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α-Tocopherol content in trout oil

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The content of α-tocopherol in 40 trout oils of the species *Oncorhynchus mykiss* (rainbow trout), corresponding to different growth stages is studied by means of high-performance liquid chromatography with UV detection. No significant difference was found ($P > 0.05$) between sexes in adult samples, while the growth stage significantly ($P < 0.05$) influenced the content of α-tocopherol, the highest value corresponding to the youngest trout. All oils exceed the 0·4 mg α-tocopherol/g PUFA recommended by the National Research Council, Washington, DC, USA.

INTRODUCTION

Many epidemiological studies have attempted to determine the consequences of fish-oil-rich diet and its possible role in preventing cardiovascular diseases (Deslypere, 1992; Capron, 1993).

Fish oil has appreciable amounts of certain long-chain polyunsaturated fatty acids of the *n*-3 series (LC-PUFA, *n*-3) such eicosapentaenoic (EPA, $C_{20:5}$) and docosahexaenoic (DHA, $C_{22:6}$), which are responsible for the beneficial effects on lipoproteic metabolism and eicosanoid formation. They also give a protective effect against atherosclerosis (Kinsella, 1990; McNamara, 1992).

Nevertheless, an excessive increase of LC-PUFA in the diet, can produce negative effects, due to their oxidation. The intake of oxidised oils could transform all beneficial effects into a threat to health (Fischer, 1990).

Therefore, it is necessary to monitor the quality of fish oil, giving special attention to its natural antioxidant content, e.g α-tocopherol. An increase in LC-PUFA consumption should be accompanied by an increase in vitamin E consumption (British Nutrition Foundation, 1992).

This paper, together with other studies carried out in the authors' Department (Satué *et al.*, 1994), extends our knowledge of the rainbow trout (*Oncorhynchus mykiss*) lipid fraction. The purpose of this study is to assess the α-tocopherol content in trout oil, considering the influence of age and sex.

Tocopherol analysis has been performed by different methods: spectrophotometric, thin layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC); this latter technique has predominated recently. Here, a reversed-phase HPLC liquid chromatography method with UV detection is developed.

Also evaluated is the ratio mg of α-tocopherol/g PUFA in trout oil, which must be sufficient to guarantee the protection against oxidation.

MATERIALS AND METHODS

Samples

Forty samples of trout provided by a fish farm were analysed. The trout were all fed on the same diet and were captured in December 1991 at the same pond to avoid seasonal and habitat differences. Fish were put in polyethylene bags, immediately frozen and stored at –20°C until analysed. The samples were divided into four groups depending on their size and sex.

- Group A: 6 trout of 15–35 g
- Group B: 11 trout of 140–200 g
- Group C: 12 female trout (0·63–2·63 kg)
- Group D: 11 male trout (1·05–2·40 kg)

Analysis

Oil extraction

The trout were defrosted, gutted, and the head, skin and bones removed. The flesh was then ground with an electric grinder and samples were kept under nitrogen and protected from light during the extraction process. Fat was extracted with chloroform–methanol (2 : 1, v/v) using the Folch–Lees method (Folch *et al.*, 1957). The oil obtained was kept at –20°C and sealed under nitrogen in dark-glass vials until analyses were performed. Total fat content in samples was determined by the Soxhlet method.

Fatty acid analysis

Fatty acids were methylated using boronfluoride/methanol (BF_3-MeOH) following the technique described by Morrison and Smith (1964) and a 5 μl aliquot of the methyl esters formed was injected in a Perkin–Elmer Sigma 300 GC equipped with a flame-ionisation detector (FID) and a cyanosilicone capillary column SP-2330 (Supelco) of 30 m length × 0·25 mm i.d. × 0·20 μm film thickness. Signals were integrated with a HP3396A (Hewlett Packard) integrator. The oven temperature program ranged from 160°C (hold for 1 min) to 260°C (hold 3 min) with a rise of 4°C/min. Injector and detector temperatures were both set at 260°C.

Fatty acids were identified by relative retention times comparing with adequate standards; quantitation was based on an internal standard method, using methyl laurate as internal standard. More detailed information about this method can be found in a paper published previously (Satué *et al.* 1994).

Tocopherol analysis

The method proposed in this paper is a modification of a protocol developed by Zonta and Stancher (1983) and consists in an alkaline hydrolysis at room temperature with continuous stirring for 12–14 h; afterwards, the insaponifiable matter is extracted with ethyl ether, concentrated and redissolved in methanol.

Qualitative analysis of α-tocopherol content in samples was performed in a Perkin–Elmer Series 3 LC with a Perkin Elmer LC55B UV detector set at 294 nm. Signals were integrated with an HP3390A.

The analytical column used was a Spherisorb ODS2 (250 mm × 4·6 mm i.d. × 5 μm particle size, Tracer Analitica). Isocratic elution was carried out with methanol/water (95 : 5, v/v) at a flow rate of 1 ml/min.

For quantitative analysis, a calibration curve was calculated by linear regression with a constant amount of δ-tocopherol (internal standard), not present in our samples, combined with concentrations of the α-tocopherol standard ranging from 0 to 500 μg/ml; a correlation coefficient of 0·9999 was obtained. The internal standard was always analysed along with the samples and peak area ratios were used for calculation following the internal standard method.

The Statgraphics package (STSC Inc. and Statistical Graphics Corporation, version 4.0) was used for statistical treatment of results.

RESULTS AND DISCUSSION

Figure 1 shows a typical fatty acid chromatogram of trout oil. Twenty-four fatty acids were separated and identified, from C14 : 0 (myristic acid) to C22 : 6, *n*-3 (docosahexaenoic acid).

Figure 2 shows a typical tocopherol chromatogram of trout oil. The α-tocopherol was well separated from the internal standard, δ-tocopherol.

In regard to the suitability of the proposed method for α-tocopherol analysis, a detection limit of 50 ng

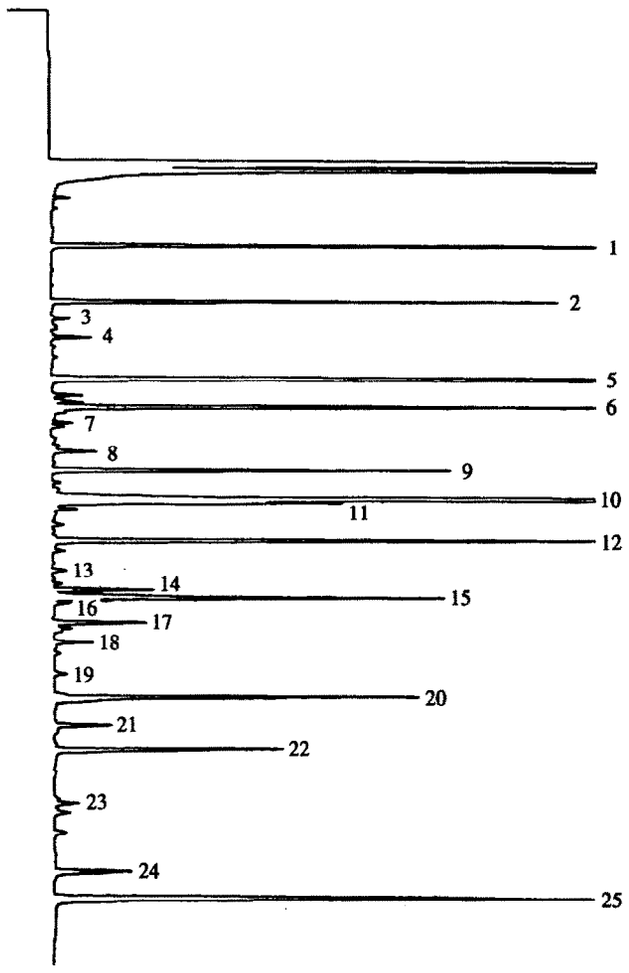

Fig. 1. Chromatogram of the fatty acid profile of a trout oil. Peaks 1, C12 : 0 (internal standard); 2, C14 : 0; 3, C15 : 0; 4, C15 : 1; 5, C16 : 0; 6, C16 : 1 *n*-7; 7, C17 : 0; 8, C17 : 1*n*-8; 9, C18 : 0; 10, C18 : 1 *n*-9; 11, C18 : 1 *n*-7; 12, C18 : 2 *n*-6; 13, C20 : 0; 14, C18 : 3 *n*-3; 15, C20 : 1 *n*-9; 16, C20 : 1 *n*-7; 17, C18 : 4 *n*-3; 18, C20 : 2 *n*-6; 19, C22 : 0; 20, C22 : 1 *n*-11; 21, C20 : 4 *n*-3; 22, C20 : 5 *n*-3; 23, C22 : 4 *n*-6; 24, C22 : 5 *n*-3; 25, C22 : 6 *n*-3.

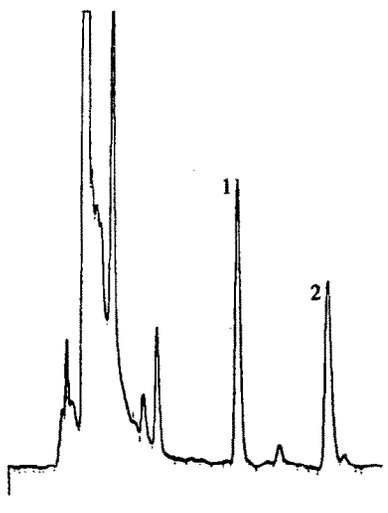

Fig. 2. Chromatogram of trout oil showing internal standard δ-tocopherol (1) and α-tocopherol (2) peaks.

ensures a good sensitivity, according to the criteria established by Kateman and Pijpers (1981). Repeatability was measured by calculating standard deviation (σ_{n-1} = 4·85) and variation coefficient (CV = 3%) of 10 repeated analyses. The CV was lower than that calculated from the formula proposed by Horwitz (1982) for intra-laboratory analysis and compounds present in a ppm range.

The standard addition method was used to test the accuracy; five levels of α-tocopherol standard were added to the samples in order to obtain the recovery for the five different levels of concentration. The study was run with two replicate samples injected twice each. The mean recovery was 95·69% (Table 1).

Table 2 displays values of the fatty acid as well as vitamin E content of the trout oil.

The results show no significant differences in α-tocopherol content ($P > 0{\cdot}05$) between sexes in the adult samples, while there are differences ($P < 0{\cdot}05$) between growth stages. The youngest trouts showed the highest α-tocopherol content (X = 350 μg/g), followed by the medium ones and the adult females and males (Table 2). The enzymatic systems responsible for free radical scavenging deteriorate with aging. According to the studies which show that antioxidant addition expands life expectation (Harman, 1988), it is not surprising to find higher α-tocopherol content in the youngest specimens.

Finally, the ratio of mg α-tocopherol/g PUFA shows a diminishing trend with age (Table 2), despite the reduction of PUFA along with the α-tocopherol. This ratio in small trout is significantly higher ($P < 0{\cdot}05$) than in the trout that are considered of suitable size for consumption. The mean values for the three groups included in this category, are close but we still found statistically significant ($P < 0{\cdot}05$) differences between sexes and between medium trout and males. There are no statistically significant ($P > 0{\cdot}05$) differences between

Table 1. α-Tocopherol recovery

α-Tocopherol (μg/ml added)	Recovery[a] X% (n = 2)
79·1	97·8
95·5	95·7
143	98·5
191	96·4
382	90·1

[a]Mean, 95·69%; SD, 3·32; CV, 3·47%.

Table 2. Fatty acid, α-tocopherol content and α-tocopherol/ PUFA ratio in the four groups of trout oil analysed (mean ± SD)

	Group A (n = 6)	Group B (n = 11)	Group C (n = 12)	Group D (n = 11)
Total fat (g)	12·16 (4·63)	20·78 (6·23)	39·40 (8·36)	29·31 (6·70)
Fatty acids (mg/kg)				
C14 : 0	36·0 (1·69)	41·5 (1·66)	41·3 (4·33)	33·7 (5·09)
C15 : 0	1·67 (0·13)	2·18 (0·14)	1·86 (0·28)	1·68 (0·21)
C16 : 0	157·45 (7·47)	157 (9·12)	181 (10·78)	150·19 (14·70)
C17 : 0	2·60 (0·38)	2·86 (0·25)	2·50 (0·40)	2·17 (0·36)
C18 : 0	29·5 (1·38)	28·5 (2·04)	38·3 (2·91)	31·2 (4·04)
C20 : 0	2·55 (0·21)	2·35 (0·18)	2·05 (0·36)	2·18 (0·12)
C22 : 0	3·67 (0·34)	3·17 (0·34)	2·89 (0·44)	3·19 (0·58)
Total saturates	233 (9·57)	238 (12·84)	70·6 (17·47)	224 (21·46)
C15 : 1	3·49 (0·80)	4·16 (0·29)	4·01 (0·41)	3·35 (0·59)
C16 : 1, n–7	43·8 (3·28)	47·9 (3·15)	63·0 (6·15)	54·3 (7·81)
C17 : 1	5·06 (0·68)	5·98 (0·57)	5·45 (0·86)	4·86 (0·65)
C18 : 1, n–9	178 (7·43)	178 (10·31)	221 (16·40)	211 (17·77)
C18 : 1, n–7	28·0 (1·99)	26·7 (1·66)	26·3 (8·22)	26·3 (1·96)
C20 : 1, n–9	46·9 (1·92)	50·9 (3·37)	48·8 (3·57)	57·1 (4·32)
C20 : 1, n–7	2·00 (0·13)	2·14 (0·26)	2·49 (1·26)	2·68 (0·05)
C22 : 1, n–11	50·6 (3·28)	56·0 (5·03)	49·1 (4·36)	53·6 (5·65)
Total monounsaturates	357 (14·29)	238 (20·96)	419 (20·34)	411 (19·68)
C18 : 2, n-6	90·9 (4·29)	88·5 (3·59)	69·7 (5·83)	92·6 (8·68)
C18 : 3, n-3	13·4 (0·52)	16·1 (0·67)	12·7 (1·21)	14·4 (1·54)
C18 : 4, n–3	11·6 (1·21)	13·2 (0·83)	10·8 (1·02)	9·70 (2·93)
C20 : 2, n–6	6·26 (0·32)	6·66 (0·37)	5·46 (0·56)	7·12 (0·69)
C20 : 4, n–6	7·92 (0·57)	10·7 (0·72)	8·74 (1·37)	8·77 (0·90)
C20 : 5, n–3	34·5 (3·57)	36·2 (1·78)	31·7 (2·44)	33·4 (2·64)
C22 : 4, n–6	5·07 (0·63)	5·13 (0·50)	5·06 (2·12)	4·56 (0·77)
C22 : 5, n–3	12·2 (0·98)	11·9 (1·26)	12·9 (2·05)	19·7 (3·95)
C22 : 6, n–3	172 (23·47)	134 (12·68)	106 (15·03)	140 (21·10)
Total polyunsaturates	353 (30·34)	322 (18·51)	266 (24·53)	330 (34·02)
Tocopherols (μg/g)				
α-tocopherol	359 (28·92)	224 (31·18)	190 (32·28)	191 (31·95)
mg α-tocopherol/g PUFA	1·02 (0·08)	0·69 (0·08)	0·71 (0·09)	0·58 (0·09)

medium trout and females, which can be explained considering the higher depletion of PUFA in females, giving a higher ratio even though vitamin E content is lower than in group B. Nevertheless it should be pointed out that all groups exceed the RDA (0·4 mg α-tocopherol/g PUFA) (NRC, 1989); therefore fish oil can be considered nutritionally adequate with respect to vitamin E/fatty acids.

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