Tocopherols and Tocotrienols in Finnish Foods: Fish and Fish Products

E.-L. SYVÄOJA* and **K. SALMINEN**, Valio Finnish Co-operative Dairies' Association, Research and Development Department, Kalevankatu 56 B, SF-00180 Helsinki 18, Finland, and **V. PIIRONEN, P. VARO, O. KEROJOKI** and **P. KOIVISTOINEN**, Department of Food Chemistry and Technology, University of Helsinki, SF-00710 Helsinki 71, Finland

ABSTRACT

A high performance liquid chromatographic method with fluorescence detection was used to determine the content of tocopherols in Finnish fish species. Samples were obtained from different sea and lake areas. Autumn- and spring-caught fish were analyzed separately.

The vitamin E of all species consisted almost entirely of α-tocopherol. Muscle and roe contained relatively high levels of α-tocopherol. High- and medium-fat fish contained 2 mg and low-fat fish 1 mg/100 g on average. In high-fat fish the tocopherol:fat ratio was lower than in low-fat fish.

Between-species differences in tocopherol content were observed, as were between-sample differences within the same species. Fish caught in the spring, the spawning season of most species, had a higher tocopherol content (and a lower fat content) than those caught in the autumn. Marine fish had a higher tocopherol (and fat) content than lake fish of the same species.

INTRODUCTION

In marine animals α-tocopherol has been found to be the principal tocopherol (1-5). Each species has characteristic tocopherol levels in its tissue, which, since fish are unable to synthesize the vitamin, are related to diet (3). Watanabe et al. (6) reported the rapid accumulation by rainbow trout of dietary α-tocopherol and a very slow accumulation of β-, γ- and δ-tocopherol. There was no interconversion between α- and the other tocopherols.

Little information can be found in the literature on the tocopherol contents of the fish species common in Finnish lakes and seas. Ackman and Cormier (3) found 0.24 and 1.16 mg α-tocopherol/100 g light and dark fatty cod muscle, corresponding to 0.30 and 0.63 mg/g fat. The same authors' and McLaughlin and Weihrauch's (7) figures for the cod-related species pollack, haddock and ling were 0.8 mg/100 g. Leth (8) and Kovacs et al. (9) reported 0.45 mg/100 g in cod, and Granroth et al. (5) 1.2 mg/100 g in autumn Baltic herring. Higashi et al. (10) studied the vitamin E content of salmon and found 1.9 mg α-tocopherol/100 g. A single reported figure for rainbow trout was only 0.2 mg/100 g (7).

The study reported here is part of a research project to survey in detail the vitamin E content of Finnish foods. In the present study the tocopherol content of fish caught in Finnish lakes and sea areas for food was determined; fresh fillets, liver, roe and frozen fish were analyzed. In addition, seasonal effects were examined. The vitamin E content of some samples of preserved fish also was determined.

MATERIALS AND METHODS

Fish Samples

In the sampling process the object was to obtain a truly representative sample which would provide average compositional data for each fish species. For this purpose a sample of each species was taken from each Finnish sea area, and these samples were pooled. In the same way pooled samples of some lake fish species were obtained from the various important lake areas in southern and central Finland.

Sea fish were purchased during the spring (May-June) and autumn (October-November) fishing seasons from a wholesale dealer in Helsinki. The sea areas represented were the Archipelago, the Gulf of Finland and the Gulf of Ostrobothnia. Most of the lake fish were obtained during the autumn fishing season from local fish markets in Lahti, Lappeenranta, Mikkeli and Säkylä.

All fish were transported in ice to the laboratory. Each sample consisted of at least three whole fish and weighed 2-3 kg: each bulked sample thus weighed 6-9 kg (sea) or 4-9 kg (lake). The fish were scaled and cleaned. The skin was removed from burbot and cod only. One-half of each fish, consisting of fresh or thawed fillets, was ground with a Moulinex mixer and a portion of the well-mixed homogenate weighed out immediately. Any fillets which had to be stored before grinding were kept at −22 C in sealed bags free of air for no longer than 1 or 2 weeks. With each fish species sea and lake samples were analyzed separately. Sample processing was conducted in near-darkness.

The blast-frozen and the deep-frozen Baltic herring fillets were obtained from the manufacturer (Kalayhtymä, Raisio, Finland). The commercial deep-frozen and preserved fish foods were bought from five different supermarkets. The bulked samples were prepared from at least 8 tins or jars from the 4 to 5 manufacturers. Fish preserved in oil or marinade were drained completely before analysis.

Aliquots of homogenates of fish samples (10-20 g), ascorbic acid (1.2 g), distilled water (40 ml) and 99.5% ethanol (100 ml) were mixed under nitrogen in an erlenmeyer flask. After 30 min 10-20 ml potassium hydroxide (50%) was added and the flask was purged further with nitrogen. The sample was allowed to saponify at room temperature overnight using magnetic stirring (11). Then the tocopherols and tocotrienols were extracted with n-hexane (3×, 100 ml). The combined hexane extracts were washed with distilled water (3×, 100 ml) and solvent was evaporated. The tocopherols were dissolved in hexane, and an aliquot was injected into the HPLC column. Other details in the preparation of the samples have been reported (12). The optimum proportion of 50% KOH was tested with Baltic herring and found to be 10 ml/10 g fish, giving recoveries of added α-, β-, γ- and δ-tocopherol of 98, 96, 101 and 85%, respectively, for the entire analysis including saponification. δ-tocopherol probably would be partially degraded under these conditions, but this is of no consequence since, as will be evident from the following, this tocopherol was not detected in any fish.

High Performance Liquid Chromatography

The instrument was a Hewlett Packard Model 1084B liquid chromatograph equipped with a programmable variable-wavelength detector with scan capacity, autosampler and integrator. The 25 × 0.4 cm ID 5 μm LiChrosorb Si60 silica column (Merck) was preceded by a 4 × 0.4 cm ID 30-40 μm Perisorb A guard column (Merck). The reagents were

*To whom correspondence should be addressed.

those used by Piironen et al. (12). Elution was with 8% di-isopropylether in hexane for 3 min, then 8 to 17% in 22 min. The flow rate was 2.5 ml/min, the column temperature 30 C and the solvent reservoirs were maintained at 50 C. The column was washed with 30% di-isopropylether in hexane at 3.5 ml/min. The eluants were dried with Na_2SO_4, filtered through a Millipore 0.45 μm FH membrane and deaerated with the instrument's vacuum system, the vacuum being released through a $CaCO_3$ column.

The eluate was monitored with a Perkin Elmer M3000 variable fluorescence spectrometer, excitation 290 nm (slit 10) emission 325 nm (slit 15).

Preparative HPLC of Tocopherol Standards

Impure γ-tocopherol was a gift from Hoffman La Roche, and β- and δ-tocopherols were components of Eastman vitamin E 4-50 mixed tocopherols (Eastman Chemical Products Inc., Kingsport, Tennessee). Pure material for use as tocopherol standards was obtained with the HP 1084B, a 25 × 1 cm ID 7 μm LiChrosorb Si60 column (Hewlett Packard) and 100-200 μl injections. The column (30 C) was eluted with 5% di-isopropylether in hexane at 5-10 ml/min and the eluate monitored at 295 nm. The tocopherol fractions were rechromatographed and concentrated, once or several times, to 99-100% purity, as measured with the analytical column. The pure preparations were sealed in ampules under nitrogen and stored at −22 C.

Identification and Quantitation

The tocopherols were identified by their retention times and their UV-visible spectra recorded with the HP variable-wavelength detector. Quantitation was based on fluorescence peak areas of tocopherol standards. Tocotrienols were quantified with the corresponding tocopherol standards (13). Portions of standard mixture were injected and regression equations of the following form calculated by means of a (Wang 2200 MVP) computer: $Y = A + BX$, where Y = amount of tocopherol/injection (μg/μl); X = peak area (as given by the integrator); A,B = constants characteristic for each tocopherol analyzed under the particular conditions.

RESULTS AND DISCUSSION

Typical separations of tocopherols in standards and fish samples are shown in Figure 1A and B. All tocopherols as well as tocotrienols could be separated in less than 30 min.

Tocopherol levels in fish from sea areas caught in the spring and autumn fishing seasons and from lake areas caught in the autumn fishing season are given in Table I.

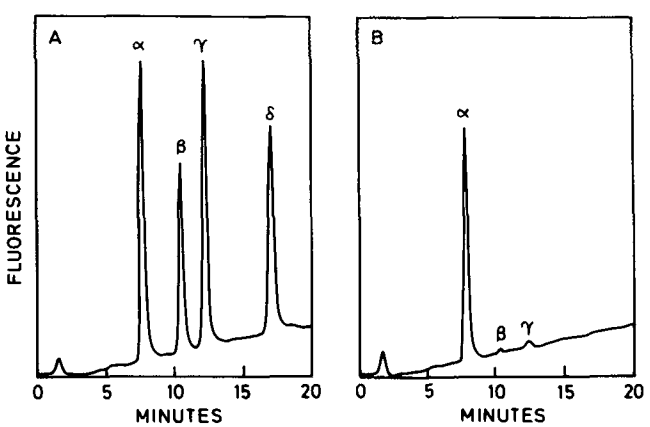

FIG. 1. HPLC separation of α-, β-, γ-, and δ-tocopherols in a standard mixture (A) and a typical fish sample (B).

TABLE I

The Tocopherol (α-T, β-T, γ-T) and Fat Content of Fillets of Sea Fish Caught in the Spring and Autumn Fishing Seasons and Lake Fish Caught in the Autumn Fishing Season

Fish[a]	Spring sea fish					Autumn sea fish					Autumn lake fish				
	α-T mg/100 g	β-T mg/100 g	γ-T mg/100 g	Fat g/100 g	α-T/fat mg/g	α-T mg/100 g	β-T mg/100 g	γ-T mg/100 g	Fat g/100 g	α-T/fat mg/g	α-T mg/100 g	β-T mg/100 g	γ-T mg/100 g	Fat g/100 g	α-T/fat mg/g
Burbot	0.85	–	0.01	0.76	1.12	0.75	–	0.01	0.78	0.96	–	–	–	–	–
Pike	1.01	–	–	0.75	1.35	0.80	–	–	0.73	1.10	0.66	–	–	0.81	0.81
Cod	1.05	–	–	0.85	1.24	0.95	–	–	0.62	1.53	–	–	–	–	–
Perch	1.50	0.01	0.01	1.02	1.47	1.44	–	–	0.61	0.89	0.89	–	–	1.18	0.75
Pikeperch	1.35	–	–	1.28	1.05	1.16	–	–	1.27	0.91	–	–	–	–	–
Baltic herring	2.45	0.01	0.01	6.90	0.36	1.48	–	0.01	7.44	0.20	–	–	–	–	–
Bream	2.91	–	–	6.80	0.43	2.30	–	–	7.40	0.31	1.08	–	–	4.14	0.26
Vendace[b]	1.96	–	–	8.00	0.25	–	–	–	–	–	1.21	–	–	5.22	0.23
Vendace	1.45	–	0.02	3.62	0.40	–	–	–	–	–	–	–	–	–	–
Whitefish	2.66	0.01	0.04	5.99	0.44	2.68	–	0.04	7.42	0.36	2.63	0.01	0.03	4.26	0.62
Salmon	2.02	–	0.02	12.50	0.16	2.43	–	0.01	12.60	0.19	–	–	–	–	–
Rainbow trout[c]	1.89	0.01	0.07	9.94	0.19	1.43	–	0.02	16.60	0.09	–	–	–	–	–

[a]Burbot (*Lota lota*), Pike (*Esox lucius*), Cod (*Gadus morhua callarias*), Perch (*Perca fluviatilis*), Pikeperch (*Stizostedion lucioperca*), Baltic herring (*Clupea harengus membras*), Bream (*Abramis brama*), Vendace (*Coregonus albula*), Whitefish (*Coregonus* spp.), Salmon (*Salmo salar*), Rainbow trout (*Salmo gairdneri*).

[b]Lakefish.

[c]Cultivated; contained traces of α-tocotrienol.

Tocopherol contents of the some roe and liver samples are shown in Table II. The results are the mean values of 2 to 6 determinations.

96.5-100% of the total tocopherol of muscle, roe and liver consisted of α-tocopherol. Only traces or small amounts of β- and γ-tocopherols were found. Rainbow trout was exceptional in being the only one found to contain α-tocotrienol.

There were considerable within and between-species differences in tocopherol content. The greatest within-species variation was found in bream (0.99-2.91 mg α-tocopherol/100 g fresh weight) and Baltic herring (1.3-3.05 mg), and the smallest in whitefish (2.63-2.68 mg), cod (0.95-1.05 mg) and burbot (0.75-0.85 mg). Low-fat fish showed less within-species variability in tocopherol content than did high-fat fish.

The lower the fat content of the fish the lower was the α-tocopherol content. Levels of 1.03 ± 0.08 (mean ±S.E.) mg per 100 g on average were found in the low-fat fish (pike, burbot, perch, pikeperch and cod), in which the total fat content was 0.97 ± 0.09 g/100 g fillet. The medium- (bream, whitefish, Baltic herring and vendace) or high-fat species (salmon and rainbow trout), in which the total fat contents were on average 6.32 ± 0.36 and 12.83 ± 1.07 g/100 g fillet respectively, had levels averaging 1.98 ± 0.13 and 1.96 ± 0.16 mg α-tocopherol/100 g. Farmed rainbow trout was exceptional, having the highest fat content recorded in this study (average 13.4 g/100 g) but only 1.66 mg α-tocopherol/100 g. Also, it has been observed that low-fat fish had lower levels of tocopherol than high-fat fish (3,4,8).

Viewed as a component of fat or total lipid, tocopherol had its highest levels in low-fat fish: on average 1.1 mg/g fat, with 0.3 mg in the medium-fat and 0.16 mg in the high-fat species.

In almost all species tocopherol contents in spring-caught fish were higher than in autumn-caught. Of the sea fish, pike, pikeperch, bream, burbot and cod caught in the autumn have a 10-20% lower, and Baltic herring almost a 40% lower, tocopherol content than in the spring (Table I). In the salmon alone the tocopherol content was higher in the autumn than in the spring. Of the freshwater fish, vendace had an autumn season level some 40% lower than that in the spring. This species, however, showed the same seasonal variation in fat content so that the tocopherol:fat ratio in the autumn was the same as that in the spring. Generally speaking, in spite of the higher fat content, the tocopherol content and thus the tocopherol:fat ratio (mg/g fat) were lower in the autumn. Since most fish spawn in the spring, it can be concluded that tocopherol levels reach their peak during the spawning season. (In the autumn-spawning species the tocopherol contents in the autumn were higher than [in the case of salmon] or the same as [whitefish] those in the spring.) Hardy and Mackie (14) reached the same conclusion, suggesting that the high levels result from the need to slow down fat metabolism. Seasonal variation in tocopherol level has been observed as well (3). Evidently other seasonal factors, such as dietary composition and temperature, play a part. Sexual maturity may have considerable effects on tocopherol content at different seasons of the year.

Marine and freshwater fish of the same species had different tocopherol contents. The α-tocopherol content of marine fish in every group (low-, medium- and high-fat species) was higher than that of freshwater fish (Table I). The levels in perch, bream and pike caught in fresh water during the same autumn season were, respectively, 40, 50 and 20% lower than those caught in the sea. Sea and lake whitefish had the same tocopherol content, while lake vendace had a higher content than sea vendace caught at the same time. The lower levels in lake fish (the exception was whitefish with no marine vs. freshwater difference) were due, in part at least, to their lower fat contents. This is a reflection of nutrient supply, which in Finnish lakes is generally inferior to that in the sea (15). According to Love (16), the contents of other vitamins, too, such as ascorbic acid, pyridoxine and niacin, are lower in lake fish than in marine fish.

The α-tocopherol content in liver and roe was considerably higher than in muscle (Table II). The same was found by Ackman and Cormier (3) and McLaughlin and Weihrauch (7) for cod liver (22 and 16 mg/100 g), Watanabe et al. (6) for rainbow trout liver (1.7-6.1 mg/100 g) and Leth (8) for cod roe (10 mg/100 g).

Since many of the tocopherol analyses reported here had to be made on stored frozen fillet samples, the effect of storage (at −22 C) on the tocopherol content of blast frozen fish fillet was studied. The results (Table III) showed that the tocopherol content of the Baltic herring was well maintained for two mo and then began to decrease slowly. After six mo, more than 70% of the tocopherol content of fish was still present. Other causes of variation in tocopherol content were changes in fat content and also the sex of the fish and their sexual maturity. Stored subsamples, that means 250 g blast-frozen fish fillets, were not equal in those respects.

The results of analyses on commercial deep-frozen and preserved fish foods are shown in Table IV. The date of preparation was not stated on the package, but at least the deep-frozen Baltic herring had a tocopherol content very close to that of the fresh, spring-caught fish. The oil used with the preserved herring and sprat had a major effect on the tocopherol content of the fish product, β-, γ- and δ-tocopherol originating entirely from the oil.

The significance of fish as a source of vitamin E depends naturally on fish consumption. This, according to statistics published by the Finnish Game and Fisheries Research Institute, was 26 kg per person per year in Finland in 1982. Thus, the daily supply of the vitamin will be about 1.0 mg per person. This represents about 10% of the total dietary

TABLE II

Tocopherol Content of Fish Roe and Liver

Sample	α-T mg/100 g	β-T mg/100 g	γ-T mg/100 g	Fat g/100 g	α-T/fat mg/g
Roe vendace	8.9	–	–	14.70	0.61
Roe whitefish	17.37	–	–	12.70	1.37
Roe Baltic herring	5.04	–	–	3.28	1.54
Liver burbot	16.14	–	–	48.08	0.34

TABLE III

Effect of Storage (−22 C) on the α-tocopherol Content of Blast-Frozen Baltic Herring Fillets

Months of storage	α-T mg/100 g	Fat g/100 g	α-T/fat mg/g
0	2.39	5.64	0.42
0.5	2.62	5.55	0.47
1.5	2.11	6.96	0.30
2	2.79	8.01	0.35
3	1.89	5.25	0.36
4.5	1.78	7.07	0.25
6	1.91	7.16	0.27

TABLE IV

Tocopherol Contents of Deep-Frozen Fillets and Preserved Fish Products

Sample	Tocopherols mg/100 g				Fat g/100 g
	α-T	β-T	γ-T	δ-T	
Pollack, deep-frozen	0.78	–	–	–	0.84
Red fish, deep-frozen	0.90	–	–	–	4.56
Baltic herring, deep-frozen	2.89	–	–	–	8.11
Baltic herring fish fingers	2.55	–	3.54	–	14.10
Herring, marinated	1.55	tr	–	–	9.16
Sprat, in oil, preserved	2.31	0.04	3.80	1.18	19.00
Sprat, preserved	2.51	–	–	–	9.91

vitamin E measured with Finnish men (17) and also about 10% of the 1980 Recommended Daily Allowance (18).

Fish consumption varies widely over the different parts of Finland and seasons of the year. It is higher along the coasts and in the lake areas than in other parts of the interior, and higher in the east than in the west. Certain Lapps may eat as much as 300 g of fish daily (19), which will provide no less than one half of the total recommended allowance of vitamin E.

ACKNOWLEDGMENT

This study was supported financially by the Foundation for Nutrition Research, Finland.

REFERENCES

1. Pennock, J.F., R.A. Morton and D.E.M. Lawson, Biochem. J. 73:4 (1959).
2. Braekkan, O.R., G. Lambertson and H. Myklestad, Fiskeridir. Skr. Ser. Teknol. Unders. 4:1 (1963).
3. Ackman, R.G., and M.G. Cormier, J. Fish. Res. Board Can. 24:357 (1967).
4. Engelhardt, F.R., J.R. Geraci and B.L. Walker, Ibid. 32:807 (1975).
5. Granroth, B., A. Mustranta and H. Boström, Turkistalous 11:472 (1977).
6. Watanabe, T., M. Wada, T. Takeuchi and S. Arai, Bull. Jap. Soc. Sci. Fish. 47:1455 (1981).
7. McLaughlin, P.J., and J.L. Weihrauch, J. Am. Diet. Assoc. 75:647 (1979).
8. Leth, T., Husholdningsrådets Tekniske Meddelelser 1:21 (1975).
9. Kovacs, M.I.P., R.G. Ackman and P.J. Ke, J. Can. Diet. Assoc. 39:178 (1978).
10. Higashi, H., K. Terada and T. Nakahira, Bitamins 45:113 (1972), Ref. Chem. Abstr. 77:284 (1972).
11. Mankel, A., Dtsch. Lebensm. Rundsch. 75:77 (1979).
12. Piironen, V., P. Varo, E.-L. Syväoja, K. Salminen and P. Koivistoinen, Intern. J. Vit. Nutr. Res. 54:35 (1984).
13. Thompson, J.N., and G. Hatina, J. Liq. Chromatog. 2:327 (1979).
14. Hardy, R., and P.R. Mackie, J. Sci. Food Agric. 20:193 (1969).
15. Koli, L., Suom. Luonto 40:232 (1981).
16. Love, R.M., The Chemical Biology of Fishes, Vol. 2, Academic Press, London and New York, 1980, p. 947.
17. Piironen, V., P. Varo, E.-L. Syväoja, K. Salminen, P. Koivistoinen and H. Arvilommi, Intern. J. Vit. Nutr. Res. 54:41 (1984).
18. Food and Nutr. Bd. Recommended Dietary Allowances, 9th rev. edn., Natl. Acad. Sci., Washington, DC, 1980, p. 63.
19. Pekkarinen, M., Suom. Luonto 40:220 (1981).

[Received December 14, 1984]

Study of Oxidation by Chemiluminescence. IV. Detection of Low Levels of Lipid Hydroperoxides by Chemiluminescence

Y. YAMAMOTO, E. NIKI*, R. TANIMURA and Y. KAMIYA, Department of Reaction Chemistry, Faculty of Engineering, University of Tokyo, Hongo, Tokyo 113, Japan

ABSTRACT

Sodium hypochlorite (NaOCl) induced decomposition of organic hydroperoxides gave strong chemiluminescence. Chemiluminescence intensity reached its maximum a few seconds after the addition of sodium hypochlorite and decreased to the background level in three min. Good linear relationships were observed between total chemiluminescence counts in three min and the amounts of hydroperoxides. This chemiluminescence method can be applied to the detection of low levels of lipid hydroperoxides.

INTRODUCTION

The oxidation of lipids has received renewed attention recently in connection with the deterioration of foods and oils and with peroxidation in biological systems (1,2). Lipid hydroperoxide is the primary product of lipid oxidation. Therefore, the detection of low levels of lipid hydroperoxides is very important to estimate the progress of oxidation. However, a convenient method has not been established that is sensitive enough for the analysis of trace amounts of lipid hydroperoxide.

It has been observed that weak chemiluminescence arises during the autoxidation of many organic compounds (3) and biological molecules (4). We have reported recently (5-8) that the chemiluminescence arises from the radical-induced decomposition of hydroperoxides and that this method can be applied to estimate antioxidant activity and extent of oxidation. The chemiluminescence method is a very sensitive method, and its application has been studied (4,7,9,10). In the course of our study on chemiluminescence, we extended this method to the detection of low levels of lipid hydroperoxides.

*To whom correspondence should be addressed.

EXPERIMENTAL PROCEDURES

Materials

Tetralyl hydroperoxide (TOOH) was prepared by the autoxidation of tetraline and recrystallized from pentane *tert*-Butyl hydroperoxide (BOOH) and di-*tert*-butyl peroxide (BOOB) were distilled under reduced pressure. Methyl linoleate hydroperoxide (18:2 LOOH) and methyl linolenate hydroperoxide (18:3 LOOH) were prepared by the oxidation of methyl linoleate and methyl linolenate, respectively, with soybean lipoxygenase and purified with silica gel column chromatography, using hexane and ether as eluents. The purity of hydroperoxides was determined by TLC and HPLC (11). 18:2 LOOH and 18:3 LOOH gave only one spot on TLC, indicating that they were free from methyl linoleate and linolenate. It was found by HPLC analysis that methyl 13-hydroperoxy-9-*cis*,11-*trans*-octadecadienoate (12) and methyl 13-hydroperoxy-9-*cis*,11-*trans*,15-*cis*-octa-