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α-Tocopherol Oxidation in Fish Muscle during Chilling and Frozen Storage

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The oxidation of α-tocopherol (TH) in chilled and frozen fish muscle was determined using high-performance liquid chromatography−atmospheric pressure chemical ionization−mass spectrometry. TH oxidation byproducts were identified as α-tocopherolquinone (TQ), 5,6-epoxy-α-tocopherolquinone (TQE_1), and 2-3-epoxy-α-tocopherolquinone (TQE_2). The concentration of TH decreased significantly during storage while those of TQ, TQE_1, and TQE_2 increased noteworthy. The relative amounts of TH and its oxidized products were significantly related with the extent of oxidation produced in postmortem fish, and the ratio TQ/TH is suggested as an index of oxidative stress in fish muscle. The effect of phenolic antioxidants supplementation on retarding TH oxidation was also studied. Data suggested that the addition of 100 ppm of caffeic acid, hydroxytyrosol, and propyl gallate could regenerate endogenous TH from its oxidized forms resulting in an antioxidant synergy consistent with the reduction of lipid oxidation observed in fish muscle supplemented with phenolic compounds.

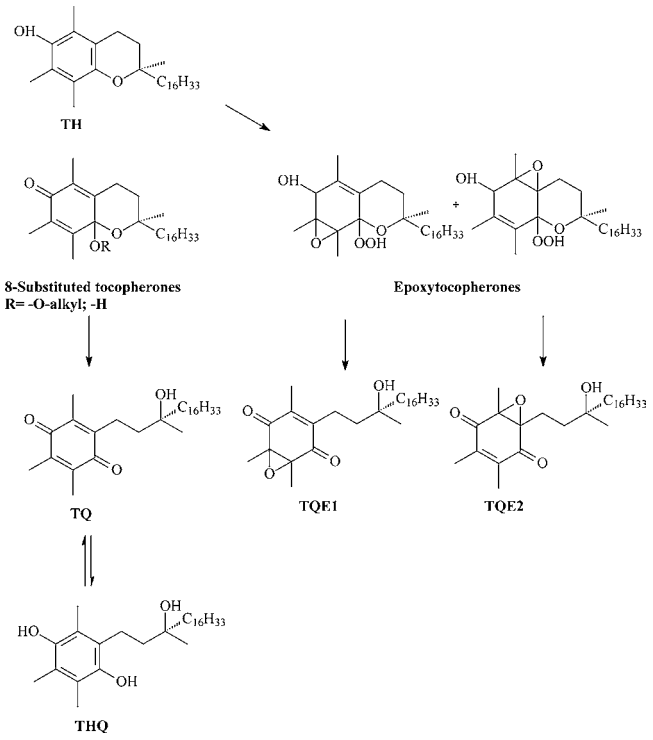
KEYWORDS: α-Tocopherol; tocopherolquinones; oxidation; fish storage

INTRODUCTION

Fish tissues have an efficient antioxidant system that stabilizes its high content in unsaturated lipids (*1*, *2*). α-Tocopherol (TH) or vitamin E is a lipophilic antioxidant of fish muscle and takes an important role, like antioxidant, in vivo (*3*, *4*). Its content is highly related with the stability of lipids and oils (*5*). However, in postmortem muscle, the antioxidant mechanisms lose efficiency due to antioxidant consumption in the oxidative process and the lack of any source of fresh antioxidants (*6*). Recent works suggest that TH is the last defense of fish muscle to inhibit oxidation and its reduction below a critical level leads to lipid oxidation (*7*). TH can neutralize free radicals via its phenol group and through different mechanisms reduces lipid peroxidation (*8*). A number of different reaction products of TH have been identified in biological systems (**Scheme 1**) (*9*, *10*). The first oxidized product of TH, metabolically produced by trapping peroxyl radical, is a tocopheroxyl radical (*5*). The reaction of this radical with lipid peroxides yields mainly an unstable product, 8-substituted tocopherone, which readily hydrolyzes in acidic conditions to tocopherolquinone (TQ). TQ can be eventually reduced reversibly into tocopherolhydroquinone (THQ). Another way currently accepted is the production of isomeric epoxytocopherones, which further hydrolyze in acidic conditions to epoxyquinones: 5,6-epoxy-α-tocopherolquinone (TQE_1) and 2-3-epoxy-α-tocopherolquinone (TQE_2).

The major reaction products identified in biological samples are TQ, THQ, TQE_1, and TQE_2 (*11*, *12*). TQ has been considered an important index of antioxidant activity because it can reflect the oxidative stress on the fatty acids due to TH oxidation (*13*). The TQ/TH ratio in red blood cells has been used to assess the antioxidant status of humans (*13*) and to test the oxidative potency of prooxidants in red cell membranes (*12*). On this basis, a few studies have determined the oxidation of TH in food. Faustman et al. (*14*) measured the TH oxidation

Scheme 1. Oxidation Pathways for TH



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byproducts in postmortem beef muscle and in bovine microsomes. They concluded that the decreasing concentrations of TH and the increasing concentrations of its oxidized products are consistent with the antioxidant role for TH in chilled muscle. Bergamo et al. (*15*) have used the ratio TQ/TH as an index to follow oxidation in dairy products and to examine the influence of processing on fatty acid composition. In fish products, the studies were focused on the antioxidant role of TH and its relation with lipid oxidation (*16*, *17*).

TH and its oxidation byproducts have been analyzed by gas chromatography−mass spectrometry after derivatization (*10*, *14*). Gauttier et al. (*18*) and Mottier et al. (*11*) developed methods based on liquid chromatography−tandem mass spectrometry (LC-MS/MS) with atmospheric pressure chemical ionization mode (APCI) and electrospray ionization (ESI). The lack of protonation sites on TH makes more difficult its analysis by LC-MS using ESI than the employment of APCI-MS (*19*).

The aim of this work was to identify TH oxidation byproducts in fish muscle during postmortem storage. TH and its oxidized products were identified by high-performance liquid chromatography (HPLC)-APCI-MS, and their relative amounts were determined in chilled and frozen fish muscle. The yield of TH oxidation was related with the oxidative stress of fish lipids. The effect of phenolic antioxidants supplementation on TH oxidation was also investigated.

MATERIALS AND METHODS

Materials. Fresh Atlantic mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*) were supplied by a local market. TH, streptomycine sulfate, propyl gallate, and caffeic acid were purchased by Sigma (St. Louis, MO). All chemicals and solvents used were either analytical or HPLC grade (Merck, Whitehouse Station, NJ). 3,4-Dihydroxyphenylethanol (hydroxytyrosol) was kindly provided by the Instituto de la Grasa (CSIC, Sevilla).

Chilled Minced Mackerel. Eight kilograms of fresh Atlantic mackerel (*S. scombrus*), 20−24 different fish, was deboned and eviscerated, and the white muscle was separated and minced. Streptomycine sulfate (200 ppm) was added for inhibiting microbial growth. Caffeic acid and propyl gallate were added at a concentration of 100 ppm (w/w). Portions of 10 g of fish muscle were placed into plastic bags. Control samples in the absence of antioxidants and samples with antioxidants were kept refrigerated at 4 °C. Duplicate samples were taken at different sampling times. The experiment was done twice.

Frozen Minced Horse Mackerel. Ten kilograms of fresh Atlantic horse mackerel (*T. trauchurus*), 25−30 different fish, was deboned and eviscerated, and the white minced muscle was obtained as described above. Hydroxytyrosol and propyl gallate were added at a concentration of 100 ppm (w/w). Portions of 10 g of fish muscle were introduced into a plastic box and were initially kept to −80 °C during 6 h to obtain a faster freezing. Then, control samples and samples with antioxidants were stored at −10 °C. Duplicate samples were taken at different sampling times. The samples were thawed at room temperature for 1 h before of analysis. The experiment was done twice.

Frozen Horse Mackerel Fillets. Twenty kilograms of fresh Atlantic horse mackerel (*T. trauchurus*) was filleted to get skin-on-fillets (20−25 g). A 0.5 mL amount of an aqueous hydroxytyrosol solution (5 mg/mL) was pulverized on each fresh fillet to achieve a final concentration of 100 ppm. Control fillets were prepared by spraying 0.5 mL of water. Each fillet was introduced into a plastic box, and they were initially kept to −80 °C during 6 h to obtain a faster freezing. Then, control samples and samples with hydroxytyrosol were stored at −10 °C. Triplicate samples were taken at different sampling times. The samples were thawed at room temperature for 1 h before of analysis. The experiment was done twice.

Extraction of TH and Its Oxidation Byproducts from Fish Muscle. TH and its oxidation byproducts were extracted using a modification of Burton et al. (*20*). Minced muscle (1 g) was homogenized in 3 mL of chilled 5 mM sodium phosphate buffer, pH 8.0. Four milliliters of 0.1 M sodium dodecyl sulfate was added to the homogenate and vigorously shaken for 1 min. Then, 8 mL of absolute ethanol was added and shaken for 1 min. Two milliliters of hexane was then added, and the mixture was shaken for 1 min. After a brief chilled centrifugation, the top hexane phase was recovered and the aqueous phase was washed with 1 mL of hexane. The hexane layer was dried under a stream of nitrogen. Lipid soluble antioxidants were extracted from the oily drop that remains with 1 mL of methanol. Finally, methanol was evaporated under a stream of nitrogen and 300 μL of absolute ethanol was added. Samples were analyzed in duplicate.

Analysis of Tocopherol and Its Oxidized Products. Analyses were performed by HPLC (P4000, ThermoFinnigan, San José, CA) coupled with a diode array detector (SpectraSystem UV6000LP, ThermoFinnigan) and a Mass Spectrometer (LCQ DEX XPPlus, ThermoFinnigan) using APCI in positive ionization mode. Operating conditions were fixed with a discharge voltage at 4.10 kV, a capillary voltage at 75.67 V, a discharge current at 4.0 μV, and a vaporizer temperature of 402.0 °C. High purity nitrogen was used as the sheath gas with a flow rate of 60 unit/min. Full scan spectra were obtained over the range of 200−1000 u, with a scan duration of 5 μs and a vacuum of 2.21×10^{-5} Torr.

TH and its oxidized products were separated on a Waters C18 column (150 mm × 2.1 mm i.d., 3.5 μm particle size, Milford, MA) with a flow rate of 0.1 mL/min using MeOH (A) and 2-propanol (B) as the mobile phase. The linear gradient program was as follows: 100% A for 2 min, 100 to 60% A between 2 and 20 min, 60 to 30% A between 20 and 40 min, and 30−100 A% between 40 and 42 min. Peaks were identified by their retention times (*21*), absorption peaks, and molecular and diagnostic fragment ions according to Gautier et al. (*18*), Mottier et al. (*11*), and Lauridsen et al. (*22*).

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 5 g of fish 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, where 7 showed absolutely fresh and ≤1 showed putrid (*23*).

Peroxide Value. The peroxide value of fish muscle was determined by the ferric thiocyanate method (*24*) and was expressed as mequiv oxygen/kg lipid. Analyses were performed in duplicate.

TBARS. The thiobarbituric acid index (mg malonaldehyde/kg muscle) was determined according to Vyncke (*25*). Analyses were performed in duplicate.

Statistical Analysis. The data were compared by one way analysis of variance (*26*), and the means were compared by a least squares difference method (*27*). Significance was declared at $p < 0.01$.

RESULTS AND DISCUSSION

Effect of Storage Conditions on the Yield of TH Oxidation Byproducts. Table 1 shows the amounts of TH and its oxidized products in minced mackerel muscle in the absence of phenolic antioxidants during storage at 4 °C. The level of TH was maintained high during the first 2 days at 4 °C, and after that, its concentration decreased gradually with time. The first significant oxidation of TH took place by the third day at the same time as a significant increase of the amounts of TQ, TQE_1, and TQE_2 was evident. At the end of the storage, TQ, TQE_1, and TQE_2 were significantly accumulated in fish muscle. THQ was not observed after 11 days at 4 °C. TH accounted for approximately 98% of total tocopherol at day 0 and decreased up to 38% at day 11. After 7 days at 4 °C, TQ constituted the greatest relative portion of tocopherol equivalents, 46%. Both epoxyquinones, TQE_1 and TQE_2, were generated during fish chilling storage in contrast with results of Faustman et al. (*14*) in beef steaks in which only the increase of TQE_2 was significant after 5 days at 4 °C. Our data demonstrated that both epoxyquinones were formed during the oxidation of TH although

Table 1. Concentrations of TH and Its Oxidation Byproducts (μg/g Muscle), Peroxides (mequiv O_2/kg Lipid), and TBARS (mg MDA/kg muscle) in Mackerel Minced Muscle during Storage at 4 °C (Mean ± Standard Deviation)[a]

day	TH	TQ	TQE_1	TQE_2	peroxides	TBARS	TQ/TH
0	3.662 ± 0.281 a	0.050 ± 0.002 a	0.028 ± 0.002 a	0.012 ± 0.002 a	1.845 ± 0.065 a	0.160 ± 0.540 a	0.014 ± 0.000 a
1	3.439 ± 0.206 a	0.086 ± 0.004 b	0.028 ± 0.000 a	0.023 ± -0.002 b	1.515 ± 0.145 a	0.825 ± 0.395 a	0.025 ± 0.003 a
3	0.951 ± 0.161 b	0.680 ± 0.031 c	0.089 ± 0.000 b	0.212 ± 0.026 cd	18.050 ± 5.850 b	2.965 ± 0.835 b	0.731 ± 0.091 b
5	0.866 ± 0.055 b	0.797 ± 0.024 e	0.094 ± 0.005 b	0.352 ± 0.166 d	41.205 ± 5.085 c	4.175 ± 1.385 bc	0.923 ± 0.032 c
7	0.590 ± 0.016 c	0.720 ± 0.021 d	0.093 ± 0.003 b	0.175 ± 0.003 c	65.150 ± 11.620 d	6.240 ± 2.220 c	1.222 ± 0.070 d
11	0.653 ± 0.034 c	0.755 ± 0.006 d	0.103 ± 0.001 c	0.190 ± 0.001 cd	44.270 ± 1.260 c		1.159 ± 0.050 d

[a] Values in each column with the same superscript letter were not significantly different ($p < 0.01$).

Table 2. Concentrations of TH and Its Oxidation Byproducts of Minced Fish Muscle (μg/g Muscle), Peroxides (mequiv O_2/kg Lipid), and TBARS (mg MDA/kg) during Storage at –10 °C (Mean ± Standard Deviation)[a]

weeks	TH	TQ	TQE_1	TQE_2	peroxides	TBARS	TQ/TH
0	2.367 ± 0.012 a	0.085 ± 0.000 a	0.000 ± 0.000 a	0.000 ± 0.016 a	2.865 ± 2.238 a	0.549 ± 0.048 a	0.036 ± 0.005 a
2	2.286 ± 0.297 a	0.279 ± 0.036 b	0.090 ± 0.026 b	0.053 ± 0.002 b	3.401 ± 0.101 a	0.773 ± 0.196 a	0.252 ± 0.130 b
4	0.022 ± 0.020 b	0.910 ± 0.016 c	0.122 ± 0.008 b	0.078 ± 0.001 c	27.398 ± 0.002 b	0.536 ± 0.048 a	41.454 ± 0.895 c
5	0.022 ± 0.019 b	1.017 ± 0.001 d	0.145 ± 0.004 c	0.082 ± 0.010 c	80.756 ± 3.939 c	2.092 ± 0.328 b	46.350 ± 0.885 c
7	0.022 ± 0.084 b	1.003 ± 0.014 d	0.100 ± 0.019 b	0.091 ± 0.025 c	103.855 ± 12.534 d	1.879 ± 0.154 b	45.707 ± 3.807 c
9	0.022 ± 0.136 b	1.045 ± 0.018 d	0.106 ± 0.015 b	0.081 ± 0.008 c	120.597 ± 24.800 d	3.224 ± 0.222 c	47.619 ± 6.209 c

[a] Values in each column with the same superscript letter were not significantly different ($p < 0.01$).

the increase of the concentration of TQE_2 was significantly higher than the formation of TQE_1. Furthermore, the amounts of TQE_1 continued to increase significantly during the whole experiment and the levels of TQE_2 were maintained rather constant after 3 days at 4 °C.

TH of frozen horse mackerel minced muscle in absence of antioxidants was oxidized with time at −10 °C, resulting in TQ, TQE_1, and TQE_2 (**Table 2**). The amount of TH was maintained high during the first 2 weeks. By the 4th week, the concentration of TH dropped severely, revealing an important oxidation. As a result, a notable increment of the concentrations of TH oxidation byproducts, TQ, TQE_1, and TQE_2, occurred. TQ was significantly accumulated in fish muscle during the whole experiment, and its relative concentration increased from 3.5% of total tocopherol equivalents at the 0th week up to 83.4% at the 9th week. In agreement with results of chilled fish muscle, TQE_1 and TQE_2 were also produced during the oxidation of TH. The relative amounts of TH decreased from 96.5% of total tocopherol equivalents at week 0 to 1.8% at week 9.

Effect of Storage Condition on Oxidative Stress Extent. The relative amounts of TH and its oxidation byproducts in chilled fish muscle were consistent with the antioxidant function of TH and therefore with the extent of lipid oxidation (**Table 1**). Sensory analysis demonstrated that during the first 2 days at 4 °C, fish samples showed a very fresh odor. A loss of sensory quality occurred by the third day in which the panelist indicated the first detection of a clear rancid odor. By the 7th day, the sensory score indicated that samples were very rancid. According to the sensory analysis, the formation of peroxides and TBARS occurred significantly by the third day. The detection of rancid odors and the significant formation of lipid oxidation products by the third day corresponded with the remarkable decrease of TH concentration and the increase of TQ, TQE_1, and TQE_2. The ratio between the concentration of TQ/TH, recently suggested as an index of oxidative stress in dairy products (*15*), was consistent with the formation of off-flavors, peroxides, or TBARS and could be associated with the oxidative stress of fish muscle (**Table 1** and **Figure 1A**). As regards to epoxytocopherolquinones, the levels of TQE_1 were significantly related with the formation of peroxides during oxidation of fish muscle but not the levels of TQE_2.

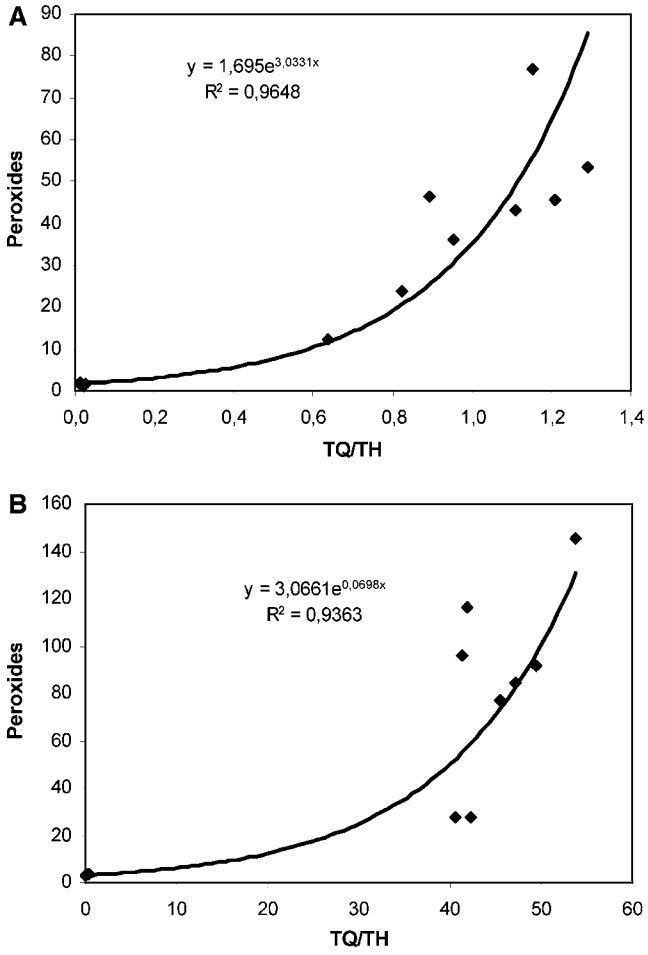

Figure 1. Relation between the ratio of TQ/TH and the formation of peroxides. (**A**) Mackerel minced muscle stored at 4 °C. (**B**) Horse mackerel minced muscle stored at –10 °C.

The recovery of total TH plus its oxidation byproducts in chilled fish was great whereas the samples were not oxidized (97.4% at day 1) (**Table 1**). However, when peroxide values increased significantly by the third day, the recovery was

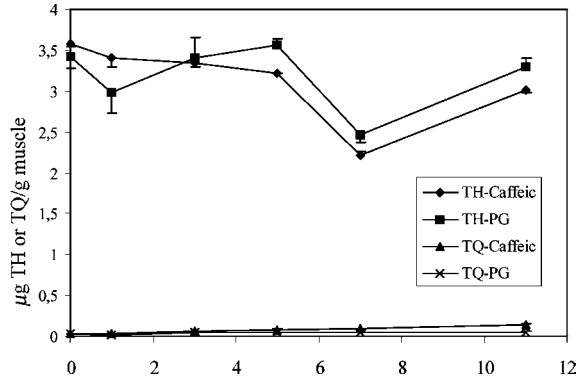

Figure 2. Concentrations (μg/g muscle) of TH and TQ in minced mackerel supplemented with caffeic acid and propyl gallate during storage at 4 °C.

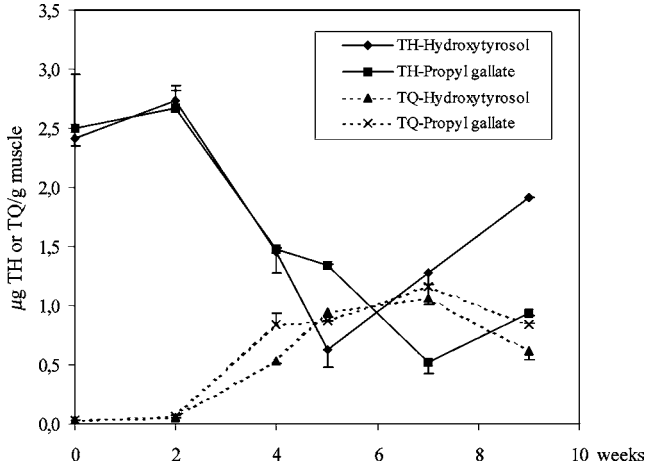

Figure 3. Concentrations (μg/g muscle) of TH and α-TQ in minced horse mackerel muscle supplemented with hydroxytyrosol and propyl gallate during storage at –10 °C.

approximately 51%. The recovery of TH plus its oxidation byproducts was 47.9% at the end of the experiment. Faustman et al. (*14*) also reported poor recovery in oxidized beef steaks during chilling storage due to further degradation of TH to other oxidation products not identified in the chromatographic analysis.

Accordingly, with results obtained during the chilled experiment, the yield of TH oxidation in frozen minced muscle in absence of antioxidants was highly related with the extent of lipid oxidation (**Table 2**). The sensory quality of frozen fish was high during the first 2 weeks at −10 °C. The values of peroxides and aldehydes were also relatively constant between 0 and 2 weeks of storage. By the fourth week, TH was highly oxidized and its oxidation corresponded with a sensory score indicating the first detection of a rancid odor and a significant formation of peroxides. The following weeks were characterized by sensory scores revealing clearly rancid odors and high amounts of peroxides. The ratio TQ/TH was again an index of the oxidative stress in fish muscle (**Table 2** and **Figure 1B**). The recovery of total tocopherol equivalents decreased from 100 to 44% during the experiment. This fact agrees with the poor recovery observed above in highly oxidized chilled fish and in chilled beef (*14*).

Effect of Phenolic Antioxidants Supplementation on TH Oxidation. Figure 2 shows the degradation of TH of chilled fish supplemented with caffeic and propyl gallate. The rate of TH oxidation in these samples was lower than in their corresponding controls in the absence of phenolics (**Table 1)**. These results agree with data previously published, which showed a retard in the consumption of TH in frozen fatty fish supplemented with grape procyanidins and propyl gallate (*7*). The concentration of TH of chilled fish in the presence of caffeic acid decreased slowly during the first 5 days. After that, there was a depletion of about 31% at the 7th day. Unexpectedly, by the 11th day, we registered a new increase of TH concentration. These results were confirmed in samples supplemented with propyl gallate. There was a significant reduction of TH after 7 days, about 38%. In agreement with results obtained in samples with caffeic acid, a new increase of TH concentration was registered by the 11th day. Therefore, the relative amounts of TH in samples treated with caffeic acid and propyl gallate were maintained upper than 90% after 11 days of storage. Parallel with these results, there was negligible formation of peroxides after 11 days at 4 °C. In both systems, the concentration of TQ increased gradually but its content was low as compared to nonoxidized samples. We have not observed a specific value of TQ/TH to direct TH regeneration during the chilling experiment. The values of TQ/TH corresponding to day 7 were 0.039 ± 0.006 and 0.019 ± 0.002 for caffeic acid and propyl gallate samples, respectively.

Figure 3 illustrates the oxidation of TH in frozen minced muscle in the presence of hydroxytyrosol and propyl gallate after 9 weeks at −10 °C. The depletion of TH in these samples was slower than in controls (**Table 2**). TH of samples supplemented with hydroxytyrosol was gradually oxidized between 2 and 5 weeks at −10 °C, whereas the concentration of TQ and peroxides increased considerably (2.453 ± 1.981 mequiv O_2/kg lipid at week 0 up to 24.930 ± 13.511 mequiv O_2/kg lipid at week 5). In latter weeks, we detected a new increase of the levels of TH. This result was also confirmed in fish samples supplemented with propyl gallate. TH was progressively oxidized during the first 7 weeks at −10 °C while significant increases of TQ and peroxides (2.600 ± 1.152 mequiv O_2/kg lipid at week 0 up to 42.328 ± 13.687 mequiv O_2/kg lipid at week 7) were detected. By the 11th week, a new increase of TH was remarkable. In both cases, the possible

Table 3. Concentrations of TH and TQ of Frozen Fillets in the Absence and in the Presence of Hydroxytyrosol (μg/g Muscle) during Storage at –10 °C (Mean ± Standard Deviation)[a]

weeks	TH–control	TQ–control	TH–hydroxytyrosol	TQ–hydroxytyrosol
0	2.476 ± 0.192 a	0.000 ± 0.000 a	2.381 ± 0.329 a	0.000 ± 0.000 a
1	1.355 ± 0.420 b	0.225 ± 0.105 b	2.599 ± 0.158 a	0.000 ± 0.000 a
3	0.944 ± 0.355 b	0.636 ± 0.281 bcd	2.504 ± 0.120 a	0.000 ± 0.000 a
5	0.057 ± –0.066 c	0.677 ± 0.079 cd	2.459 ± 0.501 a	0.025 ± 0.044 a
7	0.067 ± 0.042 c	0.706 ± 0.107 cd	1.535 ± 0.244 b	0.183 ± 0.016 b
10	not detected	0.533 ± 0.096 c	1.315 ± 0.483 b	0.139 ± 0.091 b
13	not detected	0.692 ± 0.140 cd	0.388 ± 0.057 c	0.403 ± 0.074 c
17	not detected	0.804 ± 0.053 d	0.923 ± 0.205 b	0.326 ± 0.079 c

[a] Values in each column with the same superscript letter were not significantly different ($p < 0.01$).

regeneration of TH occurred when values of TQ/TH changed from 0.4 in the fourth week up to 1.5 in the fifth week in samples with hydroxytyrosol and from 0.7 in the fifth week up to 2.3 in the seventh week for samples with propyl gallate.

The data obtained in frozen minced muscle were corroborated with results corresponding to frozen fish fillets (**Table 3**). TH of samples in absence of hydroxytyrosol decreased gradually with time at −10 °C. TH of samples containing hydroxytyrosol was high during the first five weeks at −10 °C, and its first decrease was detected by the 7th week. By the 13th week, we detected a minimum value followed by a new increment at the 17th week. The concentration of TQ was not significantly different in these last weeks.

Therefore, data obtained during frozen storage of fish muscle supplemented with phenolic antioxidants confirmed the results obtained in the chilled experiment. It seems that TH can be repaired in fish muscle by the addition of 100 ppm of caffeic acid, hydroxytyrosol, and propyl gallate. In the last years, there have been some evidences for a possible regeneration of TH from their oxidized forms in different systems. Moore and Ingold (*28*) have shown the conversion of orally administrated TQ into TH in humans. Tea catechins reduced α-tocopheroxyl radical to regenerate TH in sodium dodecyl sulfate micelles (*29*). Flavonoids also possess TH-repairing activity in human low densitity lipoprotein (*30*). Ascorbic acid, glutathione, and green tea catechins have also evidenced a regeneration of TH (*31*−*33*). The possible regeneration of TH observed in this work seems to be associated to the presence of phenolic antioxidants when TH is partly oxidized. In the frozen experiment, the concentration of TQ did not increase in the weeks in which TH was regenerated. This fact suggests a possible conversion of TQ into TH (**Figure 3**), although data corresponding to chilled fish muscle did not confirm this preliminary observation. Both experiments are hardly comparable. The initial amounts of TH of mackerel and horse mackerel were quite different and the extent of TH/lipid oxidation as well (**Tables 1** and **2**). As result of this, the amounts of TQ generated during the chilled and the frozen experiments with minced muscle were also very different. In chilled minced mackerel, the regeneration occurs before the propagation of oxidation, when the values of TQ were considerably low, around 0.05−0.15 μg/g muscle. However, in frozen minced horse mackerel, the oxidation of TH was very rapid and we detected the regeneration during the propagation step when the values of TQ were high, around 1 μg/g muscle. The mechanism involved in TH regeneration by phenolics is not well-established yet, and it could go through the reduction of tocopheroxyl radical or the presence of TQ (*29*). Additionally, TQ can also be degraded into other forms not detected in this analysis. More work is needed to elucidate the pathways of the reaction.

In conclusion, oxidation of TH in chilled and frozen fish muscle leads to the accumulation of TQ, TQE_1, and TQE_2, and the relative levels of TH and its oxidation byproducts in fish are consistent with an antioxidant role for TH in postmortem muscle. The ratio TQ/TH is an useful index of oxidative stress in fish during chilling and frozen storage. The protection of TH previously reported in frozen fatty fish by some phenolic antioxidants (*7*) and evidenced in this work can be related to a regeneration of TH. Phenolic antioxidants might reduce α-tocopheroxyl radical or TQs to regenerate TH. Work is now in progress to confirm and clarify such regeneration, which leads to a significant synergy between phenolic antioxidants and endogenous TH in fish muscle.

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