Halászatfejlesztés 34 - Fisheries & Aquaculture Development 34
2012 ISBN 978-963-7120-32-9

Effects of dietary alpha-tocopheryl acetate on lipid oxidation farmed rainbow trout (Oncorhynchus mykiss) fillets

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Abstract

A trout diet was supplemented with 0, 8.5, or 15 g/100 g of flaxseed oil (FO). To prevent lipid oxidation of fillets, FO-supplemented diets were also enhanced with 0, 400, and 900 mg/kg of alpha-tocopheryl acetate (α-TA). Total fat, moisture content, and lipid oxidation of fillets were determined following fish harvest on days 0, 30, 60, 90, and 120. Regardless of supplementing trout diets with FO or α-TA, no ($P>0.05$) difference of the total fat in fillets was measured. The effect of retarding lipid oxidation in fillets was recorded after supplementing trout with α-TA for 60 days. Our results indicate that regardless of FO level in trout diet, 900 mg/kg of α-TA can prevent lipid deterioration of fillets. However, to achieve more pronounced antioxidant effect in the ω-3- enhanced trout fillets, a synergetic effect of antioxidants and anaerobic packaging with α-TA supplementation should be investigated.

Keywords: Trout fillets; Aquatic foods; Lipid oxidation; Total fat; Antioxidant; Omega-3 fatty acids

Introduction

The beneficial effect of consumption of marine fish on human cardiovascular health was first reported by Bang and Dyerberg (1980) who proposed a linkage between the intake of omega-3 fatty acids (ω-3 FA) by the Greenland Eskimos and a reduced risk of acute myocardial infarction. Tamura et al. (1986) reported that the low coronary heart disease (CHD) mortality in Japan was due to a significant consumption of fish and fish-derived products. Therefore, Tamura et al. (1986) suggested that the consumption of fish is likely protective against the atherosclerotic diseases.

Flaxseed oil (FO) contains highest concentration of ALA among plant-derived oils. Therefore, FO may be used to supplement trout diets in order to increase the ω-3 FA content in fillets. However, the ω-3 FA are highly unsaturated, and therefore, susceptible to oxidation in fish fillets (Nurnberg, Kuchenmeister, Nurnberg, Ender,

& Hackl, 1999). Lipid oxidation causes meat quality deterioration, typically associated with development of rancidity. Hence, the FO-supplemented trout diets may need to be enhanced with higher concentration of an antioxidant to counteract lipid oxidation of fillets.

While increased concentration of ω-3 FAs is desirable from human health benefits standpoint, the oxidative stability of the omega-3-enhanced fillets may be compromised. Therefore, the objective of this research was to determine lipid oxidation and potential strategies to increase oxidative stability of the fillets recovered from omega-3-enhanced farmed rainbow trout fed diets supplemented with FO and α-TA.

Our research group has reported development of ω-3- enhanced rainbow trout (Oncorhynchus mykiss) (Chen et al., 2006). However, while increased concentration of o3 FAs is desirable from human health benefits standpoint, the oxidative stability of the omega-3-enhanced fillets may be compromised. Therefore, the objective of this research was to determine lipid oxidation and potential strategies to increase oxidative stability of the fillets recovered from omega-3-enhanced farmed rainbow trout fed diets supplemented with FO and α-TA.

Materials and methods

Feeding trial and fish diets

The experiment took place at Hărman Farm of Doripesco SA. 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 five tanks. Tanks were stocked with 75 rainbow trout fingerlings (age 11–12 months, average weight 240 g/fish, and average length 27 cm) per tank (size 91 x 122 x 91 cm). Rainbow trout were fed dry pelleted diets formulated with 0 (basal diet, Table 1), 8.5, or 15.0 g/100 g of FO supplementation (Table 2).

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**
Nutrient contents	
Crude protein (g/kg)	**420**
Fat (g/kg)	**70**
Metabolizable energy (MJ/kg)	**12.6**

Table 2 Total fat and fatty acid composition of experimental diets

Parameter	FO supplementation (g/100 g of diet)		
	0	8.5	15.0
	g/100 g of total fatty acids		
18:3n3	3.47±1.14 c	33.30±2.98 b	46.22±1.89 a
20:5n3	10.93±0.64 a	2.87±0.52 b	1.22±0.32 c
22:6n3	12.72±0.77 a	3.68±0.60 b	1.62±0.15 c
18:2n6	20.88±1.08 a	24.22±2.02 a	21.60±0.90 a
20:4n6	0.27±0.17 a	0.47±0.38 a	0.33±0.03 a
Total unsaturates	60.98±1.01 c	76.38±2.11 b	82.50±1.39 a
Total saturates	9.02±1.01 a	23.62±2.11 b	17.50±1.39 c
Total ω-3	27.47±1.44 c	40.08±2.07 b	49.07±2.13 a
Total ω-6	22.17±1.08 b	26.42±1.06 a	22.57±0.86 b
ω-3/ω-6	1.27±0.10 c	1.50±0.04 b	2.17±0.04 a
Total fat (g/100 g, dry basis)	14.43±2.32 a	13.59±0.82 a	24.86±0.65 b

Data are given as mean7SEM (n Ľ 6). Mean values in horizontal rows with different letters indicate significant differences (Least Squared Difference test; Po0.05).

Each level of the FO supplementation was also enhanced with 0, 400, or 900 mg/kg α-tocopheryl acetate (α-TA). Hence, there were nine treatment diets. The dietary treatments were randomly assigned to the tanks in each level of the raceway system. The basal diet was supplemented with 0, 8.5, and 15.0 g/100 g FO. The supplementation did not affect the protein content in the diets, and therefore, the diets were isonitrogenous. Fish were hand fed to satiation twice a day for 120 days. Feed was stored at 4 °C.

Approximately 1500 l/min of spring water flowed through the raceway system. It was aerated entering the system and half the 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 maintained on a natural photoperiod.

Sample preparation

One fish in each tank per level was harvested randomly on days 0, 30, 60, 90, and 120 and then killed by a blow to the head. All harvested fish were stored at 4 °C before trout were filleted to obtain boneless and skinless butterfly fillets. Fillets were homogenized in a laboratory blender Homogenized samples were placed in nylon vacuum pouches, labeled, vacuum packed and stored at -80 °C until analyzed. These sample preparation steps were performed on the same day when fish were harvested.

Total fat (g/100 g)

Fat content in fillets was determined according to Soxhlet extraction method (AOAC, 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 g of fat per 100 g of fillets on dry basis.

Thiobarbituric acid reactive substance (TBARS)

Oxidative rancidity of fillets was measured by a 2-TBARS assay of malondialdehyde (MDA) as described by Jaczynski and Park (2003). Three drops of antioxidant (Tenox 6) and 3ml of thiobarbituric acid (TBA) were added to 0.2 g of homogenized fillet sample. Then, 17 ml trichloroacetic acid reagent was added. The solution was flushed with nitrogen and closed. A blank was prepared in the same manner, but without the sample. The tubes were boiled for 30 min, and then cooled. The colored solution (15 ml) was centrifuged at 5000 x g for 15 min. A clear, colored supernatant was transferred to a cuvette, and the absorbance was measured at 535 nm using a UV/VIS spectrophotometer (Waters 490 E). 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.

Statistical analysis

The experiments were conducted using a 3x3 factorial design (Steel & Torrie, 1980). The interaction effect (FO x α-TA) and main effect (FO and α-TA) were analyzed. A significant difference was used at 0.05 probability level and differences between treatments were tested using the least significant difference (LSD) test. At least twelve fillets (n = 12) from each treatment (three fillets per level in the raceway system composed of four levels) were randomly obtained and analyzed. At least six diets (n = 6) from each treatment were randomly obtained and analyzed. All statistical analyses of data were performed using SAS Institute (2002).

Results and discussion

During the feeding period of 120 days, no ($P>0.05$) FO x α-TA interactions were determined for the total lipid concentration in trout fillets. Partial replacement of fat in the basal diet with FO or supplementing the basal diet with α-TA did not ($P>0.05$) affect the total lipid concentration in trout fillets (Tables 3 and 4).

The higher lipid concentration in fish has been shown to result from the fish diets with a higher fat concentration (Alvarez et al., 1998; Wang et al., 2005). However, Regost et al.(2001) reported that the total lipid concentration in trout fillets was not changed by feeding diets containing different amount of fat. These results are similar to ours. Another study was performed by Steffens, et al (1999). They fed rainbow trout isonitrogenous diets with two levels of dietary fat at 13 and 24 g/100 g. Steffens et al. (1999) indicated that the increased dietary fat does not affect lipid concentration of rainbow trout fillets. However, the 24 g/100 g fat diet resulted in significantly greater concentration of visceral fat. Therefore, Steffens et al.'s (1999) results are similar to ours and those of Regost et al. (2001). Gelineau, et al (2001) fed rainbow trout four diets with increasing levels of lipids (15, 20, 25, and 30 g/100 g). This is most likely why in our study the FO and α-TA did not ($P>0.05$) have a significant effect on lipid concentration in trout fillets.

Table 3 Fat in trout fillets as affected by feed supplementation with flaxseed oil (FO)

Feeding period (day)	FO supplementation (g/100 g of diet)		
	0	8.5	15.0
	g of fat /100 g of fillets		
0	1.91±0.80	3.79±1.15	4.43±1.32
30	2.66±0.31	2.31±0.51	4.13±0.78
60	5.29±0.52	4.92±0.39	6.24±0.58
90	3.83±0.68	4.26±0.45	4.01±0.43
120	2.04±0.43	2.12±1.18	1.62±0.43

Data are given as mean7SEM (n Ľ 12). Values are given as g/100 g (dry basis).

Table 4. Fat in trout fillets as affected by feed supplementation with α-tocopheryl acetate (α-TA)

Feeding period (day)	α-TA (mg/kg of diet)		
	0	400	900
	g of fat /100 g of fillets		
0	4.25±1.45	2.75±0.95	3.12±0.98
30	2.81±0.39	2.79±0.46	3.50±0.89
60	5.71±0.57	5.00±0.44	5.74±0.56
90	3.46±0.30	4.63±0.52	3.99±0.64
120	1.66±0.39	1.65±0.37	2.47±1.20

Data are given as mean±SEM (n = 12). Values are given as g/100 g (dry basis).

The effect of experimental diets on susceptibility of trout fillets to oxidation as assessed by TBARS values was also measured (Tables 5 and 6). The composition of intramuscular fatty acids in fish has been shown to reflect that of the diet (Caballero et al., 2002; Greene & Selivonchick, 1990; Morris et al., 2005; Skonberg et al., 1994). FO contains high concentration of α-linolenic acid (ALA, 18:3ω3), a polyunsaturated fatty acid. The rate of lipid oxidation in meat systems depends on the proportion of unsaturated fatty acids in the total fatty acids (Tichivangana & Morrissey, 1985) and the presence of antioxidants such as vitamin E (Lee & Dabrowski, 2003; Monahan et al., 1992), and vitamin C (Lee & Dabrowski, 2003). However, regardless of the supplemental level of α-TA, the significantly higher ($P<0.05$) TBARS value of trout fillets was only measured in the 15.0 g/100 g FO supplemented group at the 90 days of feeding, compared to 8.5 g/100 g FO supplemented and non-FO supplemented group (Table 5). In order to reduce the chances for extensive oxidation prior to analyses, trout were filleted, homogenized, vacuum-packed and stored at -80 °C on the same day when they were sampled. TBARS assay as a spectrophotometric method that relies on development of pink color resulting from a complex between malondialdehyde and 2-TBA is known for its variability due to interfering compounds that may contribute to color development. However, Ke et al.,(1984) correlated TBARS values for fish fillets with sensory attributes. These authors proposed that TBARS values for fish 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. Most of our TBARS values shown in Tables 5 and 6 were in the slightly rancid, but acceptable range; however, some were in just above the rancid range. Jittinandana et al. (2006) determined TBARS values in rainbow trout fillets obtained from fish fed diets supplemented with α-TA at 200 and 5000 mg/kg

for up to nine weeks. Following the start of the feeding period, the TBARS values of the fillets were within the rancid, but acceptable range for both levels of a-TA supplementation. However, the 5000 mg/kg level resulted in significantly lower TBARS values than the 200 mg/kg supplementation. Although the TBARS values shown by Jittinandana et al. (2006) were generally within the same slightly rancid, but acceptable range when compared to our data; they were lower than our TBARS values.

Table 5. TBARS values in trout fillets as affected by feed supplementation with flaxseed oil (FO)

Feeding period (day)	FO supplementation (g/100 g of diet)		
	0	8.5	15.0
	mg of MDA/kg of fillets		
0	1.61±0.27	1.55±0.15	1.64±0.24
30	1.76±0.14	1.67±0.22	1.47±0.09
60	1.34±0.04	1.33±0.07	1.39±0.07
90	1.30±0.05 b	1.32±0.05 b	1.44±0.08 a
120	1.63±0.11	1.52±0.09	1.66±0.11

Data are given as mean ±SEM (n = 12). Values are given as mg of malonaldehyde (MDA)/kg of fillet. Mean values in horizontal rows with different letters indicate significant differences (No (P>0.05) interaction effects between FO and α-TA on TBARS values; therefore, data reported per FO supplementation; Least Squared Difference test; P<0.05).

Our data as well the studies cited above indicate the importance of proper handling for the fish-derived food products such as for example trout fillets. Table 6 shows that regardless of supplemental levels of FO, the fillets obtained from trout supplemented with 900 mg/kg of α-TA had the lowest TBARS mean value, followed by 400 mg/kg, and the non-α-TA supplemented diet. However, the significant difference (P<0.05) was determined after feeding the fish for 60 days (Table 6).

Table 6 TBARS values in trout fillets as affected by feed supplementation with α-tocopheryl acetate

Feeding period (day)	α-TA (mg/kg of diet)		
	0	400	900
	mg of MDA/kg of fillets		
0	1.38±0.23	1.56±0.22	1.85±0.23
30	1.78±0.16	1.60±0.16	1.48±0.10
60	1.46±0.05 a	1.45±0.05 a	1.20±0.03 b
90	1.48±0.18 a	1.43±0.06 a	1.18±0.03 b
120	1.73±0.14 a	1.69±0.07 a	1.40±0.05 b

Data are given as mean±SEM (n = 12). Values are given as mg of malonaldehyde (MDA)/kg of fillet. Mean values in horizontal rows with different letters indicate significant differences (No (P>0.05) interaction effects between FO and α-TA on TBARS values; therefore, data reported per α-TA supplementation; Least Squared Difference test; P<0.05).

4. Conclusions

Regardless of feeding period, supplementing rainbow trout with FO or α-TA did not alter the total fat level of fillets. The lipid oxidation of ω-3-enhanced trout fillets was alleviated by dietary supplementation of trout with α-TA at 900 mg/kg starting at 60 days of feeding. However, synergistic effect of a-tocopherol with other anti-

oxidants and aerobic packaging on lipid stability of trout fillets should be further investigated in order to reduce rancidity development. The dietary supplementation of trout with 15.0 g/100 g of FO resulted in almost 1.6 - fold increase of total ω3 FA mainly due to increased concentration of ALA with a concurrent decrease of EPA and DHA fatty acids.

Acknowledgements
Researches realised within the project POSDRU/6/1.5/S/26 funded of the Social European Fund by the Sectorial Operating Programm "DEVELOPMENT OF THE HUMAN RESOURCES 2007 – 2013"
Thanks to Mr. Dorin Crizbăşan , the owner of Doripesco SA Hălchiu for his support in this study.
Thanks to "HAKI" Institute for Fisheries, Aquaculture and Irrigation Szarvas Hungary, for technical assistance.

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