THE EFFECTS OF DIETARY LINSEED OIL AND α-TOCOPHERYL ACETATE ON FATTY ACIDS CONTENT AND LIPID OXIDATION OF RAINBOW TROUT (Oncorhynchus mykiss) FILLETS DURING ABUSIVE-TEMPERATURE STORAGE

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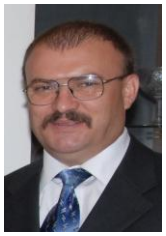

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REZUMAT. Modificările chimice de ω-3, care consolidează fileurile de păstrăv curcubeu de crescătorie dezvoltate prin modificarea dietei cu ulei de in (LO) şi alfa-tocoferil acetat (α-TA) au fost stabilite pe parcursul depozitării la temperatura de 8 ^{0}C timp de 6-8 zile. Indiferent de perioada de depozitare şi metodele de ambalare, conținutul de substanțe acide reactive superioare hiobarbiturice (TBARS) şi conținutul de α-tocoferoli inferiori în fileuri depozitate provenite din grupuri suplimentate –LO. Metoda de ambalare (vid față de non-vid) nu a afectat profilul acizilor grași (FAP), dar ambalarea în vid a suprimat oxidarea lipidelor (TBARS) în timpul depozitării. Cu toate acestea, α-tocoferolul, spre deosebire de ambalarea în vid, protejază ω-3 FA în fileurile testate. Indiferent de perioada de depozitare, fileurile stocate obţinute de la păstrăv hrănit suplimentar cu 15% LO şi 900 ppm TA, au înregistrat cele mai mari proporții de FA nesaturați, ω-3 FA total şi acid alfa-linolenic (ALA), precum şi cea mai mică proporţie de FA saturați si acid docosahexaenoic (DHA), în comparaţie cu cele din alte grupuri de experiment.

Cuvinte cheie: Ulei de semințe de in, acetat de Alpha-tocopheryl, file de păstrăv, valori TBARS, ; acizi grași ω-3, acizi grași ω-6

ABSTRACT. Chemical changes of ω-3-enhanced farmed rainbow trout fillets developed by dietary modification with linseed oil (LO) and alpha-tocopheryl acetate (α-TA) were determined during 8 ^{o}C storage for 6 or 8 days. Regardless of storage period and packing methods, higher thiobarbituric acid-reactive substances (TBARS) and lower α-tocopherol content were measured in stored fillets from LO-supplemented groups. Packaging method (vacuum vs. non-vacuum) did not affect fatty acid profile (FAP), but vacuum packaging suppressed lipid oxidation (TBARS) during storage. However, α-tocopherol unlike vacuum packaging protected ω-3 FA in the tested fillets. Regardless of storage period, stored fillets obtained from trout supplemented with 15% of LO and 900 ppm of a-TA had the highest proportions of unsaturated FA, total ω-3 FA, and alpha-linolenic acid (ALA) as well as the lowest proportions of saturated FA and docosahexaenoic acid (DHA) when compared with those from other treatment groups.

Keywords: Linseed oil; Alpha-tocopheryl acetate; Trout fillet; TBARS values; ω-3 fatty acids; ω-6 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). This report also showed that 34.2% of American adults had one or more types of CVD in 2003 and over 0.9 million people died due to CVD in 2003. Omega-3 fatty acids (ω-3 FA) in fish and fish-derived food products can reduce the risk of CVD. Hence, consumption of at least two fish servings per week is recommended by the American Heart Association (Krauss et al., 1996). Institute of Medicine (2002) also suggested intake levels of omega-3 PUFA that were set for a-linolenic acid and based on median intakes in the US as 1.6 and 1.1 g/d for men and women, respectively.

It is well known that lipid oxidation is one of the major problems in fish-derived food products due to flavor deterioration and loss of nutritional value. Lipid oxidation typically results in a formation of aldehydes (acids, hydrocarbons, and epoxides), alkyl radicals (hydrocarbons, alcohols)

and semialdehydes (or oxo-esters) (Ladikos & Lougovois, 1990; Nawar, 1996).

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, et al., 2002).

2. MATERIALS AND METHODS

2.1. Feeding trial and diets

The experiment took place at Hărman Farm of Doripesco SA. A gravity-fed flow-through raceway system composed of eight tanks. Tanks were stocked with 50 rainbow trout fingerlings (age 11–12 months, average weight 240 g/fish, and average length 27 cm) per tank (size 91 x 122 x 91cm).

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

Rainbow trout were fed dry pelleted diets formulated with 0 (basal diet, Table 1), or 15.0 g/100 g of LO supplementation (Table 2). Each level of the LO supplementation was also enhanced with 0 or 900 mg/kg α-tocopheryl acetate (α-TA). Hence, there were four treatment diets. The supplementation did not affect the protein content in the diets, and therefore, the diets were isonitrogenous. Fish were hand fed to near satiation twice a day for 120 days. Feed was stored at 4 ^{0}C.

Table 2. *Fatty acid compositiona of experimental diets*

Parameter %	LO supplementation	
	0%	15%
% Fatty acid in total fatty acids		
18:3n3	3,47	46,2
20:5n3	11,0	1,22
22:6n3	12,7	1,62
18:2n6	20,9	21,6
20:4n6	0,27	0,33
Total unsaturates	61,0	82,5
Total saturates	39,0	17,5
Total ω-3	27,5	49,1
Total ω-6	22,2	22,6

Approximately 900 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 ^{0}C during the feeding trial. Fish were fed 6 days each week and maintained on a natural photoperiod. Fish were hand fed to near-satiation twice a day for 120 days.

2.2. Sample preparation

Trout 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 ^{o}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, the fillets were either placed in nylon vacuum pouches, labelled and vacuum packed or the fillets were placed on plastic trays and aerobically over-wrapped (nonvaccum) with a typical household plastic wrapping. The vaccum and non-vacuum packed fillets were subjected to storage at 8 ^{o}C for 6 or 8 days. Following the storage period, fillets were homogenized in a laboratory blender, placed in nylon vacuum pouches, labelled, vacuum packed and stored at -80 ^{o}C until analyzed. The storage periods were selected based on correlation between TBARS values in fish and rancidity development reported by Ke, et al., (1984). The objective was to analyze the fillets when the rancidity development was slightly in progress (6 days) and more advanced (8 days).

2.3. Measurement of thiobarbituric acid-reactive substances (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 3 ml 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.

2.4. Lipid extraction and fatty acid analysis

Lipids were extracted from a fillet sample using methodology described by Folch, et al., (1957) and the extracted lipids were used for analysis of fatty acid profile. According to the procedure of Fritsche and Johnston (1990), fatty acids were

transmethylated by the addition of 4ml of 40 g/l methanolic H_2SO_4 and heated in a 90 ^{0}C water bath for 60 min. The mixture was saponified by transferring through a Na_2SO_4 filled glass Pasteur pipette and subsequent drying under N_2 in a 60 ^{0}C water bath for 60 min. The fatty acid methyl esters (FAME) were resuspended in filtered isooctane. The FAME were analyzed by using a gas chromatograph (Agilent 6890N - GC) and a flame ionization detector fitted with a OB 225 capillary column (30m length, 0.25mm inside diameter). Injection and detection temperature was maintained at 220 ^{0}C and column temperature was 190 ^{0}C. The fatty acids were identified by comparing their retention times with known standards and references (Ackman, 1980). Peak area and the amount of each fatty acid were computed by an integrator using the Agilent Camp Station software.

2.5. Statistical analysis

The experiment was conducted using a 2 x 2 factorial arrangement of treatment in a randomized block design. The interaction effect (LO x α-TA), main effect (LO 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) from each experimental treatment and six feeds (n = 6) from each experimental diet were randomly obtained and analyzed. All statistical analyses of data were performed using SAS (2002).

3. RESULTS AND DISCUSSION

3.1. The lipid oxidation

Stored fillets from non-LO and α-TA-supplemented groups had lower thiobarbituric acid-reactive substances (TBARS) values than FO and non-α-TA supplemented ones, respectively (Table 3). Vacuum packed fillets also showed lower thiobarbituric acid-reactive substances (TBARS) values than the non-vacuum packed ones (Table 3).

Table 3. *TBARS values in trout fillets as affected by feed supplementation (linseed oil (LO) and α-tocopheryl acetate (α-TA)) and packing method during temperature-abused refrigerated storage (8^{o}C)*

Storage period (d)	% LO supplementation		α-TA (ppm)		Packing method	
	0	15	0	900	Vacuum	Non-vacuum
mg of MDA/kg of fillets						
6	1,97	3,25	2,88	2,33	1,95	3,27
8	2,55	4,44	3,81	3,18	1,94	5,05

Higher concentration of unsaturated FA in the stored fillets was determined for the fillets obtained from trout fed 15% of FO supplemented diets compared with those fillets from trout without FO supplementation (Table 4). PUFA in fish are oxidized easily in the aerobic conditions (Stammen, et al., 1990). A vacuum packing prevents trout fillets from access to air, and hence, better maintains lipid stability during storage (Arashisar et al., 2004). Therefore, lipid oxidation of fish fillets can be reduced via exclusion of oxygen from the package and increased concentration of antioxidants. Tables 3 show that higher TBARS values were determined for fillets that concurrently had lower content of vitamin E. For fillets obtained from trout supplemented with 15% of LO (higher content of ω-3 PUFA), lower vitamin E was probably caused by increased consumption of the antioxidant to prevent lipid oxidation during storage. It could also be explained that higher lipid oxidation (TBARS values) in the 15% FO treatment group (Table 3) might have expended more vitamin E, resulting in the initiation of lipid oxidation. Trout without α-TA supplementation yielded fillets with lower vitamin E content, which resulted in higher oxidation (TBARS) of the fillets during storage when compared to trout supplemented with α-TA at 900 ppm (Table 4). Regardless of the dietary treatment (LO and α-TA), trout fillets stored under aerobic conditions (non-vacuum packing) had lower vitamin E contents, and hence, higher TBARS values compared with the fillets stored under anaerobic conditions (vacuum packing).

3.2. Fatty acid composition

Packing method did not affect fatty acid (FA) composition of stored fillets. In general, lower concentration of saturated FA and higher concentration of unsaturated FA were determined in the stored fillets that were obtained from trout fed diets supplemented with LO when compared with those obtained from trout without LO supplementation (Table 5). These differences were particularly evident for fillets obtained from trout supplemented with15% LO and 900 ppm α-TA and stored for 8 days at 8^{o}C. Linseed oil (LO) contains 53.3% of ALA and 12.7% of linoleic acid (LA, 18:2n6). Due to the high concentration of ALA in FO, partial replacement of fat in the basal diet with FO resulted in higher concentration of ω-3 FA in fillets.

Table 4 shows that the increased concentration of ω-3 FA in the fillets is also retained during storage, particularly when the stored fillets are supplemented with vitamin E. Therefore, when trout diet is supplemented with LO to enrich the fillets with the ω-3 FA, the diet should concurrently be enhanced with α-TA in order to prevent degradation of those ω-3 FA in the fillets during storage.

Table 4. *Fatty acid profile of trout fillets as affected by feed supplementation with linseed oil (LO) and α-tocopheryl acetate (α-TA) during temperature-abused refrigerated storage (8°C)*

Fatty acids	Storage period (d)	Treatment (% LO : α-TA (ppm))			
		0:0	0:900	15:0	15:900
% Fatty acid in total fatty acids					
Saturated	6	35,5	41,9	37,3	33,03
	8	38,4	39,7	36,3	21,72
Unsaturated	6	64,5	58,2	64,6	69,7
	8	61,6	60,3	63,7	78,3
ALA	6	18,7	12,9	25,7	33,6
	8	19,0	114,1	24,3	41,7
EPA	6	0,89	0,50	0,64	0,19
	8	0	0	0	0
DHA	6	15,2	18,2	14,3	11,1
	8	15,1	19,7	14,2	11,0
LA	6	6,99	10,9	10,5	10,3
	8	17,9	17,5	12,8	10,9
AN	6	0,61	0,40	0,34	0,29
	8	0,45	0,45	0,76	1,27
Σω-3	6	34,8	31,6	40,6	45,0
	8	34,1	32,2	38,5	52,7
Σω-6	6	23,7	30,3	26,2	22,9
	8	34,5	38,4	28,8	24,8

While vacuum packing prevents lipid oxidation (TBARS, Table 3), as mentioned above it has no effect on protecting the ω-3 FA. Table 4 shows main ω-3 and ω-6 FA, respectively, of stored fillets that were obtained from trout fed LO and α-TA-supplemented diets for up to 4 months (120 days). During temperature-abused refrigerated storage the highest concentration of ALA was determined in fillets obtained from trout supplemented with 15% of LO and 900 ppm of α-TA when compared with other dietary treatments. Fillets from LO supplemented diets also had higher ALA concentrations than non-LO supplemented diets (Table 4). However, regardless of α-TA supplementation, fillets obtained from trout supplemented with LO had lower concentration of docosahexaenoic acid (DHA, 22:6n3) than those without LO supplementation (Table 4). No difference in concentration of linoleic acid (LA, 18:2n6) in stored fillets was determined among the four dietary treatments (Table 4). However, the highest concentrations of arachidonic acid (AN, 20:4n6) were measured in fillets stored for 8 days that were obtained from trout supplemented with 15% of FO and 900 of ppm α-TA (Table 4).

4. CONCLUSION

Regardless of storage length, LO-supplemented group had a higher TBARS values in the fillets, but lower TBARS values were determined for α-TA-supplemented and vacuum packed groups. This phenomenon might be explained by the fact that reduction of lipid oxidation occurred concurrently with the depletion of α-tocopherol in fillets. In the fatty acid profile (FAP) of stored fillets, packing method did not show any effects; however, LO and α-TA supplementations showed interaction effects. Generally, regardless of storage length, the fillets obtained from trout fed LO and α-TA supplemented diets had the lowest proportion of saturated FA and total ω-6 FA, as well as the highest proportion of unsaturated FA, total ω-3 FA, and ALA, compared to those from other treatment groups. Based on this study, the vacuum packing showed a good reduction of lipid oxidation and in order to maintain ω-3 FA during storage of trout fillets obtained from LO supplemented trout, the feed should be also supplemented with α-TA.

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