

Dietary supplementation versus direct postmortem addition of α -tocopherol acetate on fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage

^{1*}Zakipour Rahimabadi, E., ¹Jasour, M.S., ²Ehsani, A., ³Rahnama, M. and ¹Arshadi, A.

¹Department of Fisheries, Faculty of Natural Resources, University of Zabol, 98615-538 Zabol, Iran

²Department of Biotechnology and Pathobiology and Quality Control, Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, Iran

³ Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Zabol, 98615-538 Zabol, Iran

Abstract: This study was designed to compare the effects of α -tocopherol acetate which added to diet (0, 300 and 500 mg/kg) in 58 days trial and direct addition (200 mg/kg flesh) after slaughtering on fatty acid composition of rainbow trout fillets during 12 days of refrigerated storage. Fish were fed experimental diets twice daily at 8:00 and 17:00 h, 7 days a week. The present study illustrated that dietary α -tocopherol acetate showed no statistically difference ($P>0.05$) in fillet composition parameters and content of the major fatty acid groups of fish fillets after 8 weeks experiment. In general, there was decrease in the proportion of MUFA and PUFA, and an increase of SFA in all treatments during refrigerated storage ($P<0.05$). However, during storage period, the fatty acid composition was not affected by the postmortem addition of vitamin E, but fish that fed supplemented diets with α -tocopherol acetate had less change in fatty acids in comparison to the control diets at the end of refrigerated storage ($P<0.05$). There were no significant differences in FA composition ($P>0.05$) between the fillets of fish fed diets containing different levels of α -tocopherol during storage period.

Keywords: Rainbow trout, α -tocopherol acetate, refrigerated storage, fatty acid composition

Introduction

Fish lipids are characterized by long chain fatty acids and high degree of un-saturation with multiple double bonds such as omega-3 fatty acids (Jittrepotch *et al.*, 2006). This character caused intensive scientific investigations regarding to health benefits of fish consumption and also fish deterioration due to lipid oxidation and hydrolysis. 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 with high content of n-3 PUFA such as fish are also more prone to lipid oxidation (Chen *et al.*, 2007). Lipid oxidation is a major cause of rancidity and flavor deterioration of muscle food by both enzymatic and non-enzymatic process (Shahidi, 1997) that leads to changes in the quality of food such as taste, texture, shelf life, appearance and nutritional value (Jittrepotch *et al.*, 2006).

Numerous strategies such as using refrigerator and freezer and also adding antioxidants are available to minimize the undesirable effects of lipid oxidation

and to prevent quality loss during post-slaughter storage. It has been found that tissue vitamin E has protective role against lipid oxidation (Machlin, 1984). Lipid oxidation of PUFA is possibly initiated due to the depletion of tissue vitamin E in meat systems (Ajuyah *et al.*, 1993). Vitamin E plays an important role in preventing the oxidation of unsaturated lipids in fish muscle by scavenging free radicals which are involved in the initiation and propagation of lipid oxidation (Machlin, 1984; Pope *et al.*, 2002). α -tocopherol acetate (α -TA), a vitamin E derivative, is a potent biological antioxidant and usually used as an antioxidant to reduce lipid oxidation in food (Zhang *et al.*, 2007; Chen *et al.*, 2007). As a natural component it is considered safe for use according to current consumer preferences (Zuta *et al.*, 2007).

The composition of fish fillets, lipid content and fatty acid composition usually reflects the dietary lipid and composition of fish diet (Bell, 1988; Chen *et al.*, 2008). Dietary supplementation α -tocopherol acetate has been reported to improve the stability of tissue lipids in fish fillets (Pirini *et al.*, 2000; Yeldiz *et al.*, 2006; Chen *et al.*, 2007; Ortiz *et al.*, 2009; Hosseini *et al.*, 2010). However, it has reported that

*Corresponding author.
Email: e_zakipour@yahoo.com

antioxidant effects of α -tocopherol on the fish are not uniform. It has also reported that α -TA did not show any antioxidant effect on the white muscle lipids (Poza *et al.*, 1988). The fatty acid composition of muscle also reflects the diet composition. The results of Turchini *et al.* (2007) showed that diets containing higher amounts of n-6 fatty acids were responsible for an increased level of n-6 fatty acids in the fish flesh. While, Yildiz *et al.* (2006) has reported that dietary vitamin E levels did not influence the fatty acid composition of the fish fillets. Comparison studies on the effects of α -tocopherol acetate through diet and direct addition after slaughtering on lipid stability of fish during storage period, have demonstrated that post-mortem addition of α -tocopherol improved lipid stability of tilapia hamburgers during frozen storage (Helena and Silvia, 2007).

However, the reports regarding direct comparisons of endogenous and exogenous vitamin E effects on body composition and fatty acid composition is rare in fish. Therefore, this study was designed to compare the effects of α -tocopherol acetate through diet and direct addition after slaughtering on proximate analysis and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage.

Material and Methods

Experimental design

This study was conducted to compare between dietary supplementation versus post-mortem addition of α -tocopherol on body and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) fillets during 12 days storage at refrigerator. 360 fish (with initial mean weight of 95 ± 3 g) randomly were distributed amongst 9 tanks and assigned to one of three experimental treatments (three replicates for each treatment). After acclimatization for two weeks, the fish were randomly allocated to nine tanks (30 per each tank), raised at a temperature of $15 \pm 1^\circ\text{C}$ in 4-5 m³ (30×45×540 cm) at the aquaculture facilities of Artemia institute (Urmia University, Iran). In this stage, experiment had three treatments including: E0 (control group, samples with commercial diet), E300 (300 mg/kg α -TA in diet) and E500 (500 mg/kg α -TA in diet). At the end of experiment, after 58 days, all fish in a tank were collected and immediately killed by ice-shocking. Samples were washed with tap water, descaled, beheaded and filleted. In this stage, fish fillets in E0 group were divided in two homogenous groups and forth treatments were formed by adding 200 mg vitamin E /kg on one fillet group (E200, samples with commercial diet and 200

Table 1. Major basal ingredients content (% of dry matter basis) of the commercial diets

Moisture	10
Crude protein	42
Crude fat	12
Ash content	13
Crude cellulose	3.7
Phosphor	0.85

mg vitamin E /kg after filleting). These samples were treated with α -tocopherol solution (α -tocopherol-ethanol 70%-distilled water) with concentration 200 mg α -tocopherol / kg flesh. Each two fillets from each trial groups were packed separately in polythene bags, and stored in a refrigerator at 4°C for 12 days. All samples were taken for proximate analysis at day 0 and 58 of feeding trial and for fatty acid composition analysis every 6 days (day 0, 6 and 12) of storage at 4°C .

Feeding trial and diets

Fish with near uniform biomass were purchased from a local aquaculture farm located at Urmia (Azarbaijan Gharbi, Iran) and transported to aquaculture facilities of Artemia Institute (Urmia University, Iran) and immediately disinfected by 5 % salt solution. For two weeks, fish were adapted to new condition and feed a commercial diet (Faradaneh Co., Esfahan, Iran). The proximate compositions of the main dietary ingredients were given in Table 1. α -tocopherol acetate was supplied as all-rac- α -tocopherol acetate (Merck, Darmstadt, Germany). After acclimatization, fish randomly were distributed amongst 9 tanks (three experimental treatments with three replicates for each treatment). The experimental diets differed only in the relative amounts of α -TA (0, 300 and 500 mg/Kg feed). Soybean oil (3%) was used for adding α -TA in diets. To avoid lipid oxidation, the diets were stored at -18°C until fed to the fish. Fish were fed experimental diets to apparent satiation, twice daily at 8:00 and 17:00 h, 7 days a week. Supplemental aeration was provided to maintain dissolved oxygen near saturation in each tank at water flow rate of 14 ± 1 L/min. Temperature and dissolved oxygen were measured daily ($15 \pm 1^\circ\text{C}$ and 10 ± 0.5 mg/L, respectively).

Muscle composition analysis

For determining the fish fillets composition (crude protein, crude fat, ash and moisture), twelve fish at the beginning of the trial, and 27 fish (9 fish in each treatment) were killed at the end of the trial and then samples minced for analysis. Crude protein was calculated by converting the nitrogen content determined by Kjeldahl's method (AOAC, 2005). Crude lipid was determined by ether extraction using a

Soxhlet method (AOAC, 2002). The moisture content was determined by drying the meat in oven at 105 °C until a constant weight was obtained (AOAC, 2002). Ash content was determined by drying the samples in a furnace at 550 °C for 12 h (AOAC, 2002).

Lipid extraction for fatty acid analysis

The procedure used for the lipid extraction was based on Kinsella *et al.* (1977). About 50 g of fish muscle were homogenized in a warring blender for 2 min with a mixture of 50 ml chloroform and 100 ml methanol. One volume of chloroform (50 ml) and one volume of distilled water (50 ml) were added to the mixture and blended for 30 sec, respectively. The homogenate was then filtered, and the filtrate collected, and transferred to a separatory funnel to allow for phase separation. The lower fraction was collected and filtered. It was then transferred to a rotary evaporator (Heidolph Laborta, 4003, Germany) for evaporation. The sample was then collected for the fatty acid analysis.

Preparation of FA methyl esters and GS analysis

Lipid samples were converted to their constituent fatty acid methyl esters by the method of Christie (1981). Analysis of fatty acid methyl esters was performed on Agilent GC (model 6890 N, USA) equipped with Polaris FID detector. The capillary column used was a DB-WAX, universal column 30 m lengths and 0.25 mm i. d., with a wide range of applications of food analysis. N2 was used as a carrier gas at flow rate 1 ml/min. The injector and detector temperature were as 230 and 300 °C, respectively. The oven temperature program was as: started with 100 °C for 2 min and then increased with a ratio of 30 °C/min to 182 °C and held for 5 min, then increased with a ratio of 2 °C/min to 220 °C and held for 5 min

Table 2. Proximate composition (% of wet matter basis) of rainbow trout fillet at the start and end of experiment

Fillet composition parameters (%)	Beginning of experiment	End of experiment		
		treatments		
		control	300 mg α -TA	500 mg α -TA
Crude Protein	17.73 \pm 0.20 ^A	17.70 \pm 0.70 ^A	17.65 \pm 0.30 ^A	17.61 \pm 0.36 ^A
Crude Fat	7.10 \pm 0.80 ^A	7.36 \pm 0.10 ^A	7.45 \pm 0.90 ^A	7.83 \pm 0.19 ^A
Ash	1.20 \pm 0.05 ^A	1.22 \pm 0.07 ^A	1.19 \pm 0.06 ^A	1.20 \pm 0.08 ^A
Moisture	75.27 \pm 0.48 ^A	75.07 \pm 0.56 ^A	75.17 \pm 0.66 ^A	75.06 \pm 0.4 ^A

Values within rows with different superscripts are significantly different ($P < 0.05$); data are as Mean \pm SD.

and increased to a final temperature 230 °C with a ratio of 3 °C/min and held for 3 min. FAMES were identified and quantified by comparison with the RT and peak areas of Supelco standards.

Statistical analysis

A complete randomized design (CRD) was used in this experiment. All data were presented as mean \pm standard division (SD) and were subjected to one way analysis of variance (ANOVA). The one-way ANOVA was used to analyze the effect of treatments on the control and also time of storage on each treatment The Turkey's test was used for mean comparison when a significant variation was found by the ANOVA test. The significance of results was at 5%. The software used was Minitab, release 14.

Results and Discussion

Proximate analysis

Proximate analysis results (day 0 and 58 of feeding period) are presented in Table 2. The protein and lipid content of fish at day 0 of experiment were 17.73 and 7.10 %, respectively. After 8 weeks of feeding period, lipid content of fish samples increased to 7.36, 7.45 and 7.83 % in treatments with 0, 300 and 500 mg of α -TA/Kg feed, respectively. As shown in Table

Table 3. Main muscle fatty acid composition (g/100 g of total fatty acids) of rainbow trout, fed the trial diets at 0, 6 and 12th days refrigerated storage

	Day 0			Day 6				Day 12			
	E0	E300	E500	E0	E300	E500	E200	E0	E300	E500	E200
C14:0	4.54 \pm 0.43 ^{AB}	4.43 \pm 0.21 ^{AB}	4.32 \pm 0.12 ^{AB}	4.49 \pm 0.35 ^{AB}	4.29 \pm 0.16 ^{AB}	4.27 \pm 0.19 ^{AB}	4.47 \pm 0.28 ^{AB}	5.68 \pm 0.66 ^{AB}	4.68 \pm 0.73 ^{AB}	4.57 \pm 0.98 ^{AB}	5.63 \pm 0.53 ^{AB}
C14:1n5	0.36 \pm 0.03 ^{AB}	0.39 \pm 0.36 ^{AB}	0.39 \pm 0.53 ^{AB}	0.51 \pm 0.34 ^{AB}	0.54 \pm 0.29 ^{AB}	0.54 \pm 0.65 ^{AB}	0.53 \pm 0.39 ^{AB}	0.44 \pm 0.63 ^{AB}	0.44 \pm 0.12 ^{AB}	0.46 \pm 0.43 ^{AB}	0.44 \pm 0.03 ^{AB}
C16:0	18.08 \pm 1.09 ^{AB}	18.15 \pm 1.03 ^{AB}	18.08 \pm 0.85 ^{AB}	18.34 \pm 0.67 ^{AB}	18.22 \pm 0.83 ^{AB}	18.16 \pm 0.46 ^{AB}	18.24 \pm 0.42 ^{AB}	18.92 \pm 0.43 ^{AB}	18.62 \pm 0.88 ^{AB}	18.63 \pm 0.58 ^{AB}	18.98 \pm 0.93 ^{AB}
C16:1n7	7.19 \pm 0.49 ^{AB}	7.21 \pm 0.94 ^{AB}	7.20 \pm 0.73 ^{AB}	7.14 \pm 0.65 ^{AB}	7.16 \pm 0.78 ^{AB}	7.15 \pm 0.53 ^{AB}	7.16 \pm 0.13 ^{AB}	6.63 \pm 0.45 ^{AB}	6.70 \pm 0.55 ^{AB}	6.78 \pm 0.73 ^{AB}	6.60 \pm 0.50 ^{AB}
C18:0	3.71 \pm 0.92 ^{AB}	3.61 \pm 0.53 ^{AB}	3.61 \pm 0.13 ^{AB}	3.66 \pm 0.29 ^{AB}	3.56 \pm 0.38 ^{AB}	3.56 \pm 0.35 ^{AB}	3.66 \pm 0.42 ^{AB}	4.16 \pm 0.38 ^{AB}	3.96 \pm 0.43 ^{AB}	3.93 \pm 0.52 ^{AB}	4.96 \pm 0.53 ^{AB}
C18:1n9	24.49 \pm 1.33 ^{AB}	24.67 \pm 1.12 ^{AB}	24.70 \pm 0.93 ^{AB}	24.44 \pm 0.83 ^{AB}	24.62 \pm 0.36 ^{AB}	24.65 \pm 0.65 ^{AB}	24.47 \pm 0.47 ^{AB}	23.06 \pm 0.79 ^{AB}	23.56 \pm 0.82 ^{AB}	23.63 \pm 1.23 ^{AB}	23.06 \pm 1.03 ^{AB}
C18:1n7	2.60 \pm 0.34 ^{AB}	2.68 \pm 0.45 ^{AB}	2.68 \pm 0.35 ^{AB}	2.55 \pm 0.25 ^{AB}	2.63 \pm 0.53 ^{AB}	2.63 \pm 0.13 ^{AB}	2.54 \pm 0.25 ^{AB}	2.32 \pm 0.43 ^{AB}	2.42 \pm 0.63 ^{AB}	2.44 \pm 0.55 ^{AB}	2.33 \pm 0.23 ^{AB}
C18:2n6	11.79 \pm 0.67 ^{AB}	11.85 \pm 0.90 ^{AB}	11.90 \pm 0.98 ^{AB}	11.74 \pm 0.46 ^{AB}	11.80 \pm 0.39 ^{AB}	11.83 \pm 0.65 ^{AB}	11.76 \pm 0.75 ^{AB}	10.94 \pm 0.76 ^{AB}	10.15 \pm 0.83 ^{AB}	11.20 \pm 0.99 ^{AB}	10.74 \pm 1.01 ^{AB}
C18:3n3	2.20 \pm 0.40 ^{AB}	2.28 \pm 0.69 ^{AB}	2.30 \pm 0.63 ^{AB}	2.16 \pm 0.63 ^{AB}	2.23 \pm 0.56 ^{AB}	2.25 \pm 0.75 ^{AB}	2.14 \pm 0.36 ^{AB}	2.05 \pm 0.96 ^{AB}	2.11 \pm 0.56 ^{AB}	2.17 \pm 0.73 ^{AB}	2.08 \pm 0.43 ^{AB}
C20:0	0.22 \pm 0.19 ^{AB}	0.19 \pm 0.23 ^{AB}	0.18 \pm 0.09 ^{AB}	0.17 \pm 0.03 ^{AB}	0.32 \pm 0.03 ^{AB}	0.27 \pm 0.03 ^{AB}	0.16 \pm 0.05 ^{AB}	0.81 \pm 0.09 ^{AB}	0.51 \pm 0.03 ^{AB}	0.45 \pm 0.05 ^{AB}	0.75 \pm 0.02 ^{AB}
C20:1n9	0.50 \pm 0.23 ^{AB}	0.54 \pm 0.31 ^{AB}	0.60 \pm 0.05 ^{AB}	0.45 \pm 0.76 ^{AB}	0.49 \pm 0.28 ^{AB}	0.55 \pm 0.36 ^{AB}	0.55 \pm 0.53 ^{AB}	0.46 \pm 0.85 ^{AB}	1.56 \pm 0.63 ^{AB}	1.49 \pm 0.93 ^{AB}	1.48 \pm 0.51 ^{AB}
C20:2n6	2.56 \pm 0.62 ^{AB}	2.60 \pm 0.12 ^{AB}	2.60 \pm 0.43 ^{AB}	2.51 \pm 0.65 ^{AB}	2.55 \pm 0.95 ^{AB}	2.55 \pm 0.28 ^{AB}	2.51 \pm 0.15 ^{AB}	1.50 \pm 0.86 ^{AB}	2.10 \pm 0.83 ^{AB}	2.05 \pm 0.75 ^{AB}	1.40 \pm 0.23 ^{AB}
C20:3n3	1.13 \pm 0.11 ^{AB}	1.22 \pm 0.42 ^{AB}	1.17 \pm 0.12 ^{AB}	1.08 \pm 0.36 ^{AB}	1.17 \pm 0.15 ^{AB}	1.12 \pm 0.49 ^{AB}	1.06 \pm 0.68 ^{AB}	0.96 \pm 0.84 ^{AB}	1.05 \pm 0.56 ^{AB}	1.06 \pm 0.63 ^{AB}	1.02 \pm 0.20 ^{AB}
C20:4n6	0.17 \pm 0.12 ^{AB}	0.21 \pm 0.13 ^{AB}	0.21 \pm 0.11 ^{AB}	0.12 \pm 0.30 ^{AB}	0.16 \pm 0.13 ^{AB}	0.16 \pm 0.22 ^{AB}	0.13 \pm 0.13 ^{AB}	0.08 \pm 0.12 ^{AB}	0.14 \pm 0.21 ^{AB}	0.15 \pm 0.03 ^{AB}	0.11 \pm 0.01 ^{AB}
C20:5n3	4.69 \pm 0.19 ^{AB}	4.73 \pm 0.73 ^{AB}	4.77 \pm 0.56 ^{AB}	4.64 \pm 0.31 ^{AB}	4.68 \pm 0.56 ^{AB}	4.70 \pm 0.75 ^{AB}	4.62 \pm 0.43 ^{AB}	4.24 \pm 0.61 ^{AB}	4.49 \pm 0.45 ^{AB}	4.68 \pm 0.23 ^{AB}	4.23 \pm 0.57 ^{AB}
C22:0	1.17 \pm 0.20 ^{AB}	1.10 \pm 0.16 ^{AB}	1.06 \pm 0.63 ^{AB}	1.12 \pm 0.37 ^{AB}	1.05 \pm 0.85 ^{AB}	1.01 \pm 0.75 ^{AB}	1.10 \pm 0.35 ^{AB}	2.02 \pm 0.03 ^{AB}	1.42 \pm 0.03 ^{AB}	1.35 \pm 0.03 ^{AB}	1.92 \pm 0.03 ^{AB}
C22:1n9	0.39 \pm 0.19 ^{AB}	0.44 \pm 0.14 ^{AB}	0.45 \pm 0.13 ^{AB}	0.34 \pm 0.03 ^{AB}	0.39 \pm 0.03 ^{AB}	0.40 \pm 0.09 ^{AB}	0.36 \pm 0.07 ^{AB}	0.33 \pm 0.34 ^{AB}	0.36 \pm 0.12 ^{AB}	0.39 \pm 0.20 ^{AB}	0.36 \pm 0.21 ^{AB}
C22:6n3	15.39 \pm 1.18 ^{AB}	15.49 \pm 0.86 ^{AB}	15.46 \pm 0.96 ^{AB}	15.34 \pm 0.99 ^{AB}	15.44 \pm 0.68 ^{AB}	15.41 \pm 0.53 ^{AB}	15.32 \pm 0.73 ^{AB}	14.62 \pm 0.20 ^{AB}	15.32 \pm 0.40 ^{AB}	15.44 \pm 0.10 ^{AB}	14.42 \pm 0.40 ^{AB}

Different superscripts a, b & c indicate significant ($P < 0.05$) differences between each treatments within the same storage period. Different superscripts A, B & C indicate significant ($P < 0.05$) differences between same treatments within the different storage period. Data are given as mean \pm SEM

Table 4. Major muscle fatty acid groups (g/100 g of total fatty acids) of rainbow trout during refrigerated storage

	Day 0			Day 6				Day 12			
	E0	E300	E500	E0	E300	E500	E200	E0	E300	E500	E200
Total SFA	27.03 ^{ab} (0.6)	26.64 ^{ab} (0.3)	26.31 ^{ab} (0.4)	27.38 ^{ab} (0.8)	26.80 ^{ab} (1.2)	26.75 ^{ab} (0.8)	27.37 ^{ab} (1.03)	31.97 ^{ab} (0.8)	28.89 ^{ab} (0.9)	28.53 ^{ab} (0.5)	32.09 ^{ab} (0.9)
Total MUFA	35.23 ^{ab} (1.1)	35.82 ^{ab} (0.5)	35.92 ^{ab} (1.03)	35.60 ^{ab} (0.9)	35.64 ^{ab} (0.5)	35.71 ^{ab} (0.8)	35.42 ^{ab} (1.1)	34.00 ^{ab} (1.3)	35.03 ^{ab} (0.9)	35.05 ^{ab} (1.1)	34.15 ^{ab} (1.2)
n-6 PUFA	14.20 ^{ab} (0.7)	14.15 ^{ab} (0.6)	14.26 ^{ab} (0.3)	14.23 ^{ab} (0.5)	14.24 ^{ab} (0.7)	14.35 ^{ab} (0.9)	14.29 ^{ab} (0.3)	12.30 ^{ab} (0.9)	13.29 ^{ab} (0.5)	13.27 ^{ab} (0.9)	12.25 ^{ab} (0.7)
n-3 PUFA	23.18 ^{ab} (1.2)	23.41 ^{ab} (0.6)	23.40 ^{ab} (0.5)	23.09 ^{ab} (1.03)	23.30 ^{ab} (0.9)	23.78 ^{ab} (0.03)	23.02 ^{ab} (0.2)	21.56 ^{ab} (0.6)	22.80 ^{ab} (0.6)	23.23 ^{ab} (0.9)	21.60 ^{ab} (0.5)
Total PUFA	37.55 ^{ab} (0.3)	37.50 ^{ab} (0.8)	37.66 ^{ab} (0.9)	37.35 ^{ab} (0.4)	37.54 ^{ab} (0.3)	37.13 ^{ab} (0.7)	37.31 ^{ab} (0.5)	33.86 ^{ab} (0.9)	36.09 ^{ab} (1.01)	36.50 ^{ab} (1.3)	33.85 ^{ab} (1.1)
Total UFA	72.97 ^{ab} (0.5)	73.56 ^{ab} (0.3)	73.69 ^{ab} (0.3)	72.62 ^{ab} (0.9)	73.21 ^{ab} (0.4)	73.36 ^{ab} (0.5)	73.70 ^{ab} (0.4)	68.04 ^{ab} (0.3)	71.11 ^{ab} (0.5)	71.50 ^{ab} (0.7)	68.01 ^{ab} (0.8)

Different superscripts small letter (a, b & c) indicate significant ($P < 0.05$) differences between each treatments within the same storage period.

Different superscripts capital letter (A, B & C) indicate significant ($P < 0.05$) differences between same treatments within the different storage period.

Data are given as mean & (SEM)

2, although lipid content of whole body increased with increasing dietary α -TA levels, no statistically differences were observed in fillet composition parameters including total lipid, protein, ash and moisture by dietary α -TA after 8 weeks experiment. These results closely agree with those of Safarpour *et al.* (2011) who reported that diets containing different level of vitamin E did not significantly affect whole body composition of beluga. Hosseini *et al.* (2010) also reported that, compared with control group, dietary α -TA supplementation did not led to change the body composition of juvenile beluga. Jitinandana *et al.* (2006), Yeldiz *et al.* (2006), Pirini *et al.* (2000) and Charyapechara *et al.* (2003) have also found that dietary vitamin E did not affect fish muscle composition. In contrast, Sau *et al.* (2004) found significantly difference in crude protein of rohu, but not differences in lipid or ash contents among the treatments. Moreover, Huang *et al.* (2003) reported that dietary vitamin E levels influenced on the muscle protein content of hybrid tilapia *Oreochromis niloticus* \times *O. aureus* and protein content of fish fed the lowest level of vitamin E was significantly lower than fish fed other diets, although it did not affect the moisture, lipid, and ash level in this species.

Fatty acid profile

The fatty acid composition of the fish fillet is shown in Table 3. Eighteen fatty acids were detected in control group (E0, samples with commercial diet). Fatty acid profile of rainbow trout showed considerable amounts of palmitic, oleic, linoleic and docosahexaenoic acid (Table 3). In control group, polyunsaturated fatty acids (PUFA) were the most abundant fatty acids (37.55% of total fatty acids), which followed by monounsaturated fatty acids (MUFA, 35.23% of total fatty acids) and saturated fatty acids (SFA, 27.03 % of total fatty acids). In this trial, fatty acid composition of fillets showed

no significant differences due to the experimental diets, which were supplemented with different levels of vitamin E ($P > 0.05$) (Table 3). The content of MUFAs slightly increased and instead the content of SFAs decreased at the end of feeding trial with different levels of vitamin E (Table 4). According to the results, dietary DL- α -tocopherol acetate did not affect the FA composition of the fresh muscle. These results confirmed previous findings that different α -tocopherol acetate levels in the diet did not affect the fatty acid composition of fish. Yeldiz *et al.* (2006) observed that fatty acid profiles of the rainbow trout fed 100, 300 and 500 mg/kg vitamin E had no changes by dietary α -tocopherol acetate. They concluded that the fatty acid composition of fish fillets reflects the fatty acid composition of the diets. This observation is also supported by Jitinandana *et al.* (2006) and Hosseini *et al.* (2010) who found that dietary vitamin E did not affect fish muscle FA composition. Watanabe and Takashima (1977) found a marked decrease in 18:2n-6 level in all tissues of carp due to α -tocopherol deficient. In our study, differences in the content of 18:2n-6 level was not significant ($P > 0.05$) between control group (E0) and dietary supplemented α -tocopherol groups (E300 and E500).

The results showed that refrigerated storage had a significant effect on fillet fatty acid composition during the 12 days of refrigerated storage. In general, there were decreases in the proportion of MUFA and PUFA and increase in the content of SFA in all treatments during refrigerated storage (Table 4). Fish fillets had significantly ($P < 0.05$) higher SFA content at day 12 in all treatments compared to day 0 (fresh) and day 6. SFA content increased to 31.97, 28.89, 28.53 and 32.09 (% of total fatty acids) at day 12. The MUFA and PUFA levels of the fillets were nearly similar between day 0 and day 6 in all treatments. Changes on PUFA content at day 12 were higher than

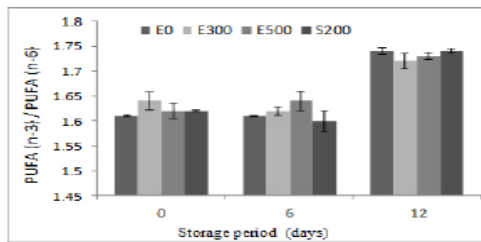


Figure 1. The ratio of (n-3) PUFA to (n-6) PUFA of rainbow trout during refrigerated storage

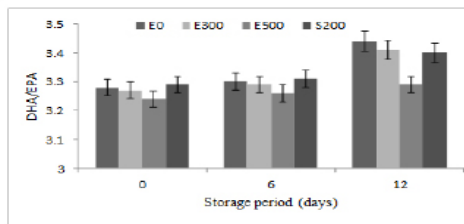


Figure 2. The ratio of DHA/EPA of rainbow trout during refrigerated storage

MUFA in all treatments. PUFA content decreased from 37.35, 37.54, 37.13 and 37.31 (day 6) to 33.86, 36.09, 36.50 and 33.85 at day 12 for E0, E300, E500 and E200 treatments, respectively. Same pattern of decreases for C22:6, C22:5, C20:3, C20:2 and C18:3 and increases for C18:0, C16:0 and C14:0 were also found in fish fillets during refrigerated storage (Table 3).

Dietary supplemented α -tocopherol (in E300 and E500 treatments) had also an insignificant ($P < 0.05$) effect on fillet fatty acid composition during the 12 days of refrigerated storage. As it showed in results, smaller decreases in the content of PUFA and MUFA were observed in E300 and E500 treatments compared to E0 and E200 at day 12 (Table 3). The highest n-3 PUFA levels were observed in E300 at day 12 which followed by E300, E200 and E0. The rate of decreases in n-3 PUFAs at day 12 in comparison with day 0 were 6.90, 2.61, 0.73 and 6.82% in E0, E300, E500 and E200, respectively. The same pattern of changes was found in total unsaturated (UFA) fatty acids. Significantly ($P < 0.05$) lower UFA content were observed for all treatments in day 12 as compared with days 0 and 6. Instead higher content of SFAs were observed in E0 and E200 at day 12 compared with E300 and E500. These results confirmed previous findings that different α -tocopherol acetate levels in the diet affect the fatty acid composition of fish during refrigerated storage. Similarly Yildiz *et al.* (2006) indicated that α -tocopherol levels of fish fillets had a significant effect on fillet fatty acid composition during the 9 days of storage at $1 \pm 0.3^\circ\text{C}$. They also reported that Total n-6 and n-3 PUFA levels of the fillet were the highest on day 0 and the lowest

on day 6 ($P < 0.05$). Hosseini *et al.* (2010) reported that although dietary DL- α -tocopherol acetate did not affect the FA composition of the *Huso huso* muscle, fed supplemented diets with vitamin E had less significant variation in FA composition in comparison to the control diets during frozen storage at $18 \pm 1^\circ\text{C}$. Jitinandana *et al.* (2006) found similar results for rainbow trout fed different levels of dietary vitamin E. In contrast, Pirini *et al.* (2000) found dietary vitamin E supplementation had no effect on general fatty acid composition, MUFA and PUFA content of experimental fish during storage.

However, the fatty acid composition was not affected by the postmortem addition of vitamin E (Tables 3 and 4). The comparison between fatty acids and fatty acid group between E 200 (samples with commercial diet and 200 mg vitamin E /kg after filleting) and E300 (300 mg/kg α -TA in diet) and E500 (500 mg/kg α -TA in diet) showed that post-slaughter vitamin E addition exhibited a slight effect compared to that of the control. Mitsumoto *et al.* (1993) compared the antioxidant potency of dietary and post-slaughter vitamin E in ground beef muscle. They found, lipid oxidation was highly suppressed ($P < 0.05$) in beef during 9 d of display by dietary rather than post-slaughter vitamin E addition, and reported that in vitro vitamin E exhibited a slight antioxidative effect compared to that of the control. Buckley and Morrissey (1992) suggested the addition of α -tocopherol during processing did not ensure sufficient positioning close to the sub-cellular membrane PUFA to execute its full antioxidant potential.

The ratio of n-3 PUFA to n-6 PUFA can be used to facilitate identification of high n-3 PUFA foodstuffs (Dunstan *et al.*, 1999). Data on n-3/n-6 PUFA ratios are given in Figure 1. The results indicated that the n-3/n-6 PUFA ratios were higher than 1 and ranged from 1.61 to 1.64 (Figure 1). The n-3/n-6 ratio in muscle lipids of common farmed fish species (rainbow trout, European catfish, pike, grass carp, crucian carp and carp) varied from 0.74 to 3.45 (Bieniarz *et al.*, 2000). The n-3/n-6 PUFA ratio of all treatments was the lowest on day 0 and the highest on 12 days. No significant ($P < 0.05$) changes in n-3/n-6 PUFA ratio was observed until day 6 of period storage, but later, n-3/n-6 PUFA ratio was significantly increased in all treatments during the period storage as a function of time (Figure 1). During storage period, the n-3/n-6 PUFA ratio was not affected by the dietary and postmortem addition of vitamin E. As fish and seafood are good sources of ω -3 fatty acids, they generally suggest balancing between ω -3 and ω -6 fatty acids and increasing the ω -3/ ω -6 ratio. Using seafood with

high content of omega-3 PUFA (a low omega-6/omega-3 ratio) can exert suppressive effects on many of diseases (Simopoulos, 2002). Figure 2 indicated the change of DHA/EPA ratios of trial treatments. As shown in Figure 2, no changes of DHA/EPA were recorded during the initial 6 days of refrigerated storage. After 6 days, irrespective of E500, the content of DHA/EPA was significantly increased in all treatments. Although There were no significant differences in DHA/EPA content ($P < 0.05$) between the lipid of fish fed 300 mg α -tocopherol and control group and groups that only received the postmortem addition tocopherol, but treatments that received 500 mg tocopherol in diets showed smaller changes when compared against the other groups during refrigerator storage (Figure 2). Also, there were no significant effects of superficial treatments of α -tocopherol on the percentage of DHA/EPA ratios.

Conclusions

Based on our results, although diets containing different level of DL- α -TA did not affect the body and fatty acid composition of fish, use of dietary DL- α -TA effectively reduce the changes of lipid composition of rainbow trout fillet, stored at 4 °C for 12 days. Surface application of DL- α -TA showed no effect in lipid content and fatty acid composition of refrigerated rainbow trout fillet. It could be concluded that dietary vitamin E provides better storage conditions than surface application and extended the shelf life of the samples.

Acknowledgements

The authors are grateful to Messrs Dr. N. Agh and Prof. S. Dragoev for their advices and unending support. Also, authors thank to Artemia and Aquatic Animals Research Institute of Urmia University for providing of facilities for this study.

References

- Ajuyah, A. O., Ahn, D. U., Hardin, R. T. and Sim, J. S. 1993. Dietary antioxidants and storage affect chemical characteristics of ω -3 fatty acid enriched broiler chicken meats. *Journal of Food Science* 58: 43–46.
- AOAC. 2005. Official Method of Analysis (17th ed). Association of Official Analytical Chemists. Washington, DC.
- AOAC. 2002. Official Method of Analysis (14th ed). Association of Official Analytical Chemists. Washington, DC.
- Bell, J. G. 1998. Current aspects of lipid nutrition in fish farming. In Black, K. D. and Pickering, A. D. (Eds.), *Biology of farmed fish*, p. 114–145. Sheffield, UK: Sheffield Academic Press.
- Bieniarz, K., Koldras, M., Kamiński, J. and Mejza T. 2000. Fatty acids and cholesterol in some freshwater fish species in Poland, *Folia Univ. Agric. Stetin.*, 214 *Piscaria*, 27, 21.
- Buckley, D. J. and Morrissey, P. A. 1992. Vitamin E and meat quality. *Animal Production Highlights*. Hoffmann-La Roche, Basel, Switzerland.
- Chaiyapechara, S., Casten, M. T., Hardy, R. W. and Dong, F. M. 2003. Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentration of lipid and vitamin E. *Aquaculture* 219: 715–738.
- Chen, Y. C., Nguyen, J., Semmens, K., Beamer, S. and Jaczynski, J. 2007. Physicochemical changes in ω -3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) muscle during refrigerated storage. *Food Chemistry* 104: 1143–1152.
- Chen, Y. C., Nguyen, J., Semmens, K., Beamer, S. and Jaczynski, J. 2008. Chemical changes in omega-3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) fillets during abusive-temperature storage. *Food Chemistry* 19: 599–608.
- Christie, W.W. 1981. The composition, structure and function of lipids in the tissues of ruminant animals. In: Christie, W. W. (Eds). *Lipid Metabolism in Ruminant Animals*, p.95-191. Oxford: Pergamon Press.
- Helena, F. S. and Silvia, L. S. 2007. Tocopherol in the lipid stability of tilapia (*Oreochromis niloticus*) hamburgers. *Food Chemistry* 105: 1214–1218.
- Hosseini, S. V., Abedian-Kenari, A., Rezaei, M. and Nazari R. M. 2010. Influence of the in vivo addition of alpha-tocopherol acetate with three lipid sources on the lipid oxidation and fatty acid composition of Beluga sturgeon, *Huso huso*, during frozen storage. *Food Chemistry* 118 (2): 341–348.
- Huang, C. H., Chang, R. J., Huang, S. L. and Chen, W. 2003. Dietary vitamin E supplementation affects tissue lipid peroxidation of hybrid tilapia, *Oreochromis niloticus* X *O. aureus*. *Comp. Biochem. Physiol. B: Biochemistry and Molecular Biology* 134: 265-270.
- Jitinandana, S., Kenney, P. B., Slider, S. D., Kamireddy, N. and Hankins J. S. 2006. High dietary vitamin E affects storage stability of frozen- refrigerated trout fillets. *Journal of Food Science* 71: 91–96.
- Jittrepotch, N., Ushio, H. and Ohshima, T. 2006. Oxidative stabilities of triacylglycerol and phospholipids fractions of cooked Japanese sardine meat during low temperature storage. *Food Science* 99: 360-367.
- Kinsella, J. E., Shimp, J. L., Mai, J. and Weihrauch, J. 1977. Fatty acid content and composition of freshwater finfish. *Journal of the American Oil Chemist's Society* 54: 424-429.
- Machlin, L. J. 1984. Vitamin E. In Machlin, L. J. (Eds). *Handbook of vitamins. Nutrition, biochemical and clinical aspects*, p. 99-145. New York: Marcel Dekker.
- Mitumoto, M., Arnold, R. N., Schaefer, D. M. and Cassens R. G. 1993 Dietary versus postmortem supplementation

- of vitamin E pigment and lipid stability in ground beef. *Journal of Animal Science* 71: 1812-1816.
- Ortiz, J., Larrain, M. A., Vivanco, J. P. and Aubourg, P. A. 2009. Rancidity development during the frozen storage of farmed coho salmon (*Oncorhynchus kisutch*): Effect of antioxidant composition supplied in the diet. *Food Chemistry* 115: 143-148.
- Pirini, M., Gatta P. P., Testi S., Trigari G. and Monetti, P. G. 2000. Effect of refrigerated storage on muscle lipid quality of sea bass (*Dicentrarchus labrax*) fed on diets containing different levels of vitamin E. *Food Chemistry* 68: 289-293.
- Pope, S. A. S., Burtin, G. E., Clayton, P. T., Madge, D. J. and Muller, D. P. R. 2002. Synthesis and analysis of conjugates of the major vitamin E metabolite, α -CEHC. *Free Radical Biology and Medicine* 33: 807-817.
- Pozo, R., Lavety, J. and Love, R. M. 1988. The role of dietary atocopherol (vitamin E) in stabilizing the canthaxanthin and lipids of rainbow trout muscle. *Aquaculture* 73: 165-175.
- Safarpour Amlashi, A., Falahatkar, B., Sattari, M. And Tolouei Gilani, M. H. 2011. Effect of dietary vitamin E on growth, muscle composition, hematological and immunological parameters of sub-yearling beluga Huso huso L. *Fish and Shellfish Immunology* 30 (3): 807- 814.
- Sau, S. K., Paul, B. N., Mohanta, K. N. and Mohanty, S. N. 2004. Dietary vitamin E requirement, fish performance and carcass composition of rohu (*Labeo rohita*) fry. *Aquaculture* 240: 359-368.
- Shahidi, F. 1997. Assessment of lipid oxidation and off-flavour development in meat and meat products. In Shahidi, F. (Eds). *Flavour of meat and meat products*, p. 247-266. London: Chapman and Hall.
- Simopoulos, A. P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother.* 56: 365-379.
- Turchini, G. M., Moretti, V. M., Mentasti, T., Orban, E. and Valfrè, F. 2007. Effects of dietary lipid source on fillet chemical composition, flavour volatile compounds and sensory characteristics in the freshwater fish tench (*Tinca tinca* L.). *Food Chemistry* 102: 1144-1155.
- Watanabe, T. and Takashima, F. 1977. Effect of α -tocopherol deficiency on carp: VI. Deficiency symptoms and changes of fatty acid and triglyceride distributions in adult carp. *Bull. Jpn. Sci. Fish.* 43: 819-830.
- Yeldiz, M., Şener, E. and Gün, H. 2006. Effect of refrigerated storage on fillet lipid quality of rainbow trout (*Oncorhynchus mykiss* W.) Fed a diet containing different levels of DL- α -tocopherol acetate. *Turkish Journal of Veterinary and Animal Sciences* 30: 143-150.
- Zhang, X. D., Wu, T. X., Cai, L.S. and Zhu, Y.F. 2007. Effects of α -tocopherol acetate supplementation in preslaughter diet on antioxidant enzyme activities and fillet quality of commercial-size *Sparus macrocephalus*. *Journal of Zhejiang University Science B* 8(9):680-685.
- Zuta, P. C., Simpson, B. K., Zhao. X. and Leclerc, L. 2007. The effect of α -tocopherol on the oxidation of mackerel oil. *Food Chemistry* 100: 800-807.