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PII: S0308-8146(97)00209-4

Food Chemistry, Vol. 62, No. 2, pp. 173–178, 1998

Printed in Great Britain
0308-8146/98 $19.00+0.00

Oxidative stability of Atlantic salmon (*Salmo salar*, L.) fillet enriched in α-, γ-, and δ-tocopherol through dietary supplementation

Kristin Hamre,[a]* Rolf Kristian Berge[b] & Øyvind Lie[a]

[a]*Institute of Nutrition, Directorate of Fisheries, P.O. Box 185, N-5002 Bergen, Norway*
[b]*Institute of Clinical Biology, Haukeland University Hospital, N-5021 Bergen, Norway*

(Received 6 June 1997; accepted 28 August 1997)

The purpose of this study was to examine the effect of dietary tocopherols on the oxidative stability of Atlantic salmon fillet. The fish were fed four diets supplemented with 150 mg kg^{-1} α-tocopherol and different combinations of γ- and δ-tocopherol (50 or 100 mg kg^{-1}) for 10 months, slaughtered, and stored for 16 days on ice, or 48 weeks at −30°C. Fillet concentrations of tocopherols at slaughter were 0.101 ± 0.001, 0.091 ± 0.004 and 0.025 ± 0.002 times feed concentration for α-, γ- and δ-tocopherol, respectively. Fillet α-tocopherol, but not γ- and δ-tocopherol, was moderately lowered during iced and frozen storage. The non-α-tocopherols protected the fillet against lipid oxidation in a dose-dependent manner during frozen storage, but appeared to act as prooxidants during storage on ice. It is concluded that α-tocopherol may be better suited than mixed tocopherols as a tool to optimize the oxidative stability of Atlantic salmon fillet.

INTRODUCTION

Vitamin E is the term of a group of lipid-soluble compounds, tocopherols and tocotrienols, which protect living organisms against free radical-mediated oxidation. These natural antioxidants are also widely used to increase the oxidative stability of foods. α-Tocopherol has the highest biological activity of the vitamin E homologues, and is retained to a greater extent than the other tocopherols in most tissues of Atlantic salmon (Hamre and Lie, 1997). Muscle and adipose tissue are exceptions, since they retain γ-tocopherol at a similar rate as α-tocopherol, and δ-tocopherol at a rate of approximately 50% that of α-tocopherol (Hamre and Lie, 1997).

When meat and fish fillet are enriched in vitamin E by supranutrial supplementation, they become better protected against forced oxidation, and sometimes show greater oxidative stability during fresh and frozen storage (Schaefer *et al.*, 1995; Boggio *et al.*, 1985; Frigg *et al.*, 1990; Gatlin *et al.*, 1992; Sigurgisladottir *et al.*, 1994*a*,*b*). Most of these studies have concentrated on α-tocopherol, whereas γ- and δ-tocopherol are sometimes claimed to be better antioxidants (Frankel, 1996).

The purpose of the present study was to examine the effect of dietary supplementation of α-, γ- and δ-tocopherol on the oxidative stability of Atlantic salmon fillet during frozen and iced storage.

*To whom correspondence should be addressed. Fax: +47 55 238095; e-mail: fett_kh@nutr.fiskeridir.no

MATERIALS AND METHODS

Materials

Herring meal (Norse-LT 94) and capelin oil (Nor Salm Oil) were obtained from Norsildmel, Oslo Norway, soya protein concentrate (SOYA CO MILL FG) from Loders Croklaan, The Netherlands, extruded wheat from Statkorn, Oslo, Norway, EPA/DHA enriched fish oil (EPAX) from Pronova A/S, Bergen, Norway. Astaxanthine (CAROPHYLL PINK), betaine (BETAINE FINE) and vitamin C (ROVIMIX STAY-C) were purchased from Hoffman-La Roche, Basel, Switzerland. *RRR*-α-tocopheryl acetate and δ-rich TOH concentrate were from Eisai Pharma-Chem Europe LTD, London, UK. Grünau GmbH, Jllertissen, Germany, delivered a mixed tocopheryl acetate (Lamevit EA 36), and esterified the δ-rich TOH concentrate with acetate.

Fish and diets

The feeding experiment was carried out at Matre Aquaculture Research Station (Institute of Marine

Research, Bergen) from May 1994 to February 1995. Data on tissue distribution and turnover of the tocopherols have been presented elsewhere (Hamre and Lie, 1997; Hamre *et al.*, 1998). Atlantic salmon (*Salmo salar*, L.) smolts (230 g) in duplicate tanks were fed four experimental diets with different combinations of α-, γ- and δ-tocopherol (Table 1). The fish were hand-fed to satiation twice daily on weekdays and once a day during weekends.

The basal experimental diet contained (added per kg): 470 g herring meal, 50 g soya protein concentrate, 240 g extruded wheat, 210 g capelin oil, 20 g EPA/DHA-enriched fish oil, 1.28 g betaine (76.8%), 3.58 g astaxanthin (1.48%) and commercial vitamin and mineral premixes for salmon, except that *all rac* α-tocopheryl acetate was reduced to 50 mg kg^{-1} dry diet. The basal diet, containing 110 g kg^{-1} of the capelin oil, was produced by Felleskjøpet Havbruk A/S, Sandnes, Norway. Vitamin E preparations originating from natural sources (*RRR*-forms) were obtained in oil form. Analysed content of α-TOH in the α-tocopheryl acetate preparation was 91.8%. The mixed tocopheryl acetate (Lamevit EA 36) contained 23.9% α-, 26.5% γ- and 4.6% δ-TOH, and the δ-rich tocopheryl acetate preparation contained 2.8% α-, 4.4% γ- and 40% δ-TOH. The experimental diets (Table 1) were produced by coating the pellets with 100 g kg^{-1} capelin oil containing the appropriate combinations of tocopheryl acetate preparations to obtain the experimental diets. Thereafter, the pellets were further coated with 20 g kg^{-1} fish oil enriched in EPA/DHA. The dry matter in the diets was 938 ± 14 g kg^{-1} and lipid accounted for 250 ± 8 g kg^{-1} of the dry matter.

Ice and frozen storage of fillet

Standardized fillet samples (three samples per tank) were taken every 1–3 months during the feeding period to monitor changes in the tocopherol status of the fish. Fillets for the storage experiments were obtained in February. Three fish per tank were slaughtered, filleted and transported in plastic bags on ice to the laboratory. The left fillet was stored in plastic bags on ice for 16 days. The right fillet was frozen and stored at −30°C for 48 weeks. Samples were taken at regular intervals according to Fig. 1 for analysis of thiobarbituric acid reactive substances (TBARS) and tocopherols.

Analytical methods

Tocopherols were analysed as described by Hamre and Lie (1997) and Lie *et al.* (1994). Briefly, the samples were homogenized and saponified in the presence of antioxidants (ascorbic acid and pyrogallol), and the unsaponifiable material was extracted with hexane. The tocopherols were analysed using normal phase HPLC and fluorescence detection. Lipid peroxidation was determined by a HPLC-based thiobarbituric acid reactive substances (TBARS) method (Yagi, 1976; Demoz *et al.*, 1994). Percent lipid and dry matter were determined according to Lie *et al.* (1988), and fatty acid composition of fillet lipids as described by Lie and Lambertsen (1991).

Statistics

The software Statistica (ver. 4.5, StatSoft inc., Tulsa OK, USA) was used for the statistical analyses. Kruskal–Wallis ANOVA and the Mann–Whitney U-test were applied to study differences between dietary groups regarding fillet tocopherol and TBARS levels. Friedman ANOVA for repeated measurements was used to detect changes in the parameters with time. The relationships between dietary and fillet tocopherol concentrations were found by linear regression. Differences and effects were considered significant at $p < 0.05$.

RESULTS

During the feeding period of 9 months, the average fish weight increased from 230 g to 1.15 kg. The mortality was negligible. After the first 6 months of feeding, the fillet tocopherol concentrations stabilized at levels dependent on the feed concentrations, and showed only minor variations thereafter. Further data on fish performance and turnover and tissue levels of tocopherols are given by Hamre and Lie (1997) and Hamre *et al.* (1998).

Fish sampled for the storage experiments weighed 1.28 ± 0.25 kg. The fillet contained 68 ± 11 g lipid kg^{-1} wet weight, with 22.3% saturated fatty acids, 43.6% monounsaturated and 32.1% polyunsaturated fatty acids, where 27.2% were n-3 fatty acids. Lipid content and fatty acid compostion of the fillet were not affected by the dietary treatments. Muscle γ- and δ-tocopherol

Table 1. Added and analysed levels of α-, γ- and δ-tocopherol equivalents (TOH equ.) in the experimental diets. The tocopherols were added as acetates (mean ± SEM, $n = 3$)

	Added (TOH equ. mg kg^{-1} dry wt.)			Analysed (mg kg^{-1} dry wt.)		
Diet	α-TOH	γ-TOH	δ-	α-TOH	γ-TOH	δ-TOH
1	150	—	—	165 ± 3	1.2 ± 0.6	0.4 ± 0.2
2	150	—	100	170 ± 6	16 ± 1	96 ± 5
3	150	50	50	164 ± 2	49 ± 1	67 ± 5
4	150	100	100	157 ± 3	88 ± 2	115 ± 10

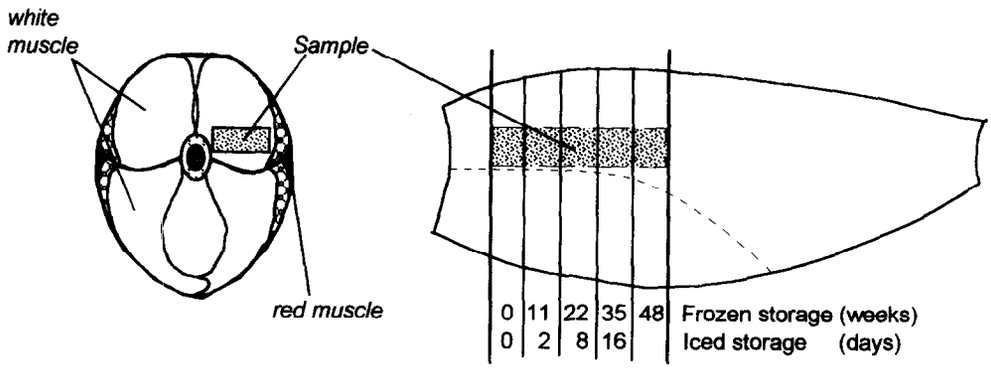

Fig. 1. Sampling procedure for stored Atlantic salmon fillet. Samples were taken from the mid section of the fillet as shown. The fillets for frozen storage were cut into individual sample slices, which were packed in separate plastic bags and frozen at −30°C. Samples slices from fillets stored on ice were obtained from whole fillet on the day of sampling. Samples for analysis were taken from above the radial bones; any red muscle tissue was removed and the samples were cut in two for analyses of TBARS and tocopherols.

concentrations were directly proportional to the feed concentrations, and amounted to 0.091 ± 0.004 and 0.026 ± 0.002 times feed concentrations, respectively. Similarly, previous studies have shown that the concentration of α-tocopherol in muscle is proportional to feed α-tocopherol concentration (Hamre and Lie, 1995; Hamre *et al.*, 1997). Fillet α-tocopherol amounted to 0.101 ± 0.001 times feed concentration in the present study (Table 1 and Fig. 2). There were no significant interactions between the tocopherols with respect to uptake in muscle tissue.

During storage on ice, fillet from all groups showed a significant decrease in α-tocopherol ($p < 0.05$, Fig. 2), occurring mainly during the first 2 days of storage (data not shown). The concentration of γ-tocopherol was unchanged in all groups, while the concentration of δ-tocopherol increased significantly during storage in fish fed diet 4 ($p < 0.05$), and was unchanged in the other groups. Fillet stored at −30°C for 48 weeks also showed an apparent decrease in α-tocopherol concentration, significant in fish fed diet 3, only ($p < 0.05$, Fig. 2). Fillet from this group also showed a decrease in γ-tocopherol

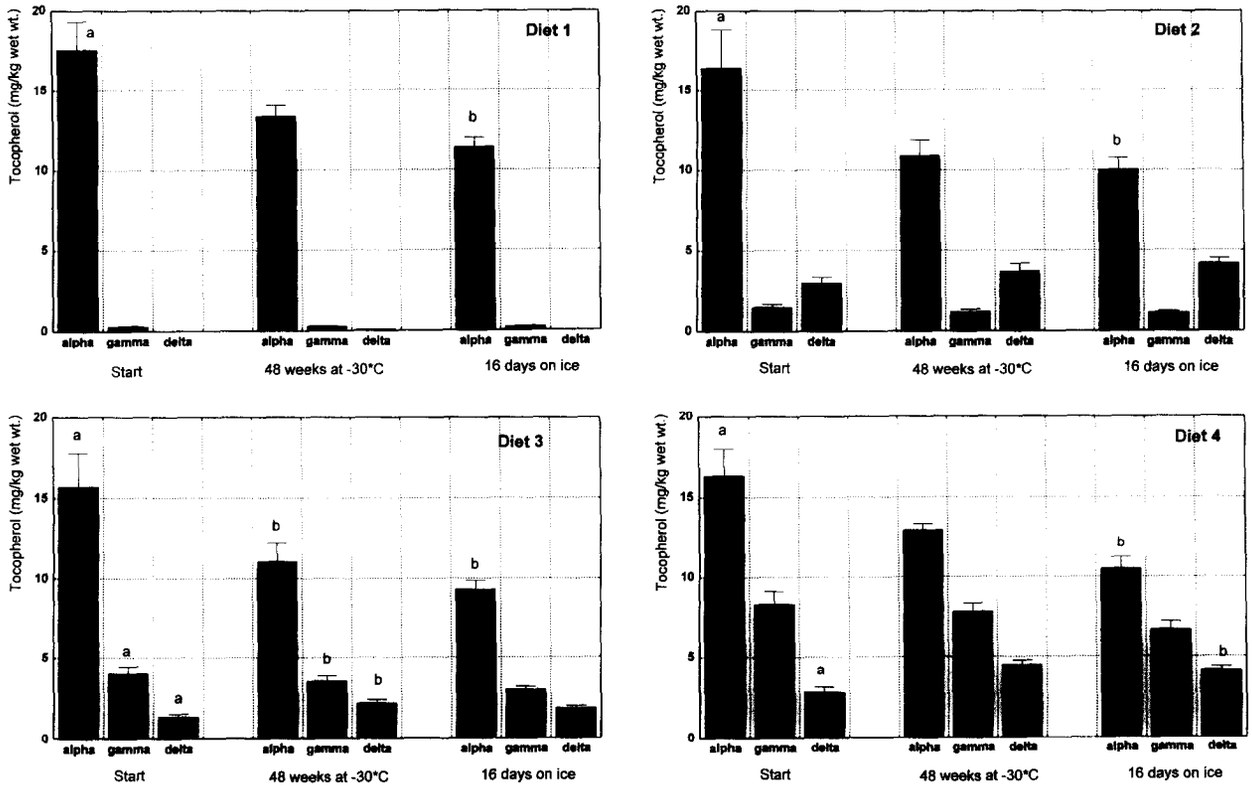

Fig. 2. Change in tocopherol (TOH) concentrations of Atlantic salmon fillet stored for 48 weeks at −30°C or for 16 days on ice. The fish were fed diets with different combinations of α-, γ- and δ-tocopherol prior to harvest (mg tocopherols added kg^{-1} dry diet: (a) Diet 1: 150 α. (b) Diet 2: 150 α + 100 δ. (c) Diet 3: 150 α + 50 γ + 50 δ. (d) Diet 4: 150 α + 100 γ + 100 δ. Different letters indicate significant change in concentration during storage (mean ± SEM, $n = 6$).

concentration and an increase in δ-tocopherol concentration ($p < 0.05$). No further changes in tocopherol concentration during frozen storage were detected.

In fillet stored on ice, increase in TBARS was detected on day 8 in fillet from fish supplemented with non-α-tocopherols ($p < 0.001$), and on day 17 ($p = 0.014$) in fish fed α-tocopherol as the only vitamin E source (Fig. 3). In fillet stored at −30°C, all groups showed a gradual and parallell increase in TBARS until 22 weeks ($p < 0.05$, Fig. 3). In week 36, fillet from fish fed diet 1, with α-tocopherol as the only vitamin E source, had higher TBARS levels than fish fed diet 4 with the highest levels of γ- and δ-tocopherol ($p < 0.05$). Fish fed diets 2 and 3 with intermediate levels of non-tocoperols had intermediate TBARS levels.

DISCUSSION

The present study confirms that γ- and δ-tocopherol are taken up by Atlantic salmon fillet. Uptake of γ-tocopherol occurs at the same rate as α-tocopherol, while δ-tocopherol appears to be retained at a lower rate than the other two. Similarly, Sigurgisladottir *et al.* (1994*a*) and Sigurgisladottir *et al.* (1994*b*) fed a diet supplemented with a natural tocopherol mix to Atlantic salmon and found uptake of the non-α-tocopherols in the fillet. Concentrations in fillet relative to feed were approximately one-third of those found in the present experiment. This can partly be explained by the difference in stability of free and esterified tocopherol in the digestive tract of salmon (Meland, 1995). Uptake of the non-α-tocopherols in the fillet contrasts with most other organs, which typically retain δ- and γ-tocopherol at < 10% and 20–40%, respectively, compared to α-tocopherol (Hamre and Lie, 1997). This gives an opportunity to produce Atlantic salmon fillet enriched in non-α-tocopherols which, according to the hypothesis of this study, may increase the oxidative stability during storage.

During storage on ice, there was an increase in concentration of δ-tocopherol in all groups. A similar trend appeared during frozen storage, but a significant increase was only found in fish fed diet 3. Since concentrations were analysed in wet tissue, this may be explained by a decrease in water content during storage. Assuming no change in fillet δ-tocopherol *content* in response to storage, would imply a somewhat larger reduction in concentration of α-tocopherol than what is actually shown by our results, and possibly a decrease of γ-tocopherol concentration. The results thus indicate that the tocopherols disappear at rates that correspond with their biological activity, i.e. $\alpha > \gamma > \delta$. This is in accordance with their peak oxidation potentials which have been reported as 860, 930 and 990 mV, respectively, versus a saturated calomel electrode (Itoh *et al.*, 1994), and with experimental results in as different systems as homogenous solutions, micelles, human lipoproteins and minced cooked turkey (Itoh *et al.*, 1994; Suarna *et al.*, 1993; Bruun-Jensen *et al.*, 1996).

The decreases in tocopherol concentrations during storage were quite modest, and similar to the results of Erickson and Thed (1994) working with tilapia, and with changes in tocopherol levels in frozen stored herring (Hamre, unpublished). Apparently, lipid oxidation was initiated in the presence of ample amounts of vitamin E, and there was no obvious link between change in fillet vitamin E concentration and accumulation of lipid oxidation products measured as TBARS. It is possible that most of the vitamin E was oxidized to form the tocopheroxyl radical during storage. The tocopheroxyl radical does not act as an antioxidant and is not distinguished from reduced tocopherol by our analytical method.

Changes in fillet TBARS levels indicate that γ- and δ-tocopherol protected frozen stored fillet against oxidation in a dose-dependent manner. It is not clear from the present experiment, whether this protection was specifically due to the presence of non-α-tocopherols, or just an effect of increasing the total tocopherol concentration. The relative antioxidant activity of tocopherol homologues has received much attention in the literature, and the results differ considerably. According to

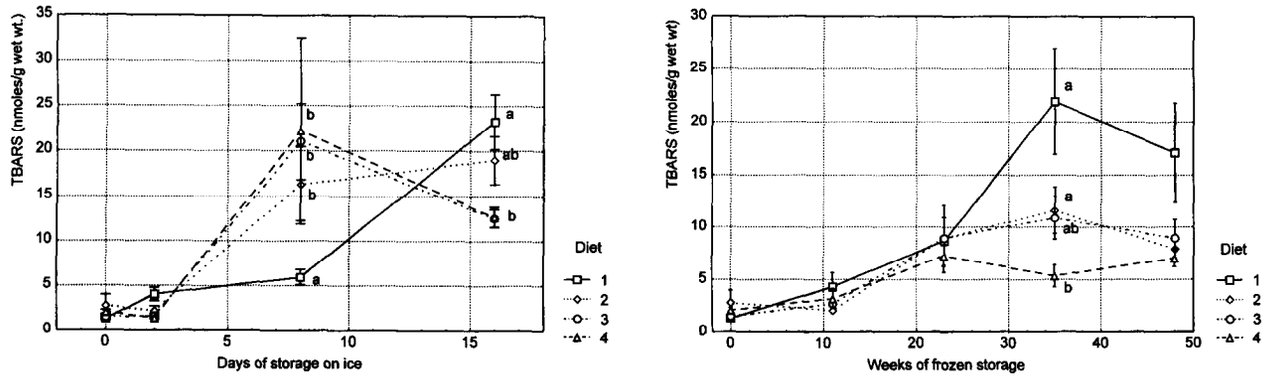

Fig. 3. Change in concentration of thiobarbituric acid reactive substances (TBARS) during (a) frozen storage and (b) storage on ice in Atlantic salmon fillet. The fish were fed diets with different combinations of α-, γ- and δ-tocopherol prior to harvest (mg tocopherols added kg^{-1} dry diet: Diet 1: 150 α. Diet 2: 150 α + 100 δ. Diet 3: 150 α + 50 γ + 50 δ. Diet 4: 150 α + 100 γ + 100 δ. Different letters indicate significant differences in TBARS levels between dietary groups (mean ± SEM, $n = 6$).

Frankel (1996) this is probably due to varying experimental conditions with respect to the unsaturated substrates used, oxidation temperature, and the methods used to measure oxidation. Changes in concentrations of individual tocopherol homologues in the present study, point to α-tocopherol as the most active antioxidant in Atlantic salmon fillets. The extra protection during frozen storage obtained by loading the fillet with γ- and δ-tocopherol, would then be due to the increased total tocopherol concentration. Additional experiments are, however, required to confirm this assumption. The results from frozen stored salmon are in agreement with previous studies that show improved oxidative stability of beef and fish fillet when supranutritional levels of vitamin E were fed prior to harvest (Schaefer *et al.*, 1995; Boggio *et al.*, 1985; Frigg *et al.*, 1990; Gatlin *et al.*, 1992; Sigurgisladottir *et al.*, 1994*a*,*b*).

In contrast to the results from frozen stored fillet, accumulation of TBARS in fillet stored on ice appeared to be stimulated by the presence of non-α-tocopherols. It is well known that tocopherols may act as prooxidants under certain conditions, especially when present at high concentrations. The concentration of total tocopherol in the fillet of fish supplemented with γ- and δ-tocopherol ranged from 20 to 30 mg kg^{-1} wet weight or 300–430 mg kg^{-1} lipid, which is low compared to concentrations that have been reported to produce prooxidant activity in oil (> 600 mg kg^{-1}; Frankel, 1996).

Taken together, the results from this study confirm that supplementation of high levels of vitamin E to Atlantic salmon before harvest improves oxidative stability of the fillet during frozen storage. They also show that α-tocopherol is retained more effectively in the fillet than δ-tocopherol, and indicate that α-tocopherol offers better protection against oxidation than γ- and δ-tocopherol. Both the non-α-tocopherols are lost from the body at a higher rate than α-tocopherol during fasting (Hamre *et al.*, 1998). Although this did not affect the relative fillet concentrations of α-, γ- and δ-tocopherol during the 4 days of fasting applied, longer fasting periods commonly used before slaughter of commercially farmed fish may well have such an effect. The preferred method to protect the fillet against lipid oxidation may therefore be by feeding high levels of α-tocopherol alone. The concentration of α-tocopherol in fillet increases linearly in response to feed concentration, and the time required for tissue concentrations to be in equilibrium with feed is approximately 3 months (Hamre and Lie, 1995; Hamre *et al.*, 1997*b*). The available data offer a possibilty to closely control the α-tocopherol level in Atlantic salmon fillet, thereby optimizing the oxidative stability.

ACKNOWLEDGEMENTS

The authors want to thank Thu Thao Nguyen, Idun Kallestad and Bjørn Netteland for excellent technical assistance. Financial support from the Norwegian Research Council is appreciated (Project no. 104617/110).

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