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Fatty acid composition of trout oil

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Fatty acid analysis of oils from farmed trout of the species *Oncorhynchus mykiss* was carried out by gas–liquid chromatography with a flame ionisation detector (FID). Results show an influence of trout age and sex in fatty acid composition of the oils analysed. Trout oil has more docosahexaenoic acid (DHA) than eicosapentaenoic acid (EPA) and, while the EPA content does not change during growth, DHA diminishes during trout development, especially in females. In the group of medium sized trout (140–200 g), the ratios of SFA:MUFA:PUFA are similar to those recommended by the American Heart Association.

INTRODUCTION

Due to its fat content and the ease with which it can be farmed, the trout is an important source of *n*-3 series long-chain polyunsaturated fatty acids (LC-PUFA).

Many studies indicate the importance of these fatty acids in the prevention of cardiovascular diseases (Kinsella *et al.* 1989; Kelly, 1991; Deslypere, 1992; McNamara, 1992; Shrapnel *et al.*, 1992; Capron, 1993) and show them to be useful in different autoimmune and inflammatory disorders (Bjerve *et al.*, 1989; Bjorneboe *et al.*, 1989; Dianzani, 1989; Lee *et al.*, 1991; Karmali, 1992; Von Schacky *et al.*, 1993). They have also been given a significant role in the correct development of foetal and newborn brain and retina; some authors have proposed them as essential in this growth state (Koletzko, 1992; Crawford, 1992).

The muscle fatty acid composition of the species *Oncorhynchus mykiss* (rainbow trout) and its variations related to growth stage and sex was studied with the aim of improving its exploitation.

MATERIALS AND METHODS

Samples

All samples were from a fish farm and they were fed on the same diet. They were classified into four groups depending on age and sex:

Group A: trouts of 15–35 g
Group B: trouts of 140–200 g
Group C: female trouts of 0·63–2·63 kg
Group D: male trouts of 1·05–2·40 kg

A total of 54 samples were studied: 11 in group A, 12 in group B, 16 in C and 15 in D.

Methods

The first step was sample preparation: the trouts were gutted, the skin and bones were removed and the muscle was ground with an electric grinder.

In order to extract the muscle fat, the Folch-Lees method (Folch *et al.*, 1957), which consists in an extraction with a mixture of chloroform–methanol (2:1, v/v), was used. The fat obtained was kept frozen at −20°C in dark-glass bottles under nitrogen.

For quantification, fat was extracted from muscle by the Soxhlet method. For fatty acid analysis, methyl ester derivatives were obtained using boronfluoride–methanol (BF_3-MeOH) as derivatisation agent, and the esters formed were separated with hexane, as described by Morrison and Smith (1964).

Separation was carried out in a gas–liquid chromatograph Perkin Elmer Sigma 300, equipped with a flame ionisation detector (FID) and a cyanosilicone capillary column SP 2330 (Supelco) of 30 m length × 0·25 mm i.d. and 0·20 μm film thickness. The chromatograph was connected to an HP 3396 A (Hewlett Packard) integrator.

The analysis was performed through a temperature programme ranging from 160°C (hold 1 min) to 260°C (hold 3 min) with a rise of 4°C/min. Injector and detector temperatures were both 260°. The carrier gas (helium) flow measured at the end of the column was 0·83 ml/min, and the split ratio was 1:108. The volume injected was 0·5 μl.

Fatty acid standards were purchased from Sigma with a purity greater than 98%. Fatty acids were identified by comparing their retention times with suitable standards and by means of the lineal regression between retention time logarithms and carbon atom numbers. A total amount of 24 fatty acids, from $C_{14:0}$ (myristic acid) to $C_{22:6}$ (docosahexaenoic acid, *n*-3),

Table 1. Response factors, related to the internal standard, found for the fatty acids identified

Fatty acids	X (n = 10)	S_{n-1}	CV (%)
$C_{14:0}$	38·6	0·52	1·35
$C_{15:0}$	1·66	0·02	1·20
$C_{15:1}$	1·86	0·02	1·07
$C_{16:0}$	166	1·94	1·17
$C_{16:1\ (n\text{-}7)}$	48·5	0·73	1·50
$C_{17:0}$	2·35	0·03	1·28
$C_{17:1}$	5·55	0·07	1·26
$C_{18:0}$	30·3	0·40	1·32
$C_{18:1\ (n\text{-}9)}$	187	2·05	1·10
$C_{18:1\ (n\text{-}7)}$	31·3	0·38	1·21
$C_{18:2\ (n\text{-}6)}$	97·2	1·09	1·12
$C_{20:0}$	2·22	0·03	1·35
$C_{18:3\ (n\text{-}3)}$	14·0	0·15	1·07
$C_{20:1\ (n\text{-}9)}$	47·8	0·51	1·07
$C_{18:4\ (n\text{-}3)}$	10·9	0·14	1·29
$C_{20:2\ (n\text{-}6)}$	6·76	0·07	1·03
$C_{22:0}$	4·17	0·05	1·20
$C_{22:1\ (n\text{-}11)}$	47·6	0·58	1·22
$C_{20:4\ (n\text{-}3)}$	8·12	0·09	1·11
$C_{20:5\ (n\text{-}3)}$	34·6	0·47	1·36
$C_{22:4\ (n\text{-}6)}$	4·30	0·06	1·39
$C_{22:5\ (n\text{-}3)}$	11·8	0·15	1·27
$C_{22:6\ (n\text{-}3)}$	164	1·98	1·21

were identified and quantified. Quantification was achieved by two methods: internal normalisation and internal standard calibration, using methyllaurate, not present in trout oil, as internal standard.

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

RESULTS AND DISCUSSION

The response factors, calculated in relation to the internal standard for the different fatty acids identified, fluctuated from 1·07 to 1·27. Quantification limits ranged between 17 ng for $C_{14:0}$ and 25 ng for $C_{18:0}$, $C_{18:1\ n\text{-}9}$ and $C_{22:6\ n\text{-}3}$.

The accuracy was adequate for all fatty acids, with variation coefficients ranging from 1·07 to 1·50 (Table 1). Recovery was, in all cases, greater than 93% (Table 2).

Table 2. Variation coefficients calculated for each fatty acid identified

Fatty acid	% Recovery $X \pm s$ (n = 10)
$C_{14:0}$	95·8 ± 3·80
$C_{15:0}$	94·1 ± 2·14
$C_{16:0}$	93·3 ± 2·19
$C_{16:1\ (n\text{-}7)}$	98·1 ± 3·99
$C_{18:0}$	94·6 ± 2·78
$C_{18:1\ (n\text{-}9)}$	96·0 ± 2·14
$C_{18:2\ (n\text{-}6)}$	97·9 ± 2·81
$C_{20:1\ (n\text{-}9)}$	95·3 ± 3·96
$C_{22:1\ (n\text{-}11)}$	96·1 ± 2·41
$C_{20:5\ (n\text{-}3)}$	94·2 ± 3·92
$C_{22:6\ (n\text{-}3)}$	96·3 ± 3·97

Table 3. Fat content, fatty acid distribution, and fatty acid content in the groups analysed

Group	A (n = 11)	B (n = 12)	C (n = 16)	D (n = 15)
Fat (%)	13·8 ± 1·7	23·8 ± 6·0	39·4 ± 7·9	30·6 ± 6·07
SFA (%)	26·0 ± 1·0	26·7 ± 0·8	29·3 ± 1·8	24·5 ± 2·4
MUFA (%)	38·6 ± 0·7	40·3 ± 0·9	44·4 ± 1·4	43·2 ± 2·0
PUFA (%)	35·4 ± 1·5	33·1 ± 1·5	26·3 ± 2·0	32·3 ± 3·3
N-3 FA (%)	24·4 ± 1·8	22·2 ± 1·4	18·0 ± 1·7	21·3 ± 2·5
N-6 FA (%)	10·9 ± 0·3	10·7 ± 0·4	8·36 ± 0·6	10·7 ± 0·9
EPA (%)	3·4 ± 0·2	3·64 ± 0·2	3·12 ± 0·2	3·22 ± 0·2
DHA (%)	16·4 ± 1·7	13·1 ± 1·1	10·3 ± 1·4	12·9 ± 2·1
EPA (g/kg)	34·4 ± 3·3	36·4 ± 1·9	32·0 ± 2·5	33·3 ± 2·5
DHA (g/kg)	170 ± 21·9	135 ± 12·7	109 ± 15·5	136 ± 21·6

Table 3 displays values of fat content, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 fatty acids, n-6 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for the groups analysed. Total fat increased during growth and attained its peak in female trout. There are statistically significant differences ($P < 0·05$) between all groups studied.

The pattern of fatty acid distribution was steady for SFA, increasing for MUFA and decreasing for PUFA. The biggest changes were always shown by female trout. Analysis of variance (ANOVA) of PUFA content reveals significant differences ($P < 0·05$) between all groups except medium-sized trout (group B) and male trout.

In group B, which includes the size of trout usually consumed (140–200 g), the ratios SFA:MUFA:PUFA are (0·7:1:0·8), close to the values recommended (1:1:1) by the American Heart Association.

In all samples analysed, the fatty acid profile indicates $C_{18:1\ n\text{-}9}$, $C_{16:0}$, $C_{22:6\ n\text{-}3}$ and $C_{18:2\ n\text{-}6}$ as the major fatty acids (Figure 1). Fatty acid composition is influenced by sex, there are significant differences ($P < 0·05$) in the major fatty acid contents.

In regard to LC-PUFA, in all samples DHA ($C_{22:6\ n\text{-}3}$) contents (109–170 g/kg) were four times higher than EPA ($C_{20:5\ n\text{-}3}$).

This finding may be important for the preparation of lipidic concentrates rich in DHA, which are used in the elaboration of new infant formulas, so they can be more like human milk. Differences in EPA content are

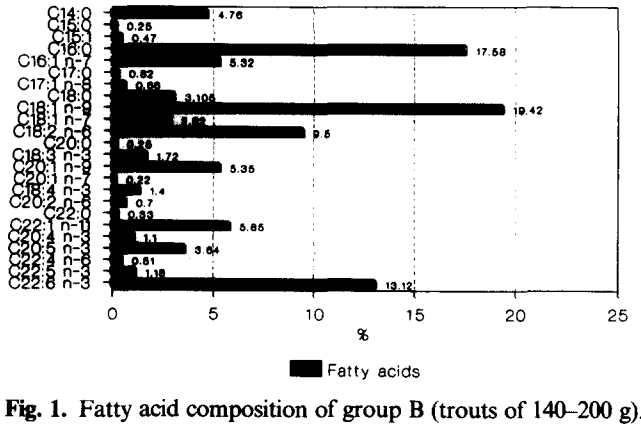

Fig. 1. Fatty acid composition of group B (trouts of 140–200 g).

small and not significant ($P < 0.05$) while differences were significant for DHA values ($P < 0.05$) between all groups. DHA clearly diminishes during trout growth, reaching the lowest value in females.

Regarding the *n*-3 and *n*-6 series fatty acid contents, it is notable that the former are more than two-fold the latter in all samples analysed; differences are statistically significant ($P < 0.05$) in both cases between male and female trout.

These results reflect, in general, bigger differences for female trout, especially in regard to unsaturated fatty acids. This may be related to egg formation, although no conclusive data were found in the literature. However, some authors point out that the main EFA (essential fatty acid) requirement of rainbow trout is for the *n*-3 series, and there are indications that longer chain forms may promote egg quality (Bromage *et al.*, 1992; Cowey, 1992).

The results also demonstrate that trout consumption provides *n*-3 series LC-PUFA, as well as balanced quantities of SFA, MUFA, and PUFA.

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