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Physicochemical changes in ω − 3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) muscle during refrigerated storage

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Abstract

Physicochemical changes of ω − 3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) fillets developed by dietary modification with flaxseed oil and α-tocopheryl acetate (α-TA) were determined during storage at 2 °C. Trout were fed experimental diets for 120 days followed by processing to obtain boneless skinless fillets. The dietary modification increased concentration of total ω − 3 fatty acids in the fillets, which enhanced chances for lipid oxidation during storage. The fillets were vacuum or non-vacuum packed and stored at 2 °C for 10 or 12 days. Dietary α-TA resulted in higher ($P < 0.05$) concentration of α-tocopherol in fillets during storage; however, it did not retard ($P > 0.05$) lipid oxidation. Vacuum packaging resulted in much lower ($P < 0.05$) TBARS and higher ($P < 0.05$) retention of α-tocopherol during storage than non-vacuum packaging. However, α-tocopherol unlike vacuum packaging better protected ω − 3 FA in the fillets during storage.
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Keywords: Flaxseed oil; α-Tocopheryl acetate; Trout fillet; Refrigerated storage; TBARS values; Unsaturated fatty acids; ω − 3 fatty acids

1. Introduction

According to the American Heart Association statistics in 2006, the cardiovascular disease (CVD) has been the number one leading cause of human death in the United States since 1990 (Thom et al., 2006). Because ω − 3 fatty acids (ω − 3 FA, also known as $n - 3$ FA) in fish and fish-derived food products can reduce the risk of CVD, consumption of at least two fish servings per week is recommended by the American Heart Association (Krauss et al., 1996). Institute of Medicine (2002) suggests intake levels for ω − 3 polyunsaturated fatty acids (ω − 3 PUFA) at 1.6 g and 1.1 g per day for men and women, respectively.

Although docosahexaenoic acids (DHA, 22:6$n - 3$) and eicosapentaenoic (EPA, 20:5$n - 3$) are considered more important than α-linolenic acid (ALA, 18:3$n - 3$) for human nutrition, Zhao et al. (2004) investigated the effects of average American diet, a linoleic acid (LN, 18:2$n - 6$) diet, and an ALA diet. These authors found that the level of C-reactive protein, a marker of inflammation strongly associated with heart disease, declined for both the LN and ALA diets, but much more significantly for the ALA diet. Therefore, Zhao et al. (2004) concluded that ALA seems to lower the CVD risk by inhibiting vascular inflammation beyond its lipid-lowering effects. Similar results to the effects of ALA on cardio-protective fatty acids (FA) in human were also reported by Harper, Edwards, DeFilipis, and Jacobson (2006). In addition, ALA can be converted to EPA and DHA in mammals (Voss, Reinhart, Sankarappa, & Sprecher, 1991). Therefore, it is likely that regardless of the chain length of ω − 3 FA, increasing overall concentration of these FA and an ω − 3 to ω − 6 fatty acid ratio (ω − 3/ω − 6 FA ratio) will contribute to human health. Although currently there is no formal dietary

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recommendation for the adequate ω − 3/ω − 6 FA ratio for humans, higher ω − 3/ω − 6 FA ratio in the human diet seems to contribute to human health.

While worldwide fish consumption has been steadily increasing, the capture of marine fish has been fairly stable (Williams, 1998). This is likely why the aquaculture production has quadrupled and currently makes up for that shortfall (Sargent & Tacon, 1999). However, the content of the ω − 3 FA in farmed rainbow trout fillets is typically lower than in wild counterparts (Hardy, 1990). Greene and Selivonchick (1990) suggested that trout maintained concentrations of the EPA and DHA at a physiologically optimum level that are related to their dietary precursor α-linolenic acid (ALA, 18:3*n* − 3). Flaxseed oil (FO) contains the highest concentration of ALA compared to other plant-derived lipid sources used in fish feeds (National Research Council, 1993). An ω − 3-enhanced rainbow trout (*Oncorhynchus mykiss*) was successfully raised in our research farm (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2006).

It is well-known that lipid oxidation is one of the major problems in fish-derived food products. Polyunsaturated fatty acids (PUFA) are more easily oxidized than saturated fatty acids, and therefore, food products enhanced with the ω − 3 PUFA are also more prone to lipid oxidation. There are potential human health risks associated with increased consumption of oxidized ω − 3 PUFA products (Fritsche & Johnston, 1990; Kubow, 1993). Another important factor to limit a more common use of ω − 3 PUFA-enhanced food products is the development of off-flavors due to the lipid oxidation that may be offensive to consumers (Waagbø, Sandnes, Torrissen, Sandvin, & Lie, 1993). The composition of fish fillets typically correlates with the composition of the fish diet. Therefore, trout diet supplemented with an antioxidant may decrease lipid oxidation of the fillets.

In order to prevent quality loss, fish-derived food products require an effective antioxidant system due to high unsaturation of fish muscle lipids (Jia et al., 1996). Fat-soluble antioxidants, such as vitamin E, play an important role in preventing the oxidation of unsaturated lipids in fish muscle (Pope, Burtin, Clayton, Madge, & Muller, 2002). The α-tocopherol has the greatest antioxidant activity among four homologue pairs (α-, β-, γ-, δ-tocopherols and tocotrienols) (Burton & Ingold, 1981). When comparing the antioxidant effectiveness among α-, γ-, and δ-tocopherols, α-tocopherol typically shows the highest rate of scavenging of lipid peroxyl and alkoxyl radicals (Kulas & Ackman, 2001). Hence, α-tocopheryl acetate (α-TA), a vitamin E derivative, is usually used as an antioxidant to reduce lipid oxidation in food. However, the loss of antioxidant efficacy in postmortem muscle is due to the depletion of the antioxidant in the muscle (Petillo, Hultin, Krzynowek, & Autio, 1998). It has also been speculated that lipid oxidation of ω − 3 PUFA is possibly initiated due to the depletion of tissue vitamin E in meat systems (Ajuyah, Ahn, Hardin, & Sim, 1993). Dietary α-TA showed antioxidant effects on ω − 3 enriched pork (Rey et al., 2001), dry-cured ham (Isabel et al., 2003), broilers (Ahn, Wolfe, & Sim, 1995), eggs (Galobart, Barroeta, Baucells, Codony, & Ternes, 2001), salmon (Scaife, Onibi, Murray, Fletcher, & Houlihan, 2001), and trout (Frigg, Probucki, & Ruhdel, 1990).

Based on the results from our research group, supplementing rainbow trout with 900 ppm of α-TA for 120 days of feeding increased α-tocopherol content in the fillets obtained from ω − 3-enhanced rainbow trout (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2007). It was also reported that supplementing α-TA in diets increased pig muscle α-tocopherol levels and improved oxidative stability after slaughter (Buckley, Morrissey, & Gray, 1995). Therefore, the addition of antioxidants to fish diets may also promote post-slaughter oxidative stability during storage and distribution of fish-derived food products. However, there are no reports concerning physicochemical changes and prevention of quality degradation during post-slaughter storage of trout enriched with ω − 3 FA via dietary modification with flaxseed oil and α-tocopheryl acetate.

2. Materials and methods

2.1. Feeding trial and diets

A gravity-fed flow-through raceway system composed of four levels was used for this study. Each level had two parallel lanes and each lane had two tanks. Tanks were stocked with 75 rainbow trout (*O. mykiss*) fingerlings per tank (91 × 122 × 91 cm). Rainbow trout were fed dry pelleted diets formulated with 0 (basal diet) or 15.0% (w/w) of flaxseed oil (FO) supplementation (Ziegler Brothers, Inc., Gardners, PA). Each level of the FO supplementation was also enhanced with 0 or 900 ppm of α-tocopheryl acetate (α-TA) (Table 1). Hence, there were four dietary treatments. The dietary treatments were randomly assigned to the tanks in each level of the raceway system. The fat in the basal diet was partially replaced with the FO in the experimental diets. The lipid replacement did not affect

Table 1
Major ingredients of the trout basal diet (g/kg)

Ingredients	(g/kg)
Wheat middlings	280
Fish meal	250
Hydrolyzed feather meal	100
Dehulled soybean meal	100
Blood meal	100
Ground extruded whole soybean	60
Corn gluten meal	50
Minerals	25
Vitamins	15
Soy lecithin	10
Yeast culture	10
Crude protein (g/kg)	42
Metabolizable energy (MJ/kg)	12.6

the protein content in the diets; and therefore, the diets were isonitrogenous. Feed was stored at 4 °C.

Approximately 1512 l per minute of spring water flowed through the raceway system. It was aerated entering the system and half way through the system to maintain a dissolved oxygen concentration above 70% of saturation. Water temperature was approximately 12 °C during the feeding trial. Fish were fed 6 days each week and maintained on a natural photoperiod. Fish were hand fed to satiation twice a day for 120 days. The animal experiments were approved by appropriate institutional authorities.

2.2. Sample preparation

The fish from the four dietary treatments were harvested on day 120 and then killed by a blow to the head. All harvested fish were stored at 4 °C before filleting. The harvested fish were filleted to obtain boneless and skinless butterfly fillets on the same day when the fish were harvested. Following filleting, six fillets were randomly selected from six different trout per dietary treatment. The fillets were either individually placed in nylon vacuum pouches (3 mil standard barrier, Koch, Kansas City, MO), labelled and vacuum packed or the fillets were individually placed on plastic trays and aerobically over-wrapped (non-vaccum) with a typical household plastic wrapping material (Aldi® clear plastic wrap, Batavia, IL). The vaccum- and non-vacuum packed fillets were subjected to storage at 2 °C for 10 or 12 days. Following the storage period, fillets were individually homogenized in a laboratory blender (Model 51BL31, Waring Commercial, Torrington, CT) and analyzed for moisture, total fat, thiobarbituric acid reactive substance (TBARS) values of fillets, and α-tocopherol content and fatty acid profiles in fillets. The storage periods were selected based on correlation between TBARS values in fish and rancidity development reported by Ke, Cervantes, and Robles-Martinez (1984). In addition, an un-trained panel monitored overall appearance and smell (non-vacuum only) of the fillets during storage in order to assess when the storage should be stooped and the fillets analyzed. The objective was to analyze the fillets when the rancidity development was slightly in progress (10 days) and more advanced (12 days).

2.3. Moisture (%) and total fat (%)

Two grams of homogenized fillets were placed onto an aluminum dish (Fisher Scientific Co., Fairlawn, NJ) and dispersed evenly across the dish. The moisture content of fillets was determined by using the oven-drying method (100 °C for 18 h) (Association of Official Analytical Chemists, 1995). Fat content in fillets was determined according to Soxhlet extraction method (Association of Official Analytical Chemists, 1995). Sample size was 5 g and extraction time was 16 h at a drip rate of approximately 10 ml/min. Extractions were performed with petroleum ether. Fat content was determined on a gravimetric basis and expressed as percent by weight on dry basis.

2.4. Measurement of thiobarbituric acid reactive substances (TBARS)

Oxidative rancidity of fillets was measured by a 2-thiobarbituric acid reactive substances (TBARS) assay of malondialdehyde (MDA) as described by Jaczynski and Park (2003). Three drops of antioxidant (Tenox 6, Eastman Chemical Div., Kingsport, Tenn., USA) and 3 mL of TBA were added to 0.2 g of homogenized fillet sample. Then, 17 mL trichloroacetic acid-HCl reagent was added. The solution was flushed with nitrogen and closed. A blank was prepared in the same manner, but without sample. The tubes were boiled for 30 min, and then cooled. The colored solution (15 mL) was centrifuged at 5000*g* for 15 min. A clear and colored supernatant was transferred to a cuvette, and the absorbance was measured at 535 nm using a UV/Vis spectrophotometer (model DU530, Beckman Instruments, Fullerton, CA). The TBARS value was calculated based on molar absorptivity of MDA (156,000 M^{-1} cm^{-1} at 535 nm) and the results were reported as mg MDA/kg of sample.

2.5. Measurement of α-tocopherol content

The content of α-tocopherol in trout fillets was measured by a modified saponification method using high pressure liquid chromatography (HPLC) described by Liu and Lee (1998). An aliquot of 0.25 g of L-ascorbic acid (Fisher Scientific, Fair Lawn, NJ) and 0.01 g of TBHQ (*tert*-butylhydroquinone, Sigma–Aldrich, St. Louis, MO) were added to approximately 1 g of a homogenized fillet sample in a test tube. Exact weight of the fillet sample was recorded and used in calculations. A freshly prepared 7.3 mL digestion solution (11% (w/v) potassium hydroxide, 55% (v/v) ethanol, 45% (v/v) distilled and deionized water) was added to the fillet sample, followed by vortexing (Vortex Genie 2, Scientific Industries, Bohemia, NY) for 30 s to dissolve the ascorbic acid. The tubes were incubated at 80 °C for 20 min with continuous and gentle shaking in order to digest the fillet sample. Immediately following the incubation, the tubes were cooled in ice slush for 10 min. An aliquot of 4 mL of iso-octane (Fisher Scientific, Fair Lawn, NJ) was added to the cooled solution and the tubes were vortexed for 2 min. Then, the tubes were allowed to stand in the ice slush for 5 min. An aliquot of 1 mL of the top clear iso-octane layer was transferred to a small tube and stored at −80 °C until the solution was injected into the HPLC (Perkin–Elmer series 4, Perkin–Elmer, Norwalk, CT) using a Rheodyne injector (Rheodyne, Rohnert Park, CA). The HPLC was equipped with a Waters Resolve C-18 spherical silica column (5 μm, 3.9 × 150 mm) (Water Guard-Pak, Waters Corp., Milford, MA) and a spectrophotometric detector (Perkin–Elmer LC-75, Perkin–Elmer, Norwalk, CT). A mixture of iso-octane/tetrahydrofuran (96/4, v/v)

was prepared and filtered (0.45 μm) daily and the mixture was used as a mobile phase. The flow rate was 1 ml/min and the injection volume was 30 μL. The α-tocopherol was detected at excitation wavelength of 296 nm and emission wavelength of 325 nm. The blank was run as described above, but without the fillet sample. Various isomeric forms of tocopherol (Sigma–Aldrich, St. Louis, MO) were also run on the HPLC as standards as well as the extraction efficiency of α-tocopherol was determined and used in calculations. An experimental standard curve was used to calculate α-tocopherol content in the trout fillets, which is reported as mg of α-tocopherol per kg of fillets.

2.6. Lipid extraction and fatty acid analysis

Lipids were extracted using methodology described by Folch, Lees, and Sloane (1957) and used for analysis of fatty acid profile. According to the procedure of Fritsche and Johnston (1990), fatty acids were transmethylated by the addition of 4 mL of 4% (w/v) methanolic H_2SO_4 and heated in a 90 °C water bath for 60 min. The mixture was saponified by transferring through a Na_2SO_4 filled glass Pasteur pipette and subsequently dried under N_2 in a 60 °C water bath for 60 min. The fatty acid methyl esters (FAME) were re-suspended in filtered isooctane. The FAME and standards were analyzed by using a gas chromatograph (Varian CP-3800 gas chromatograph, Varian Analytical Instruments, Walnut Creek, CA) and a flame ionization detector fitted with a wall-coated open tubular (WCOT) fused silica capillary column (50 m length, 0.25 mm inside diameter; Varian Analytical Instruments, Walnut Creek, CA). Injection and detection temperature was maintained at 220 °C and column temperature was 190 °C. The stationary phase was CP-Silica 88 (Varian Analytical Instruments, Walnut Creek, CA). Nitrogen was the carrier gas, and a split ratio of 10–1 was used. The fatty acids were identified by comparing their retention times with known standards (Sigma, St. Louis, MO) and references (Ackman, 1980). Peak area and the amount of each fatty acid were computed by an integrator using the Star GC workstation version 6 software (Varian Analytical Instruments, Walnut Creek, CA).

2.7. Statistical analysis

The experiment was conducted using a 2×2 factorial arrangement of treatment in a randomized block design. The interaction effect (FO × α-TA), main effect (FO and α-TA), and blocking effect (packaging method) were analyzed. All significant differences in the interaction effect, main effect, and blocking effect were tested using an ANOVA test at 0.05 probability level. When a significant difference in the interaction effect was determined, the least significant difference (LSD) test at 0.05 probability level was used to test differences between combination treatments. At least six trout ($n = 6$) and six feeds ($n = 6$) from each experimental diet were randomly obtained and analyzed. All statistical analyses of data were performed using SAS Institute (2002).

3. Results and discussion

3.1. Trout muscle fat and moisture content

There was no ($P > 0.05$) interaction between flaxseed oil (FO) and α-tocopheryl acetate (α-TA) supplementations with respect to moisture and fat contents in fillets during refrigerated storage at 2 °C (Tables 3 and 4). Generally, supplementation of the basal diet with FO and α-TA, as well as the packing methods did not change ($P < 0.05$) moisture and fat contents in fillets.

It has been demonstrated that fish raised on lipid-rich diets typically have higher fat content (Alvarez et al., 1998; Wang et al., 2005). However, our data suggest that increased dietary fat due to FO supplementation has an insignificant effect on lipid concentration of rainbow trout fillets. Regost, Arzel, Cardinal, Laroche, and Kaushik (2001) showed that feeding trout with diets containing increased concentrations of dietary fat results in significantly greater concentration of visceral fat; however, similar to our results, the muscle lipids concentration was insignificantly affected. Gelineau, Corraze, Boujard, Larroquet, and Kaushik (2001) fed rainbow trout four diets with increasing levels of lipids (15%, 20%, 25%, and 30%). They concluded similarly to Regost et al. (2001) that the increased lipid concentration in the fish body was mainly due to fat deposition in the visceral adipose tissue, and the intra-muscular fat was insignificantly affected by the concentration of the dietary fat. Trout in our study were filleted prior to the refrigerated storage; therefore, the viscera including the visceral adipose tissue was removed. In addition, the belly flaps that typically contain more fat were trimmed off during filleting. This is likely why the total fat data in our study is similar to those presented by Regost et al. (2001) as well as Gelineau et al. (2001).

Table 2
Total fat and fatty acid composition[a] of experimental diets

Parameter	% FO supplementation	
	0%	15.0%
	% fatty acid in total fatty acids[b]	
18:3$n-3$	3.47 ± 1.14	46.22 ± 1.89[a]
20:5$n-3$	10.93 ± 0.64[a]	1.22 ± 0.32
22:6$n-3$	12.72 ± 0.77	1.62 ± 0.15[a]
18:2$n-6$	20.88 ± 1.08	21.60 ± 0.90
20:4$n-6$	0.27 ± 0.17	0.33 ± 0.03
Total unsaturates	60.98 ± 1.01	82.50 ± 1.39[a]
Total saturates	39.02 ± 1.01	17.50 ± 1.39[a]
Total ω − 3	27.47 ± 1.44	49.07 ± 2.13[a]
Total ω − 6	22.17 ± 1.08	22.57 ± 0.86
ω − 3/ω − 6	1.27 ± 0.10	2.17 ± 0.04[a]
Total fat (%, dry basis)	14.43 ± 2.32	24.86 ± 0.65[a]

[a] Indicates significant differences ($P < 0.05$) between mean values of the same type of fatty acid.
[b] Data are given as mean ± SEM ($n = 6$).

Table 3
Moisture content in trout fillets as affected by feed supplementation (flaxseed oil (FO) and α-tocopheryl acetate (α-TA)) and packing method during refrigerated storage[a] (2 °C)

Storage period (d)	% FO supplementation		α-TA (ppm)		Packing method	
	0	15.0	0	900	Vacuum	Non-vacuum
	% moisture[b]					
10	72.34 ± 0.25	71.75 ± 0.47	72.40 ± 0.23	71.69 ± 0.47	72.48 ± 0.47	71.61 ± 0.43
12	71.08 ± 0.41	70.98 ± 0.28	71.64 ± 0.28*	70.42 ± 0.32	71.05 ± 0.30	71.01 ± 0.40

	FO	α-TA	FO × α-TA	Packing method
	P-value			
10	0.23	0.15	0.07	0.08
12	0.83	0.01	0.95	0.94

[a] Mean values in the main effect (FO or α-TA) and blocking effect (packing method) with * indicate significant differences ($P < 0.05$) within the same storage period.

Table 4
Fat content in trout fillets as affected by feed supplementation (flaxseed oil (FO) and α-tocopheryl acetate (α-TA)) and packing method during refrigerated storage[a] (2 °C)

Storage period (d)	% FO supplementation		α-TA (ppm)		Packing method	
	0	15.0	0	900	Vacuum	Non-vacuum
	% fat (dry basis)[b]					
10	3.12 ± 0.42	2.89 ± 0.14	3.11 ± 0.33	2.90 ± 0.29	2.87 ± 0.49	3.14 ± 0.14
12	5.25 ± 0.17	5.31 ± 0.12	5.31 ± 0.13	5.24 ± 0.16	5.19 ± 0.13	5.36 ± 0.16

	FO	α-TA	FO × α-TA	Packing method
	P-value			
10	0.62	0.65	0.99	0.56
12	0.78	0.72	0.18	0.44

[a] Mean values in the main effect (FO or α-TA) and blocking effect (packing method) with * indicate significant differences ($P < 0.05$) within the same storage period.

Although vitamin E (α-tocopherol) and its derivative α-tocopheryl acetate (α-TA) are considered fat-soluble (i.e., hydrophobic), Jittinandana, Kenney, Slider, Kamireddy, and Hankins (2006) demonstrated that supplementing rainbow trout with dietary α-TA did not affect moisture and fat contents in fillets. Similar findings were reported for Atlantic salmon (Sigurgisladottir, Parrish, Lall, & Ackman, 1994), sea bass (Gatta, Pirini, Testi, Vignola, & Monetti, 2000), and rainbow trout (Chaiyapechara, Casten, Hardy, & Dong, 2003). Our data (Table 4) is in general agreement with those reports. Moreover, not only may the dietary fat or antioxidant supplementation levels have a lesser effect on the fat deposition in fillets, but also other factors such as fish growth phase, species, nutrient interactions, environmental conditions, and others may contribute to lipid concentration in the fish muscle, and hence, in the fillets.

3.2. α-Tocopherol content and lipid oxidation

No ($P > 0.05$) interactions between dietary FO and α-TA supplementations (FO × α-TA) for α-tocopherol content and TBARS values in fillets were determined (Tables 5 and 6). Dietary supplementation of trout with 900 ppm of α-TA resulted in higher ($P < 0.05$) α-tocopherol contents in fillets during storage at 2 °C for both storage periods (Table 5). Similar effect of increased α-tocopherol content in the fillets stored at 2 °C had vaccum packing (Table 5). However, the FO dietary supplementation at 15% decreased ($P < 0.05$) concentration of α-tocopherol in the fillets at 10 days of storage; and although the difference at 12 days of storage was insignificant ($P = 0.11$), the α-tocopherol in the fillets decreased further (Table 5). Higher α-tocopherol content in trout fillets has been reported as a function of increased level of dietary α-TA (Cowey, Adron, & Youngson, 1983; Frigg et al., 1990). Similar results were also demonstrated in other animal species such as poultry (Lin et al., 1989), pig (Buckley et al., 1995), beef (Arnold, Scheller, Arp, Williams, & Schaefer, 1993), and rabbit (Lopez-Bote, Rey, Sanz, Gray, & Buckley, 1997). Akhtar, Gray, Cooper, Garling, and Booren (1999) indicated that rainbow trout fed diets supplemented with α-TA at 500 mg/kg increased concentration of α-tocopherol in the fillets. Jittinandana et al. (2006) subjected rainbow trout fillets obtained from fish supplemented with α-TA at 200 and 5000 mg/kg to 6-month frozen (−20 °C) storage followed by a 3-day refrigerated (1 °C) storage. Although the α-tocopherol content in trout fillets decreased during frozen

Table 5
α-Tocopherol content in trout fillets as affected by feed supplementation (flaxseed oil (FO) and α-tocopheryl acetate (α-TA)) and packing method during refrigerated storage[a] (2 °C)

Storage period (d)	% FO supplementation		α-TA (ppm)		Packing method	
	0	15.0	0	900	Vacuum	Non-vacuum
	mg of α-tocopherol/kg of fillets[b]					
10	83.44 ± 6.08*	64.6 ± 07.08	64.28 ± 7.14	83.77 ± 5.92*	84.77 ± 6.44*	63.28 ± 6.38
12	50.75 ± 4.13	45.52 ± 2.71	44.35 ± 1.82	51.92 ± 4.44*	56.77 ± 3.19*	39.50 ± 1.38

	FO	α-TA	FO × α-TA	Packing method
	P-value			
10	0.02	0.02	0.58	0.01
12	0.11	0.02	0.87	<0.00

[a] Mean values in the main effect (FO or α-TA) and blocking effect (packing method) with * indicate significant differences ($P < 0.05$) within the same storage period.

Table 6
TBARS values in trout fillets as affected by feed supplementation (flaxseed oil (FO) and α-tocopheryl acetate (α-TA)) and packing method during refrigerated storage[a] (2 °C)

Storage period (d)	% FO supplementation		α-TA (ppm)		Packing method	
	0	15.0	0	900	Vacuum	Non-vacuum
	mg of MDA/kg of fillets[b]					
10	3.37 ± 0.73	4.03 ± 0.85*	3.67 ± 0.82	3.73 ± 0.77	1.12 ± 0.08	6.28 ± 0.21*
12	4.09 ± 0.08	4.81 ± 0.86*	4.48 ± 0.83	4.43 ± 0.87	1.74 ± 0.11	7.17 ± 0.21*

	FO	α-TA	FO × α-TA	Packing method
	P-value			
10	0.00	0.78	0.88	<0.00
12	0.00	0.81	0.90	<0.00

[a] Mean values in the main effect (FO or α-TA) and blocking effect (packing method) with * indicate significant differences ($P < 0.05$) within the same storage period.

storage, the higher level of α-TA dietary supplementation resulted in higher α-tocopherol content in trout fillets during the storage. Our data showed similar trends; and therefore, is in agreement with the current literature.

The FO dietary supplementation at 15% and non-vacuum packing (i.e., aerobic) significantly increased ($P < 0.05$) thiobarbituric acid reactive substances (TBARS) in trout fillets during both storage periods, while dietary supplementation with α-TA had no effect ($P > 0.05$) (Table 6). The TBARS value is a very important indicator of oxidative quality for fish-derived food products. The higher TBARS value of fish-derived food products has been correlated with higher rancidity and lower consumer acceptance for these products (Ke et al., 1984). Ke et al. (1984) proposed that TBARS values for fish products below 0.58 mg/kg were perceived as not rancid; 0.58–1.51 mg/kg slightly rancid, but acceptable; and above 1.51 mg/kg were perceived as rancid. Using the Ke et al. (1984) correlation, the refrigerated storage for 10 days of the ω − 3-enhanced trout fillets under vacuum packing resulted in the "slightly rancid, but acceptable" fillets (Table 6). However, TBARS measured in the fillets following 12 day storage exceeded 1.51 mg/kg; and therefore, would be considered unacceptable. Therefore, vacuum packing of the fillets obtained from the ω − 3-enhanced trout unlike dietary supplementation of trout with α-TA seems best practice for refrigerated storage in terms of preventing rancidity onset; and hence, preventing the quality loss.

The rate of lipid oxidation in meat systems depends on the proportion of unsaturated fatty acids (FA) in total FA (Tichivangana & Morrissey, 1985), and the presence of antioxidants such as vitamin E (Lee & Dabrowski, 2003; Monahan, Buckley, Morrisey, Lynch, & Gray, 1992) and vitamin C (Lee & Dabrowski, 2003). The FO contains high concentration of α-linolenic acid (ALA, 18:3$n-3$), a polyunsaturated fatty acid (National Research Council, 1993). The experimental feed supplemented with 15% of FO contained approximately 13 times more ALA than the feed without FO supplementation (Table 2). Fig. 1 shows that higher concentration of unsaturated FA in fillets during refrigerated storage was measured in the fillets obtained from trout fed 15% FO supplemented diets compared with trout without FO supplementation. Fish rancidity due to oxidation of polyunsat-

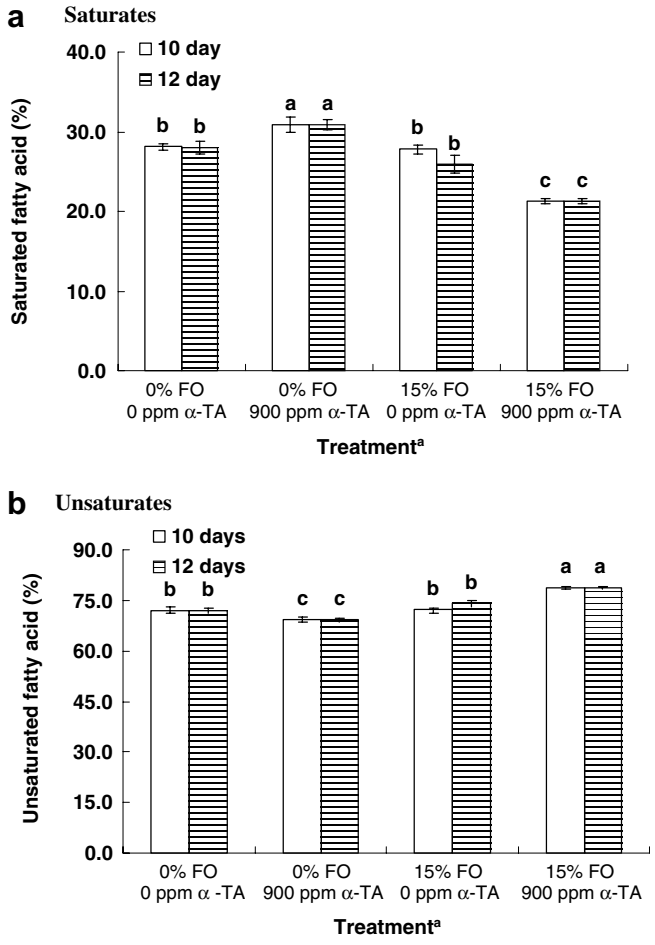

Fig. 1. Saturated and unsaturated fatty acids in total fatty acids of trout fillets as affected by feed supplementation with flaxseed oil (FO) and α-tocopheryl acetate (α-TA) during refrigerated storage (combination treatment (% FO and α-TA (ppm)) indicates percentage of FO and ppm of α-TA supplemented in the fish feed. (a) Saturates, (b) unsaturates (2 °C). Mean values in each storage period with different letters indicate significant differences (least squared difference test; $P < 0.05$).

urated FA is also highly related to the presence of oxygen (Stammen, Gerdes, & Caporaso, 1990). Restricted access to air by vacuum packing has been demonstrated to limit oxidation in various meat products including rainbow trout (Arashisar, Hisar, Kaya, & Yanik, 2004; Brewer, Ikins, & Harbers, 1992; Higgins, Kerry, Buckley, & Morrissey, 1998; Nam & Ahn, 2003). However, unlike vacuum packing, increased ($P < 0.05$) concentration of vitamin E (α-tocopherol) in trout fillets (Table 5) showed limited ($P > 0.05$) effect on preventing lipid oxidation during both storage periods in our study (Table 6). We plan to further investigate a synergistic effect of different antioxidants and vacuum packing on lipid oxidation (i.e., TBARS) of ω − 3-enhanced trout fillets.

The oxidation of ω − 3 PUFA in meat products is initiated due to depletion of tissue levels of vitamin E in the meat (Ajuyah et al., 1993). Jittinandana et al. (2006) reported that TBARS values had increased during frozen storage concurrently with a decrease of α-tocopherol content in rainbow trout fillets obtained from fish fed diets supplemented with α-TA at 5000 mg/kg. These results are similar to ours (Tables 5 and 6). This phenomenon may be explained by greater ($P < 0.05$) utilization of α-tocopherol to counteract oxidative changes leading to the partial depletion of the tissue levels of vitamin E (Table 5), which in turn, initiated lipid oxidation as confirmed by higher ($P < 0.05$) TBARS values in the fillets in 15% FO supplemented group as compared to the ones without FO supplementation (Table 6). Furthermore, the rate of vitamin E degradation increases in the presence of oxygen (Gregory, 1996). This statement also supports our data that vacuum packing retards ($P < 0.05$) depletion of α-tocopherol in fillets (Table 5) and results in lower ($P < 0.05$) TBARS values (Table 6).

3.3. Fatty acid composition

Packing method did not ($P > 0.05$) affect fatty acid (FA) composition; and therefore, the data are not shown. Flaxseed oil (FO) contains 53.3% of ALA and 12.7% of linoleic acid (LN, 18:2n − 6), yielding the highest ω − 3/ω − 6 FA ratio among plant sources (National Research Council, 1993). Due to the high concentration of ALA in FO, partial replacement of fat in the basal diet with the FO resulted in higher ω − 3 FA concentration in rainbow trout fillets (Chen et al., 2006). Refrigerated storage at 2 °C of the fillets obtained from the ω − 3-enhanced trout via dietary modification with the FO and α-TA also showed a higher ($P < 0.05$) concentration of the ω − 3 FA in the fillets (Fig. 1). The FA composition of fish diet has a direct effect on that of fish fillets (Bell, Tocher, Henderson, Dick, & Crampton, 2003; Boggio, Hardy, Babbitt, & Brannon, 1985; Cowey, 1993; Watanabe, 1982). However, the rate of lipid oxidation in meat systems depends on the proportion of unsaturated FA in the total FA (Tichivangana & Morrissey, 1985). Vitamin E is one of the most important lipid-soluble antioxidants and is usually used in the food industry. The α-tocopherol has the greatest antioxidant activity among four homologue pairs (α-, β-, γ-, δ-tocopherols and tocotrienols) (Burton & Ingold, 1981). The lower ($P < 0.05$) saturated FA (Fig. 1a) and higher ($P < 0.05$) unsaturated FA (Fig. 1b) in fillets from FO and α-TA supplemented diet at 15% and 900 ppm, respectively, were determined during refrigerated storage when compared with those from 15% FO diets, but without α-TA supplementation. The highest ($P < 0.05$) total ω − 3 FA were measured in fillets obtained from trout supplemented with FO and α-TA at 15% and 900 ppm, respectively, while the total ω − 6 FA did not change ($P > 0.05$) (Table 7). These results demonstrate that although α-TA showed limited ($P > 0.05$) protection against development of TBARS during refrigerated storage (Table 6), it did slow ($P < 0.05$) oxidation of unsaturated FA (Fig. 1), mainly ω − 3 FA (Table 7).

Highest ($P < 0.05$) concentrations of ALA and eicosapentaenoic acid (EPA, 20:5n − 3) during refrigerated storage were determined for fillets obtained from fish fed diet

Table 7
ω − 3 (ω − 3) and ω − 6 (ω − 6) fatty acids in total fatty acids of trout fillets as affected by feed supplementation with flaxseed oil (FO) and α-tocopheryl acetate (α-TA) during refrigerated storage[a] (2 °C)

Parameter	Storage period (day)	Treatment[c]			
		0% FO 0 ppm α-TA	0% FO 900 ppm α-TA	15% FO 0 ppm α-TA	0% FO 900 ppm α-TA
		% fatty acid in total fatty acids[b]			
∑ω − 3	10	24.94 ± 0.15b	22.43 ± 1.11b	17.27 ± 2.58c	33.03 ± 0.79a
	12	26.26 ± 0.86b	21.84 ± 0.99c	26.84 ± 1.43b	33.93 ± 0.53a
∑ω − 6	10	27.0 ± 01.08	27.64 ± 0.50	26.99 ± 0.90	25.15 ± 0.69
	12	26.38 ± 0.57	26.27 ± 0.76	26.85 ± 0.36	24.91 ± 0.36

[a] Mean values in a horizontal row with different letters indicate significant differences (least squared difference test; $P < 0.05$) within the same storage period.
[b] Data are given as mean ± SEM ($n = 6$).
[c] Combination treatment (% FO and α-TA (ppm)) indicates percentage of FO and ppm of α-TA supplemented in the fish feed.

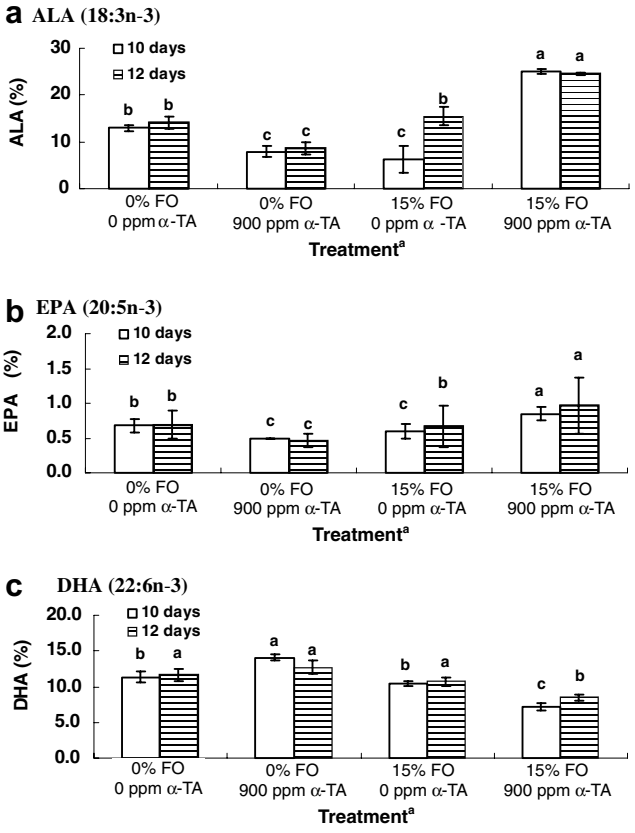

Fig. 2. ALA (18:3n − 3), EPA (20:5n − 3), and DHA (22:6n − 3) in total fatty acids of trout fillets as affected by feed supplementation with flaxseed oil (FO) and α-tocopheryl acetate (α-TA) during refrigerated storage (combination treatment (% FO and α-TA (ppm)) indicates percentage of FO and ppm of α-TA supplemented in the fish feed. (a) ALA (18:3n − 3), (b) EPA (20:5n − 3), (c) DHA (22:6n − 3)) (2 °C). Mean values in each storage period with different letters indicate significant differences (least squared difference test; $P < 0.05$).

supplemented with 15% of FO and 900 ppm of α-TA compared with other dietary treatments (Fig. 2a and b). However, regardless of FO supplementation, α-TA did not seem to affect the concentration of docosahexaenoic acid (DHA, 22:6n − 3) in stored fillets (Fig. 2c).

Trout supplemented with 15% of FO and 900 ppm of α-TA yielded fillets that had the highest ($P < 0.05$) and the lowest ($P < 0.05$) concentrations of LN and arachidonic

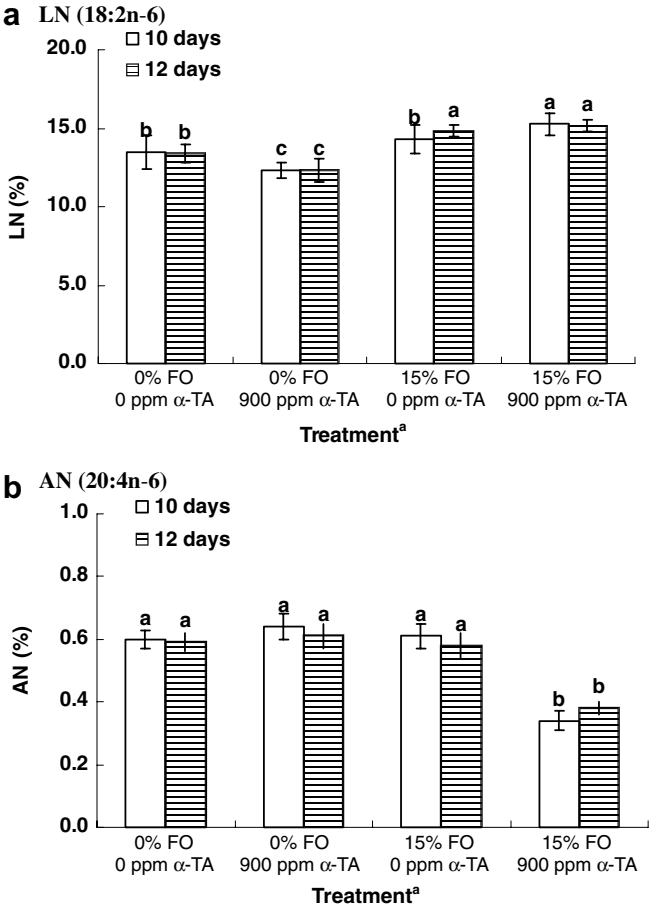

Fig. 3. LN (18:2n − 6) and AN (20:4n − 6) in total fatty acids of trout fillets as affected by feed supplementation with flaxseed oil (FO) and α-tocopheryl acetate (α-TA) during refrigerated storage (combination treatment (% FO and α-TA (ppm)) indicates percentage of FO and ppm of α-TA supplemented in the fish feed. (a) LN (18:2n − 6), (b) AN (20:4n − 6)) (2 °C). Mean values in each storage period with different letters indicate significant differences (least squared difference test; $P < 0.05$).

acid (AN, 20:4$n-6$), respectively, compared with other dietary treatments (Fig. 3a and b). Although these differences were statistically significant, they probably are of limited practical importance due to relatively small magnitude of these differences.

According to the literature, dietary vitamin E level does not appear to affect trout muscle FA composition (Frigg et al., 1990; Jittinandana et al., 2006). However, based on our results, it seems that α-TA protected unsaturated FA, mainly the ω − 3 FA of the ω − 3-enhanced trout fillets during refrigerated storage. Unlike the fillets tested in the literature cited above (Frigg et al., 1990; Jittinandana et al., 2006), the fillets in our experiments were obtained from fish with considerably higher ($P < 0.05$) concentration of the ω − 3 FA due to dietary modification with FO when compared to the fillets obtained from fish fed the basal diet (i.e., without FO) (Table 7). This significantly higher amount of ω − 3 FA in the ω − 3-enhanced trout fillets likely accounts for the differences of the antioxidant effect of vitamin E during storage between our data and those cited above (Frigg et al., 1990; Jittinandana et al., 2006).

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