of 20 kg of Atlantic horse mackerel (*Trauchurus trauchurus*) were deboned and eviscerated, and the white muscle was separated and minced to obtain a solid muscle homogenate (size, 5 mm). Streptomycine sulfate (200 ppm) was added for inhibiting microbial growth. The different antioxidant compounds, hydroxycinnamic acids and catechins, were added at concentrations ranging between 10 and 200 ppm (w/w, 0.001−0.020%) and tested in different, independent experiments. Portions of 10 g of minced muscle were placed into plastic bags. Control samples, without antioxidants, and samples with hydroxycinnamic acids were kept refrigerated at 4 °C for 6−7 days. After that time, samples began to show microbial growth. Control samples and samples with catechins were also kept refrigerated for 4 days. Duplicate samples were taken at different sampling times. Inhibition of oxidation was calculated during the propagation period of controls according to Frankel (*5*). Induction periods were calculated as the time (in days) required for a sudden change in the rate of oxidation by the method of tangents to the two parts of the kinetic curve (*28*). Each experiment was done twice.

Sensory Analysis. A total of four panelists trained in descriptive analysis of fishy off-flavors sniffed the same raw samples that were used for chemical determinations. Approximately 10 g of muscle was placed in separate sterile polystyrene Petri dishes and put on a tray of ice. The panelist concentrated on detecting rancidity/painty odors using a hedonic scale from 7 to ≤1 (*8*). The odor scores were as follows: 8, fresh seaweedy; 7, low odor; 6, stale, earthy; 5, sour, fishy, rotting orange; 4, slight paint; 3, moderate paint; 2, strong paint; and 1, putrid ammonia.

Lipid Extraction. Lipids were extracted from fish muscle according to Bligh and Dyer (*29*) and quantified gravimetrically (*30*).

Peroxide Value. The peroxide value of fish muscle was determined by the ferric thiocyanate method (*31*) and was expressed as mequiv oxygen/kg lipid. Analyses were performed in duplicate.

Thiobarbituric Acid Reactive Substances (TBARS). The thiobarbituric acid index (mg malonaldehyde/kg muscle) was determined according to Vyncke (*32*). Analyses were performed in duplicate.

Statistical Analysis. All experiments were replicated, and analyses were performed in duplicate. The data were compared by one-way analysis of variance (*33*), and the means were compared by a least-squares difference method (*34*). Significance was declared at $p < 0.05$. Correlations between the propagation rates of lipid oxidation products and the physicochemical properties of phenolics were determined by Pearson coefficients. Statistical analyses were performed with the software Statistica 6.0.

RESULTS AND DISCUSSION

Figures 1 and **2** show the different molecular structures of the compounds tested in this study. The hydroxycinnamic acids tested mainly differ in the number of hydroxilic groups, and the catechins are different in the presence of the pyrogallol moiety and the galloylated residues. **Table 1** shows their reducing and chelating capacities and their partitioning coefficients between the oily and the aqueous phases. The reducing capacity measures the ease of the compounds in donating electrons. The partitioning coefficients reflect the polarity of the molecules.

Hydroxycinnamic acids showed a very different reducing ability. These differences are more evident when the capacity is expressed per mol of phenolic. Caffeic acid donated the highest number of electrons (2.2 mol electrons/mol phenolic) followed by ferulic acid (1.9 mol electrons/mol phenolic) and chlorogenic acid (1.6 mol electrons/mol phenolic). *o*-Coumaric acid showed a low reducing ability by donating only 0.1 mol electrons/mol phenolic. The introduction of a second hydroxyl group in caffeic acid or a methoxy substitution in ferulic acid increased the number of donated electrons. Esterification of

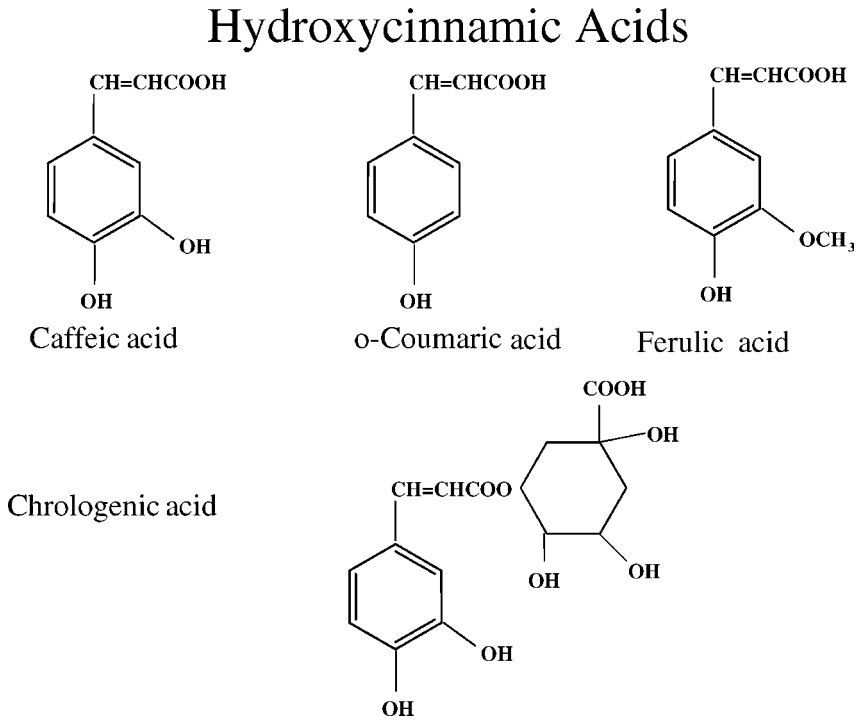

Figure 1. Molecular structures of hydroxycinnamic acids.

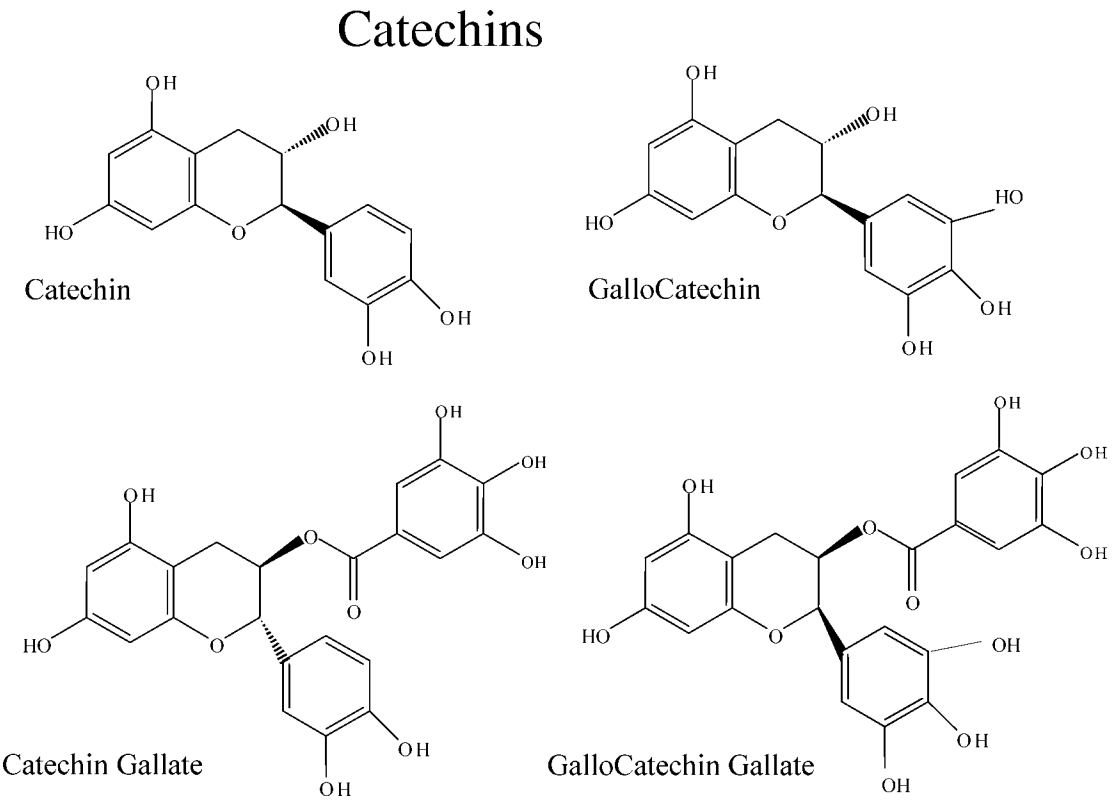

Figure 2. Molecular structures of catechins.

caffeic acid with quinic acid in chlorogenic acid reduced the donating ability of the hydroxycinnamic acids. Therefore, the extensive number of hydroxilic groups in chlorogenic acid did not increase its reducing capacity. Recently, it has been discussed that the absolute antioxidant capacity cannot be predicted simply by determining the number of hydroxyl groups (*35*). These results are in agreement with previous reported studies in which caffeic acid showed a higher 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity than ferulic acid and *p*-coumaric acids (*36*). DPPH and FRAP methods utilize the same single-electron transfer mechanism.

As regards catechins, catechin, and gallocatechin, it was found that these molecules could donate 1.7 and 2.3 mol of electrons/mol phenolic, respectively. The gallate esters, catechin gallate and gallocatechin gallate, donated a higher number of electrons per mol, 3.8 and 4.0, respectively. Different studies have demonstrated that the pyrogallol moiety provides more hydrogen atoms or electrons than the catechol group (*37*). Propyl gallate

Table 1. Reducing (μmol Electrons/mg Antioxidant) and Chelating Capacities (Calculated at 20 μM) and Partitioning Coefficients* of Hydroxycinnamic Acids and Catechins

	μmol electrons/ mg antioxidant	chelating	partitioning coefficient	partitioning[a] (% oil)
caffeic acid	12.2	79.3 ± 0.7	0.10 ± 0.04	0.30 ± 0.15
chlorogenic acid	4.5	77.5 ± 0.4	0.03 ± 0.01	3.1 ± 0.3
o-coumaric acid	0.6	–2.7	0.30 ± 0.15	22.6 ± 8.3
ferulic acid	9.8	–2.1	0.98 ± 0.08	49.6 ± 2.0
catechin	5.9	56.9 ± 1.6	0.11 ± 0.01	9.8 ± 0.8
catechin gallate	8.6	82.9 ± 0.3	0.29 ± 0.07	25.8 ± 3.2
gallocatechin	7.5	62.1 ± 1.5	0.06 ± 0.01	5.8 ± 1.1
galocatechin gallate	8.7	87.2 ± 0.0	0.12 ± 0.01	10.7 ± 0.5
propylgallate	18.9	95.2 ± 0.2	0.98 ± 0.11	49.4 ± 2.9
BHT	1.8	–0.5	1.00 ± 0.01	99.9 ± 1.2

[a] Expressed as percent in oil.

Table 2. Sensory Descriptors of Minced Horse Mackerel Muscle during Storage at 4 °C by the Supplementation of 10 ppm of Hydroxycinnamic Acids and Catechins.

Hydroxycinnamic Acids	Day 5	Day 6
control	moderate paint	moderate paint
caffeic	fresh seaweedy	fresh seaweedy
o-coumaric	moderate paint	moderate paint
chlorogenic	dresh seaweedy	slight paint
ferulic	dresh seaweedy	slight paint

Catechins	Day 2	Day 3
control	fresh seaweedy	moderate paint
catechin	fresh seaweedy	slight paint
gallocatechin	fresh seaweedy	moderate paint
catechin gallate	fresh seaweedy	moderate paint
gallocatechin gallate	fresh seaweedy	moderate paint

also showed a high reducing ability by donating 3.4 mol of electrons/mol phenolic (18.2 μmol of electrons/mg), and BHT had a low reducing ability of 0.4 mol of electrons/mol phenolic (1.82 μmol of electrons/mg).

The capacity of antioxidants for chelating metals is strongly dependent on the number of hydroxilic groups in ortho positions (*7*). In accordance with this, caffeic and chlorogenic acids showed a strong chelating capacity when compared to ferulic and *o*-coumaric acids. Ferulic acid and *o*-coumaric acids did not show a chelating capacity when employed at the 20 μM level (**Table 1**). Catechins showed significant differences in their chelating capabilities The results demonstrated a high dependence of the number of *o*-hydroxilic groups. As a consequence, catechin gallate and gallocatechin gallate were more effective chelatants than catechin and gallocatechin. Propyl gallate showed a strong chelating ability close to that of ethylenediaminetetraacetic acid used as a reference. BHT had no chelating capacity when employed at 20 μM.

As regards the partitioning behavior (**Table 1**), caffeic and chlorogenic acids were highly polar and totally found in the aqueous phase. However, ferulic acid was equally distributed between the oily and the aqueous phases. *o*-Coumaric acid was more hydrophobic than caffeic and chlorogenic acids but less hydrophobic than ferulic acid. Among catechins, the presence of the pyrogallol group increased the polarity. They showed the following order of polarity: gallocatechin > catechin = gallocatechin gallate > catechin gallate. Propyl gallate was also uniformly distributed into the oily and aqueous phases, and BHT was highly hydrophobic and totally found in the oily phase.

Effectiveness in Chilled Fish Muscle. The first batch of minced horse mackerel muscle used for the experiments with

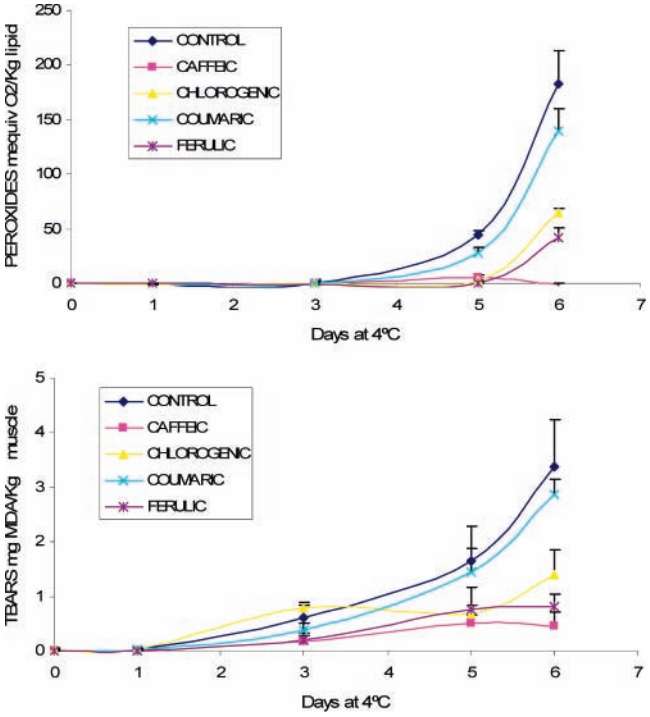

Figure 3. Hydroperoxide (**A**) and TBARS (**B**) formation during chilled storage of minced fish muscle supplemented with hydroxycinnamic acids (mean ± standard deviation of experiments performed in duplicate).

hydroxycinnamic acids showed a medium fat content of 1.3 ± 0.3%. The second batch of fish used for the experiments with catechins had higher levels: 4.4 ± 0.4% of fat. This different lipid content influenced the oxidation produced and the second group of fish oxidized faster than the first one. Because the antioxidant potency is mostly attributed to the number of phenolic and hydroxilic groups (*7*), the results are discussed here in relation to the weight concentration of the antioxidants.

Table 2 shows the sensory scores of horse mackerel muscle supplemented with 10 ppm of hydroxycinnamic acids and catechins during chilling storage. No differences in the odor of the fish homogenate supplemented with 10 ppm of phenolics were detected at time zero. Results demonstrated that control samples and those supplemented with *o*-coumaric acid lost sensory quality by the fifth day and where panelists first detected a clear rancid odor. Minced fish muscle supplemented with ferulic acid and chlorogenic acid developed rancidity by the sixth day. However, after 6 days at 4 °C, rancid odors were not detected in fish muscle supplemented with caffeic acid. In the test performed with minced fish muscle supplemented with catechins, control samples developed rancidity by the third day and only catechin was slightly able to retard the generation of rancid odors. Factors such as the lipid content, the PUFA composition, and the content and activities of muscle prooxidants such as hemeproteins and lipoxigenases have been described to influence fish lipid oxidation (*4*, *6*).

According to the generation of off-flavors, there was also a retardation of the induction periods for the formation of lipid oxidation byproducts in samples supplemented with 10 ppm of hydroxycinnamic acids. The exception to this was the sample containing *o*-coumaric acid (**Figure 3**). The amounts of byproducts formed were also lower than those formed in controls. *o*-Coumaric was the least effective antioxidant, having no efficacy in inhibiting oxidation (**Table 3**). Caffeic acid showed a high antioxidant efficiency for preserving fish muscle to oxidation and could significantly inhibit the formation of off-

Table 3. Percentage Inhibition[a] on the Formation of Peroxides and TBARS in Horse Mackerel Muscle Supplemented with 10 ppm of Hydroxycinnamic Acids and Catechins during Chilling Storage at 4 °C (Mean ± SD)[b]

Hydroxycinnamic Acids	hydroperoxides Day 6	TBARS Day 6
control	0.7 ± 0.5 a	0.1 ± 7.1 a
caffeic	100.4 ± 0.2 e	86.2 ± 5.4 e
chlorogenic	64.7 ± 32.1 d	58.8 ± 9.8 c
o-coumaric	24.1 ± 4.1 b	14.0 ± 5.3 b
ferulic	88.1 ± 11.1 d	75.5 ± 4.8 d

Catechins	hydroperoxides Day 3	TBARS Day 3
control	0.1 ± 5.4 a	0.1 ± 3.1 a
catechin	56.8 ± 13.7 c	42.8 ± 3.6 c
gallocatechin	39.9 ± 3.0 c	15.4 ± 1.6 b
catechin gallate	14.1 ± 5.7 b	−2.6 ± 14.6 a
gallocatechin gallate	20.8 ± 2.0 b	15.5 ± 1.0 b

[a] % inhibition = [(C − S)/C] × 100 where C = oxidation product formed in control and S = oxidation product formed in sample. [b] Values corresponding to a family of compounds in each column with the same letter were not significantly different ($p < 0.05$).

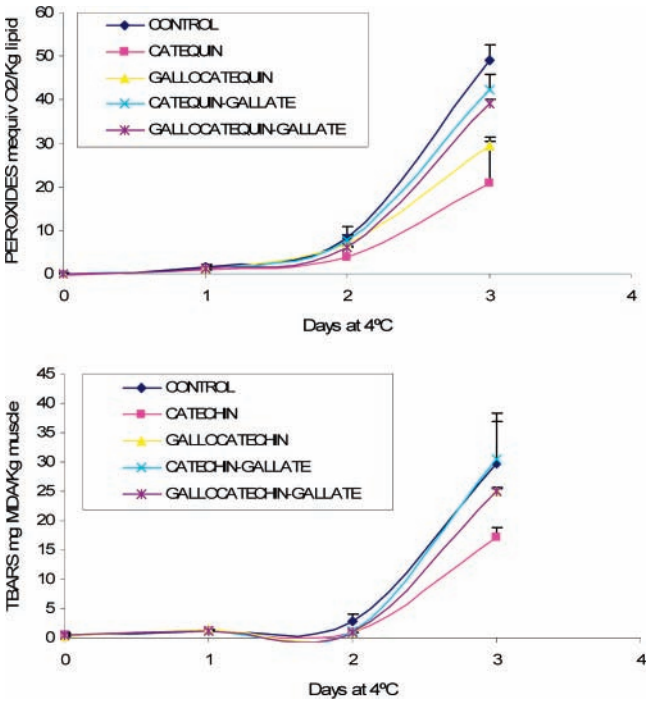

Figure 4. Hydroperoxide (**A**) and TBARS (**B**) formation during chilled storage of minced fish muscle supplemented with catechins (mean ± standard deviation of experiments performed in duplicate).

flavors, peroxides, and TBARS. The overall order of antioxidant efficiency for hydroxycinnamic acid was caffeic acid > ferulic acid = chlorogenic acid ≫ *o*-coumaric acid. These results are consistent with other studies performed on both homogeneous and heterogeneous systems, such as bulk and emulsified oils, where it was also found that caffeic acid had a high antioxidant activity when compared to other hydroxycinnamic and benzoic acids (*38*, *39*).

Catechins supplemented at 10 ppm showed a low inhibition of the formation of peroxides and TBARS in chilled horse mackerel in agreement with sensory data (**Tables 2** and **3** and **Figure 4**). A significant result was that increasing numbers of hydroxyl groups did not provide an extension in antioxidant effectiveness. The superiority of pyrogallol over catechol in the flavonoidal nucleus for displaying high radical scavenging activity (*40*) did not appear to influence the activity of catechins in fish muscle. Catechin was a better inhibitor of oxidation than gallocatechin. Catechin gallate and gallocatechin gallate showed similar inhibition on the formation of peroxides and TBARS. This last result has also been reported by He and Shahidi (*21*) in a model system composed of mackerel muscle and 20% w/w of water supplemented with catechins solved in ethanol. These authors reported a higher activity of gallocatechin than catechin. Recently, the activity of antioxidants for inhibiting oxidation has been related to the antioxidant carrier. The choice of carrier can improve incorporation of antioxidants into the oxidative sensitive sites of fish muscle (*41*). Therefore, the higher polarity of gallocatechin over catechin and the use of ethanol as antioxidant carrier could increase its antioxidant activity in a fish model system with a high content of water. When catechins are added as a solid power to the minced fish muscle, such enhancement of increment in antioxidant activity of gallocatechin over to catechin is likely to be negligible. Accordingly, recent studies have found a stronger activity of epicatechin for inhibiting the lipoxygenase initiation of oxidation in mackerel muscle than that shown by epigallocatechin (*23*).

The gallate esters were employed at lower molar concentrations than the single catechins. Ten parts per million of catechin and gallocatechin corresponded to 0.034 mmol/kg of fish muscle and 0.032 mmol/kg of fish muscle, respectively. Ten parts per million of catechin gallate and gallocatechin gallate corresponded to 0.022 mmol/kg of fish muscle and 0.021 mmol/kg of fish muscle, respectively. These differences in molar concentrations can explain the higher antioxidant activity of the gallate esters than the single forms found by He and Shahidi (*21*).

A new set of experiments were then performed to test the effect of the antioxidant concentration in fish samples supplemented with the most active compounds of each phenolic family, caffeic acid and catechin. The fat contents of these experiments that contained caffeic acid and catechin were 1.8 ± 0.2 and 2.7 ± 0.2, respectively. The inhibition achieved by the supplementation of 10−200 ppm of each antioxidant is shown in **Table 4**. There was a positive relationship between the efficiency and the concentration up to 100 ppm in both antioxidants. An increment of concentration over than 100 ppm did not provide higher efficiency for preventing fish lipid oxidation during the experiments. The increase of antioxidant activity between 10 and 100 ppm was more prominent for catechin than for caffeic acid.

The above results showed a high inhibition of rancidity in minced horse mackerel muscle achieved through the supplementation of low amounts of caffeic acid (0.001%). Caffeic acid has only recently been identified as one of the most active antioxidants in different in vitro antioxidant assays when it was compared to standard antioxidant compounds such as BHT, BHA, α-tocopherol, or trolox (*42*). Antioxidant effectiveness of caffeic acid was better than that of BHA in hydrophobic phases such as cod liver oil (*43*). Therefore, the relative effectiveness of caffeic acid against synthetic antioxidants such as propyl gallate and BHT in chilled horse mackerel muscle was then calculated. The percent of inhibition achieved by the synthetic phenolics calculated by the sixth day of oxidation was peroxides, 95.9 ± 5.2, and TBARS, 98.4 ± 1.3, for propyl gallate and peroxides, 20.0 ± 0.01, and TBARS, 33.3 ± 11.3, for BHT. Also, panelists detected rancid off-flavors in samples supplemented with BHT by the fifth day, and they did not detect

Table 4. Percentage Inhibition[a] on the Formation of Peroxides and TBARS in Horse Mackerel Muscle Supplemented with Caffeic Acid and Catechin Employed at Different Concentrations during Chilling Storage at 4 °C (Mean ± SD)[b]

Caffeic acid	hydroperoxides Day 7	TBARS Day 7
control	6.1 ± 3.2 a	0.1 ± 7.1 a
10 ppm	75.1 ± 0.2 b	63.7 ± 5.3 b
25 ppm	77.5 ± 11.3 b	59.6 ± 12.8 b
50 ppm	80.3 ± 4.0 b	54.3 ± 31.1 b
100 ppm	99.6 ± 0.3 c	98.4 ± 0.1 c
200 ppm	100.4 ± 0.5 c	100.1 ± 0.1 c

Catechin	hydroperoxides Day 3	TBARS Day 3
control	0.6 ± 0.3 a	2.0 ± 1.7 a
10 ppm	37.8 ± 15.9 b	37.1 ± 6.6 b
25 ppm	64.5 ± 8.5 c	43.1 ± 6.2 b
50 ppm	76.3 ± 8.1 c	44.1 ± 13.6 b
100 ppm	98.0 ± 1.5 d	86.3 ± 2.3 c
200 ppm	99.4 ± 0.3 d	92.4 ± 4.5 c

[a] % inhibition = [(C − S)/C] × 100 where C = oxidation product formed in control and S = oxidation product formed in sample. [b] Values corresponding to caffeic acid or catechin in each column with the same letter were not significantly different ($p < 0.05$).

Table 5. Correlations between Reducing and Chelating Capacities and Partitioning Coefficients of Phenolic Compounds and the Rates of Peroxides and TBARS Generation in Minced Horse Mackerel Supplemented with Hydroxycinnamic Acids

	hydroxycinnamic acids	
	peroxides	TBARS
reducing power	–0.97[a]	–0.96[a]
chelating activity	–0.58	–0.45
partitioning	0.22	0.01

[a] Significant correlation with $p < 0.05$.

rancid off-flavors in samples with propyl gallate after 6 days. These results showed that caffeic acid employed at 10 ppm showed similar antioxidant effectiveness in chilled horse mackerel muscle than propyl gallate and a higher effectiveness than that of BHT.

Correlations among Antioxidant Effectiveness in Fish Muscle and Physicochemical Properties. Table 5 shows the correlations between the reducing and the chelating capacities and the partitioning coefficients of hydroxycinnamic acids and the rate of generation of peroxides and TBARS during oxidation of minced horse mackerel muscle. Becasue there was a little difference in the reducing activity and the rate of oxidation of catechins supplemented at 10 ppm, the antioxidant activity of catechins was not discussed from the view of the physicochemical properties. Data corresponding to the oxidation experiments of hydroxycinnamic acids showed a significant negative correlation between the reducing capacity and the formation of peroxides and TBARS. However, the chelating capacity and the partitioning between oily and aqueous phases were not correlated with the oxidation detected in fish samples supplemented with the phenolic antioxidants. Therefore, the capacity for donating electrons seems to play a more significant role for stabilizing the antioxidant efficiency of hydroxycinnamic acids than the ability for chelating metals or the polarity. These data confirmed previous results obtained with procyanidins and hydroxytyrosol in frozen fish fillets, which indicated the importance of the total reducing power over the chelating capacity (*25*).

In conclusion, minced horse mackerel muscle was highly stabilized by the supplementation of 10 ppm of ferulic, chlorogenic, and caffeic acids. Catechins supplemented at 10 ppm were poorly effective in inhibiting lipid oxidation and therefore rancidity. The activity of catechin increased significantly when supplemented at 100 ppm. Caffeic acid has been identified as a very active antioxidant for fish muscle, and its efficacy was highly related with its capacity for donating 12.2 μmol electrons/mg antioxidant. The results of this work persist on the importance of the reducing ability of phenolic antioxidants supplemented as powers for retarding and inhibiting lipid oxidation of fish tissues.

LITERATURE CITED

(1) Ackman, R. *Marine Biogenic Lipids, Fats and Oils*; CRC Press: Florida, 1989.

(2) Lee, K. W.; Lip, G. Y. H. The role of omega-3 fatty acids in the secondary prevention of cardiovascular disease. *QJM* **2003**, *96*, 465−480.

(3) Tsuji, M.; Murota, S.; Morita, I. Docosapentaenoic acid (22:5 n-3) suppressed tube forming activity in endothelial cells induced by vascular endothelial growth factor. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2003**, *68*, 337−342.

(4) Hsieh, R.; Kinsella, J. Oxidation of polyunsaturated fatty acids: Mechanisms, products and inhibition with emphasis on fish. *Adv. Food Res. Nutr. Res.* **1989**, *33*, 233−241.

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

(6) Richards, M. P.; Hultin, H. O. Contribution of blood and blood components to lipid oxidation in fish muscle. *J. Agric. Food Chem.* **2002**, *50*, 555−564.

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

(8) Richards, M. P.; Kelleher, S. D.; Hultin, H. O. Effect of washing with or without antioxidants on quality retention of mackerel fillets during refrigerated and frozen storage. *J. Agric. Food Chem.* **1998**, *46*, 4363−4371.

(9) Shahidi, F.; Naczk, M. *Food Phenolics: Sources, Chemistry, Effects, Applications*; Technomic Publishing Co.: United States, 1995.

(10) Vareltzis, K.; Koufidis, D.; Gavriilidou, E.; Papavergou, E.; Vasiliadou, S. Effectiveness of a natural Rosemary (*Rosmarinus officinalis*) extract on the stability of filleted and minced fish during frozen storage. *Food Res. Technol.* **1997**, *205*, 93−96.

(11) Fagbenro, O.; Jauncey, K. Chemical and nutritional quality of fermented fish silage containing potato extracts, formalin or ginger extracts. *Food Chem.* **1994**, *50*, 383−388.

(12) Pazos, M.; Gallardo, J. M.; Torres, J. L.; Medina, I. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chem.* **2005**, *92*, 547−557.

(13) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

(14) Rao, M. V.; Paliyath, G.; Ormrod, D. P. Ultraviolet-band ozone-induced biochemical changes in antioxidant enzyes of *Arabidopsin thaliana*. *Plant Physiol.* **1996**, *110*, 125−136.

(15) Marinova, E. M.; Yanishlieva, N. V.; Toneva, A. G. Antioxidant activity and mechanism of action of ferulic and caffeic acids in different lipid systems. *Riv. Ital. Sostanze Grasse* **2006**, *83*, 6−13.

(16) Ates, B.; Dogru, M. I.; Gul, M.; Erdogan, A.; Dogru, A. K.; Yilmaz, I.; Yurekli, M.; Esrefoglu, M. Protective role of caffeic acid phenethyl ester in the liver of rats exposed to cold stress. *Fundam. Clin. Pharmacol.* **2006**, *20*, 283−289.

(17) Hayakawa, S.; Kimura, T.; Saeki, K.; Koyama, Y.; Aoyagi, Y.; Noro, T.; Nakamura, Y.; Isemura, M. Apoptosis-inducing activity of high molecular weight fractions of tea extracts. *Biosci., Biotechnol., Biochem.* **2001**, *65*, 459−462.
(18) Hong, J. G.; Smith, T. J.; Ho, C. T.; August, D. A.; Yang, C. S. Effects of purified green and black tea polyphenols on cyclooxygenase and lipoxygenase-dependent metabolism of arachidonic acid in human colon mucosa and colon tumour tissues. *Biochem. Pharmacol.* **2001**, *62*, 1175−1183.
(19) Shahidi, F.; Wanasundata, P. K. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 67−103.
(20) Kondo, K.; Kurihara, M.; Miyata, N.; Suzuki, T.; Toyoda, M. Mechanistic studies of catechins as antioxidants against radical oxidation. *Arch. Biochem. Biophys.* **1999**, *362*, 79−86.
(21) He, Y.; Shahidi, F. Antioxidant activity of green tea and its catechins in a fish meat model system. *J. Agric. Food Chem.* **1997**, *45*, 4262−4266.
(22) Tang, S. Z.; Sheehan, D.; Buckley, D. J.; Morrissey, P. A.; Kerry, J. P. Anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle. *Int. J. Food Sci. Technol.* **2001**, *36*, 685−692.
(23) Banerjee, S. Inhibition of mackerel (*Scombrus scombrus*) muscle lypoxygenase by green tea polyphenols. *Food Res. Int.* **2006**, *39*, 486−491.
(24) Huang, S.-W.; Hopia, A.; Schwarz, K.; Frankel, E. N.; Aeschbach, R.; German, J. B. Antioxidant activity of a-tocopherol and Trolox in different lipid substrates: Bulk oils vs oil-in-water emulsions. *J. Agric. Food Chem.* **1996**, *44*, 444−452.
(25) Pazos, M.; Alonso, A.; Fernández-Bolaños, J.; Torres, J. L.; Medina, I. Physicochemical properties of natural phenolics from grapes and olive oil by-products and their antioxidant activity in frozen horse mackerel fillets. *J. Agric. Food Chem.* **2006**, *54*, 366−373.
(26) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.
(27) Kolayli, S.; Ocak, M.; Kucuk, M.; Abbasoglu, R. Does caffeine bind to metal ions? *Food Chem.* **2004**, *84*, 383−388.
(28) Alaiz, M.; Hidalgo, F. J.; Zamora, Z. Comparative antioxidant activity of Maillard- and oxidized lipid-damage bovine serum albumin. *J. Agric. Food Chem.* **1997**, *45*, 3250−3254.
(29) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.* **1959**, *37*, 911−917.
(30) Herbes, S.; Allen, C. Lipid quantification of freshwater invertebrates: Method modification for microquantitation. *Can. J. Fish Aquat. Sci.* **1983**, *40*, 1315−1317.
(31) Chapman, R. A.; Mackay, K. The estimation of peroxides in fats and oils by the ferric thiocyanate method. *J. Am. Oil Chem. Soc.* **1949**, *26*, 360−363.
(32) Vyncke, W. Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. *Fette Wiss Technol.* **1970**, *72*, 1084−1087.
(33) Sokal, R.; Rohlf, F. *Biometry*; Freeman and Co.: San Francisco, California, 1981.
(34) Statsoft. *Statistica for Macintosh*; Statsoft and its Licensors: Tulsa, Oklahoma, 1994.
(35) Ozgen, M.; Reese, R. N.; Tulio, A. Z., Jr.; Scheerens, J. C.; Miller, A. R. Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2′-diphenyl-1-picrylhydrazyl (DPPH). *J. Agric. Food Chem.* **2006**, *54*, 1151−1157.
(36) Bratt, K.; Sunnerheim, K.; Bryngelsson, S.; Fagerlund, A.; Engman, L.; Andersson, R. E.; Dimberg, L. H. Avenantramides in oats (*Avena sativa* L.) and structure-antioxidant activity relationships principles. *J. Agric. Food Chem.* **2003**, *50*, 7022−7028.
(37) Bors, W.; Michel, C.; Stettmaier, K. Electron paramagnetic resonnace studies of radical species of proanthocyanidins and gallate esters. *Arch. Biochem. Biophys.* **2000**, *374*, 347−355.
(38) Pekkarinen, S. S.; Stöckmann, H.; Schwarz, K.; Heinonen, I. M.; Hopia, A. I. Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate. *J. Agric. Food Chem.* **1999**, *47*, 3036−3043.
(39) Satué-Gracia, 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.
(40) Furuno, K.; Akasako, T.; Sugihara, N. The contribution of the pyrogallol moiety to the superoxide radical scavenging activity of flavonoids. *Biol. Pharm. Bull.* **2002**, *2*, 19−23.
(41) Raghavan, S.; Hultin, H. O. Effect of carrier solvent polarity on selective incorporation of exogenous delta-tocopherol into muscle membranes. *J. Muscle Foods* **2005**, *16*, 117−125.
(42) Gülçin, I. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology* **2006**, *217*, 213−220.
(43) De Leonardis, A.; Macciola, V. Effectiveness of caffeic acid as an antioxidant for cod liver oil. *Int. J. Food Sci. Technol.* **2003**, *38*, 475−480.

Received for review December 4, 2006. Revised manuscript received March 7, 2007. Accepted March 12, 2007. This work was performed within the Integrated Research Project SEAFOODplus, Contract FOOD-CT-2004-506359, and the research project PPQ2003-06602-C04-03. The financing of this work by the European Union and the Spanish Ministry of Science and Technology is gratefully acknowledged.

JF063498I