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Analytical Methods

Stereospecific analysis of triacylglycerol and phospholipid fractions of four freshwater fish species: *Salmo trutta*, *Ictalurus punctatus*, *Ictalurus melas* and *Micropterus salmoides*

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Abstract

The fatty acid compositions and the positional distributions of fatty acid in triacylglycerol (TAGs) and phospholipid (PLs) fractions of four cultured freshwater fish species (*Salmo trutta, Ictalurus punctatus, Ictalurus melas* and *Micropterus salmoides*) were investigated. As regards the TAGs fraction, the *S. trutta* (trout) and *I. punctatus* (channel catfish) species had the highest % content in *n*3 polyunsaturated fatty acids (PUFAs) and the lowest *n*6/*n*3 ratio; this ratio resulted always lower than 1 in all the considered fish species. The PLs fraction of the considered fish was very interesting because of the high percentage of PUFAs, both in phosphatidylcholines and in phosphatidylethanolamines. The fatty acid distribution among the three *sn*-positions of the glycerol backbone was non-random; generally, both *n*6 and *n*3 PUFAs were mainly distributed between *sn*-2- and *sn*-3-positions of TAGs while the contents in *sn*-1-position were generally lower; in PLs fraction these fatty acids preferred the *sn*-2-position.

Keywords: Freshwater fish; Triacylglycerols; Phosphatidylcholines; Phosphatidylethanolamines; Structural analysis

1. Introduction

Fish lipids are currently under scientific investigation because of the numerous health benefits attributed to them. Cultured freshwater fish generally contain high proportions of *n*6 polyunsaturated fatty acids (PUFAs), but also good levels of eicosapentaenoic (EPA, C20:5*n*3) and docosahexaenoic (DHA, C22:6*n*3) acids (Ackman, 1967), so that the *n*3/*n*6 ratio ranges from 1 to 4 (Van Vliet & Katan, 1990); their fatty acid (FAs) composition is influenced by the FAs composition of their natural foods, in fact, as a rule, freshwater algae and aquatic insect larvae are rich in EPA, linoleic (C18:2*n*6) and linolenic (C18:3*n*3) acids (Steffens, 1997). Obviously differences in species, location, seasonal and environmental conditions may influence the fish lipids (EFSA, 2005; Leger, Bergot, Lukuet, Flanzy, & Meurot, 1977; Luzia, Sampaio, Castellucci, & Torres, 2003). Unlike marine fish, freshwater fish are able to desaturate and elongate larger quantities of dietary C18 *n*6 and *n*3 PUFAs to C20 and C22 desaturates (Kanazawa, Teshima, & Ono, 1979).

The epidemiological evidence of inverse correlation between heart disease and fish intake in Greenland Eskimos has stimulated the study of the biochemistry, physiology and metabolism of *n*3 PUFAs (Kinsella, Lokesh, & Stone, 1990; Simopoulos, 1991), in particular of EPA and DHA, involved in several biological processes (Grimm, Mayer, Mayser, & Eigenbrodt, 2002). Much attention has been focused on the possible and potential protective effects against the development of arterial thrombosis and atherosclerotic cardiovascular diseases (Calder, 2004; Lands, 1992); other positive effects of *n*3 PUFAs in

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rheumatoid arthritis (Cleland, James, & Proudman, 2003), psoriasis (Mayser, Grimm, & Grimminger, 2002), cancer (Larsson, Kumlin, Ingelman-Sundberg, & Wolk, 2004; Simopoulos, 1991) and diabetes mellitus (Malasanos & Stacpoole, 1991) have been reported. Some researchers (Fitch Haumann, 1997; Larsen et al., 1997) have shown possible positive biological properties of two other *n*3 PUFAs, the heneicosapentaenoic (HPA, C21:5*n*3) and the docosapentaenoic (DPA, C22:5*n*3) acids, present in small amounts in fish oils; HPA is incorporated into triacylglycerols (TAGs) and phospholipids (PLs) in cell culture to a similar extent as EPA and DHA; the DPA, being about a third of *n*3 PUFAs circulating in human blood, may be more powerful than EPA as antiatherogenic factor.

It is widely known that the dietary lipids are absorbed mainly as *sn*-2-MAGs and also as free FAs, produced by lipase hydrolysis on TAGs (Nestel, 1990); their absorption into the enterocytes is followed by re-esterification and incorporation of TAGs in chylomicrons, so the absorbed TAGs molecules retain the FAs in the *sn*-2-position as in the dietary TAGs, whereas the FAs in the *sn*-1- and *sn*-3-positions are randomized and partially substituted by endogenous FAs (Brindley, 1984). The intramolecular distributions of FAs among the *sn*-positions of glycerol backbone of TAGs and PLs, as well as the length and the degree of unsaturation of the carbon chain, differ considerably in different fish species. These variations reflect important metabolic and functional differences; so the molecular structures of TAGs and PLs result very important for the digestion and absorption of dietary fat, having a significant effect on lipid metabolism, on its rate and on the total quantity absorbed (Leray, Raclot, & Groscolas, 1993; Nelson & Ackman, 1988). A deeper knowledge of *sn*-positional distribution of *n*3 and *n*6 PUFAs in the glycerol structures can provide further informations on their biological effects.

In this study the FAs composition and the stereospecific analysis of TAGs, phosphatidylcholine (PCs) and phosphatidylethanolamine (PEs) fractions of four different cultured freshwater fish species, *Salmo trutta* (trout), *Ictalurus punctatus* (channel catfish), *Ictalurus melas* (Italian catfish) and *Micropterus salmoides* (largemouth bass), were investigated.

2. Materials and methods

2.1. Samples and reagents

Four cultured freshwater fish species of commercial importance were selected: *S. trutta* (trout), *I. punctatus* (channel catfish), *I. melas* (Italian catfish) and *M. salmoides* (largemouth bass). Five individuals from each species were chosen and purchased from local fish market (fish arrived at the laboratory in ice one or two days post-capture); then, after removal of viscera, head, skin and fishbone, they were ground, homogenized with deionized water and then freeze dried.

All solvents and reagents were of analytical grade and were purchased from Carlo Erba Reagents (Milano, Italy).

2.2. Lipid extraction

Lipid fraction was extracted from freeze dried samples according to the Folch method (Folch, Lees, & Sloane Stanley, 1957). For each species, the lipid fractions extracted from representative amounts of edible muscle tissue of the five individuals were pooled and then subjected, in triplicate, to the analytical determinations reported in the following sections.

2.3. Stereospecific analysis of TAGs

The isolation of TAGs fraction by thin layer chromatography (TLC) was carried out using silica gel plates (SIL G-25, 0.25 mm, 20 cm × 20 cm, Macherey-Nagel, Germany) and petroleum ether:diethyl ether:formic acid (80:20:2, v/v/v) as mobile phase (Damiani et al., 1994); the TAGs fraction ($R_f \cong 0.9$) was scraped off and extracted with hexane:diethyl ether (1:1, v/v); then, after evaporation of the extraction solvent, an aliquot was subjected to transesterification using 2 N methanolic KOH and hexane in order to obtain the constituent fatty acid methyl esters (FAMEs), while the remaining aliquot was used to prepare the *sn*-1,3/*sn*-1,2(2,3)-diacylglycerols (DAGs) through Grignard deacylation. The *sn*-1,2(2,3)-DAGs were isolated by TLC ($R_f \cong 0.3$), using hexane:diethyl ether (1:1, v/v) as developing mixture. The *sn*-1,2(2,3)-PCs, obtained treating the *sn*-1,2(2,3)-DAG with phosphorus oxychloride and choline chloride, were isolated by TLC ($R_f \cong 0.2$) using as developing mixture chloroform:methanol:water (65:25:2, v/v/v). The hydrolysis of *sn*-1,2(2,3)-PCs was carried out adding 0.1 ml of enzymatic preparation (1 mg of phospholipase A_2 in 0.2 ml of 3 mM $CaCl_2$ solution) and maintaining under stirring for 4 h at room temperature; the hydrolysis products, *sn*-1-lysophosphatidylcholines (*sn*-1-LPCs) and free FAs ($R_f \cong 0.1$ and 0.9, respectively), were separated by TLC with chloroform:methanol:water (65:25:2, v/v/v) as developing mixture (Cossignani et al., 1994).

The FAs % compositions of TAGs and *sn*-1-LPCs were obtained by high resolution gas chromatography (HRGC) analysis, operating on FAMEs prepared by transmethylation with 2 N methanolic KOH and hexane. The free FAs were esterified using an ethereal solution of diazomethane (Christie, 2003) and also analyzed by HRGC. The HRGC analysis of FAMEs of *sn*-1-LPCs and of free FAs gave the FAs % composition of *sn*-1- (A_1) and *sn*-2-position (A_2), respectively.

The FAs % composition of the *sn*-3-position (A_3) was obtained starting from the FAs % composition of total TAGs (A_t), applying the following formula:

$$A_3 = 3 \times A_t - A_1 - A_2$$

where $A_3 =$ % intrapositional composition of FAs esterified in *sn*-3-position, $A_t =$ % total composition of FAs

esterified in all the three *sn*-positions of TAGs, $A_1 = \%$ intrapositional composition of FAs esterified in *sn*-1-position, $A_2 = \%$ intrapositional composition of FAs esterified in *sn*-2-position.

2.4. Stereospecific analysis of PCs and PEs

The isolation of the PCs and PEs classes by TLCs and the preparation of FAME derivatives of the constituent FAs were carried out according to procedures previously described (Cossignani et al., 1994). The enzymatic incubation with phospholipase A2, the isolation of *sn*-1-LPCs and FAs lipid classes by TLC and the HRGC analysis of FAMEs from *sn*-1-LPCs and FAs classes were carried out as above described for PCs synthetized from TAGs.

2.5. HRGC analysis

A DANI 1000DPC gas-chromatograph (Norwalk, CT, USA), equipped with a split–splitless injector and with a flame ionization detector (FID), was used. The separation was obtained using a CP Sil-88 column (50 m × 0.25 mm i.d., 0.25 μm f.t. from Chrompack International B.V., Middleburg, The Netherlands). The chromatograms were acquired and processed using Clarity integration software (DataApex Ltd., Prague, Czech Republic). The oven temperature was 140 °C, held 6 min, raised to 180 °C at a rate of 5 °C/min and then to 230 °C at a rate of 2 °C/min, while the injector and the FID temperatures were set at 250 °C. Carrier gas (He) flow rate was 2 ml/min.

FAs were identified by comparing the retention times of their methyl esters with three different standard FAME mixtures from Supelco (Bellefonte, PA, USA), named Supelco 37 Component FAME Mix (Catalog No. 47885-U), PUFAs Methyl Esters No. 2 (Catalog No. 47015-U) and PUFAs Methyl Esters No. 3 (Catalog No. 47085-U).

Repeated injections of standard solutions were carried out to test the analytical precision. The relative standard deviations (%RSD) were less than 5% for all the FAs, both considering the intradie precision, calculated on six repeated injections, and the interdie precision, evaluated over six days.

All the GC analyses were carried out in duplicate.

3. Results and discussion

The fat % contents of the four considered freshwater fish is reported in Table 1; the intraspecies variability in lipid content of fish is due to several factors, among which age, diet, geographical origin and season, as reported in the introduction.

Table 1
The lipid contents of fish species, mean values ± SD ($n = 5$)

	Lipid content (%)
Salmo trutta (trout)	3.0 ± 0.2
Ictalurus punctatus (channel catfish)	4.6 ± 0.2
Ictalurus melas (Italian catfish)	3.3 ± 0.4
Micropterus salmoides (largemouth bass)	3.7 ± 0.5

Tables 2–5 show the total % FAs compositions and FAs positional distributions of TAGs, PCs and PEs from *S. trutta* (trout), *I. punctatus* (channel catfish), *I. melas* (Italian catfish) and *M. salmoides* (largemouth bass), respectively. As above indicated, all the analytical procedures were carried out in triplicate and the results were expressed as mean values, mol% ± standard deviation (SD).

The most abundant FAs was the oleic acid (C18:1*n*9) in all the fish species; it ranged from 27.3% in channel catfish to 18.5% in trout. Linoleic (C18:2*n*6) and linolenic (C18:3*n*3) acids were higher in Italian catfish TAGs, 9.5% and 8.5% respectively, than in the other species; trout had the highest content of DHA (16.2%), channel catfish of EPA (12.2%), largemouth bass of DPA (3.9%). HPA was between 0.1% for Italian catfish to 0.5% for channel catfish. As regards the FAs positional distributions, the linoleic and linolenic acids were preferentially esterified in the *sn*-2-position of all fish species, with the exception of Italian catfish for linoleic acid and of trout for linolenic acid; DPA and DHA were preferentially esterified in the *sn*-2-position of Italian catfish and largemouth bass TAGs. Generally, for all the considered fish, EPA was esterified in TAGs *sn*-3-position.

In each fish species, PEs were always richer in DHA and DPA than PCs, while PCs had higher percentage of EPA than PEs. DHA was the most abundant FAs in PEs fraction of all the considered fish species, it ranged from 56.0% of trout to 22.4% of Italian catfish; also in PCs fraction the most abundant FAs was the DHA, with the exception of Italian catfish. This last fish species was rich in linoleic acid, both in PCs (8.0%) and in PEs (9.8%) fractions. EPA % content was higher in trout PCs (11.6%) than in PCs of the other species, while its % content in PEs was similar in all the considered fish species (from 6.2% of trout to 6.7% of channel catfish), with the exception of largemouth bass (4.4%). DPA, another interesting *n*3 PUFAs, was found higher in PLs than in TAGs fraction for each species; in particular DPA was more represented in PCs and PEs of largemouth bass compared to the respective PLs fraction of the other fish species.

In the same Tables 2–5 the values of the total SFAs, MUFAs, PUFAs, *n*6 PUFAs, *n*3 PUFAs and some ratios are shown together with the unsaturation index (UI) and the "average chain length" (ACL) (Barja et al., 1996). As regards the TAGs fraction, the data shown that PUFAs were more abundant in trout (10.6% of *n*6 and 34.3% of *n*3) than in the other three fish species, which were richer in MUFAs. The results of TAGs stereospecific analysis show a preference of SFAs and MUFAs for the *sn*-1-position, with the exception of Italian catfish MUFAs, which preferred the *sn*-3-position. Both the *n*6 and *n*3 PUFAs were equally distributed between *sn*-2- and *sn*-3-positions, even if in channel catfish TAGs the *n*6 PUFAs were located preferentially in the *sn*-2-, while the *n*3 PUFAs in the *sn*-3-position.

As regards PLs fractions, the SFAs were more abundant in PCs than in PEs; PUFAs content was higher in both PLs fractions than in TAGs. The trout resulted the fish species with the lowest content of *n*6 PUFAs (3.2%) in PCs and the highest content of *n*3 PUFAs (61.8%) in PEs fraction. In each fish species, SFAs and MUFAs were preferentially esterified in *sn*-1-position while total PUFAs in *sn*-2-position, considering both PCs and PEs fractions.

It has been suggested that the balance between the intake of *n*6 and *n*3 PUFAs is more important than levels of intake of individual FAs, with regard to many metabolic functions in the human body. Experimental evidences have suggest that an increased intake of *n*6 and an associated relative *n*3 deficiency represent the major risk factor for cancers, coronary heart disease and cerebrovascular disease (Calder, 2004; Kris-Etherton, Harris, & Appel, 2002; Ulbricht & Southgate, 1991). In order to improve the health status of humans, it has been recommended by FAO/WHO (1994) that the *n*6/*n*3 PUFAs ratio should be less than 4. Therefore all the considered freshwater fish species, characterized by low *n*6/*n*3 PUFAs ratios, could be very interesting for improving human nutrition. Moreover it has been reported that balancing the eicosanoids in the body is an excellent way for managing heart disease and

Table 2
Salmo trutta (trout): total and intrapositional FAs compositions of TAGs, PCs and PEs, mean values, mol% ± SD ($n = 3$)

FAs	TAGs				PCs			PEs		
	Total	*sn*-1-	*sn*-2-	*sn*-3-	Total	*sn*-1-	*sn*-2-	Total	*sn*-1-	*sn*-2-
C14:0	4.5 ± 0.1	6.5 ± 0.5	4.0 ± 2.1	3.0 ± 2.1	1.4 ± 0.2	2.2 ± 0.5	0.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	0.2 ± 0.1
C14:1*n*5	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.3	–	–	–	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C16:0	16.3 ± 0.9	28.5 ± 2.7	13.0 ± 2.8	6.5 ± 2.7	21.6 ± 0.9	38.7 ± 3.6	4.5 ± 2.3	10.9 ± 0.4	19.4 ± 1.1	2.3 ± 1.1
C16:1(*n*7 + *n*9)[a]	6.6 ± 0.2	7.4 ± 0.4	7.9 ± 2.1	3.4 ± 2.3	1.2 ± 0.1	1.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0.3 ± 0.1
C17:0	0.4 ± 0.0	0.8 ± 0.1	0.9 ± 0.4	0.0 ± 0.4	0.3 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.7 ± 0.1	0.2 ± 0.0
C17:1*n*7	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.3	0.5 ± 0.7	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	–	0.2 ± 0.1
C18:0	3.9 ± 0.2	7.6 ± 1.1	3.9 ± 0.7	0.3 ± 0.6	2.9 ± 1.6	5.3 ± 3.2	0.5 ± 0.2	5.1 ± 0.4	9.0 ± 1.0	1.3 ± 0.3
C18:1(*n*7 + *n*9)[a]	18.5 ± 0.6	23.4 ± 1.0	16.8 ± 0.9	15.8 ± 0.5	6.6 ± 0.3	7.4 ± 0.7	5.8 ± 0.5	7.1 ± 0.5	11.1 ± 1.1	3.1 ± 0.4
C18:2*n*6	7.3 ± 0.1	6.2 ± 0.6	8.4 ± 0.7	7.2 ± 0.8	1.1 ± 0.1	1.5 ± 0.1	0.6 ± 0.1	2.4 ± 0.1	4.1 ± 0.3	0.8 ± 0.3
C18:3*n*6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.1	–	–	–	–	–	–
C18:3*n*3	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	–	–	–	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1
C18:4*n*3	2.2 ± 0.1	1.6 ± 0.1	4.4 ± 1.8	−0.5 ± 2.2	0.5 ± 0.1	0.4 ± 0.5	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
C20:0	0.4 ± 0.0	0.5 ± 0.1	0.7 ± 0.4	0.0±0.5	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1
C20:1(*n*7 + *n*9 + *n*11)[a]	3.2 ± 0.3	3.1 ± 0.5	2.6 ± 0.2	3.8 ± 0.4	0.6 ± 0.1	1.0 ± 0.2	0.3 ± 0.1	1.2 ± 0.2	1.8 ± 0.8	0.6 ± 0.4
C20:2*n*6	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.7	0.2 ± 0.8	0.1 ± 0.0	0.1 ± 0.2	0.2 ± 0.1	0.3 ± 0.0	0.1 ± 0.7	0.5 ± 0.7
C20:3*n*6	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.2	0.7 ± 0.3	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.3	0.2 ± 0.2
C20:4*n*6	1.1 ± 0.1	0.5 ± 0.1	1.0 ± 0.2	1.7 ± 0.3	1.3 ± 0.2	0.7 ± 0.4	2.0 ± 0.2	1.2 ± 0.0	0.7 ± 0.2	1.6 ± 0.2
C20:3*n*3	0.1 ± 0.1	0.6 ± 0.5	1.1 ± 1.1	0.6 ± 1.0	0.1 ± 0.1	–	0.1 ± 0.0	0.1 ± 0.1	–	0.1 ± 0.0
C22:0	0.3 ± 0.1	0.2 ± 0.0	0.5 ± 0.1	0.0 ± 0.5	0.1 ± 0.1	0.1 ± 0.0	–	0.1 ± 0.1	–	0.1 ± 0.0
C22:1*n*9	0.6 ± 0.5	0.3 ± 0.1	0.7 ± 0.3	1.1 ± 1.9	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.1 ± 0.1
C20:4*n*3	1.2 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	2.1 ± 0.3	0.7 ± 0.1	1.3 ± 0.2	0.2 ± 0.1	1.4 ± 0.2	2.4 ± 0.5	0.4 ± 0.2
C20:5*n*3	10.4 ± 0.2	4.0 ± 0.9	6.9 ± 1.9	19.8 ± 2.5	11.6 ± 0.9	9.3 ± 1.1	13.9 ± 0.7	6.2 ± 0.2	6.6 ± 0.5	5.7 ± 0.4
C24:0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.2	0.0 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	0.1 ± 0.0	–
C21:5*n*3	0.4 ± 0.0	0.5 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
C22:4*n*6	0.7 ± 0.0	0.4 ± 0.1	0.7 ± 0.5	0.9 ± 0.5	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.2 ± 0.1
C22:5*n*6	0.5 ± 0.1	0.1 ± 0.1	0.3 ± 0.0	1.1 ± 0.4	0.5 ± 0.0	0.4 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.9 ± 0.0
C22:5*n*3	3.6 ± 0.3	1.9 ± 0.5	3.4 ± 1.1	5.5 ± 0.7	2.6 ± 0.2	2.0 ± 0.3	3.0 ± 0.2	4.3 ± 0.2	5.7 ± 0.8	3.1 ± 0.5
C22:6*n*3	16.2 ± 0.6	3.0 ± 1.9	19.1 ± 4.7	27.0 ± 3.7	46.0 ± 1.2	26.1 ± 3.8	65.9 ± 3.5	56.0 ± 1.6	34.3 ± 4.4	77.7 ± 2.2
∑SFA	25.8	44.3	23.2	9.8	26.4	46.9	5.7	17.2	30.0	4.1
∑MUFA	29.2	34.5	28.8	24.6	8.6	10.3	6.9	9.2	14.2	4.3
∑PUFAs	45.0	21.2	48.0	65.8	65.0	42.8	87.4	73.6	55.8	91.6
∑*n*6 PUFAs	10.6	8.3	11.6	11.9	3.2	3.1	3.9	5.2	6.2	4.1
∑*n*3 PUFAs	34.3	13.0	36.4	53.9	61.8	39.7	83.5	68.5	49.6	87.5
*n*6/*n*3 PUFAs	0.3	0.6	0.3	0.2	0.1	0.1	0.0	0.1	0.1	0.0
C20:4*n*6/C20:5*n*3	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.3
∑(*n*6 + *n*3)/∑SFA	1.7	0.5	2.1	6.7	2.5	0.9	15.5	4.3	1.9	22.3
∑*n*6 PUFAs/∑SFA	0.4	0.2	0.5	1.2	0.1	0.1	0.7	0.3	0.2	1.0
∑*n*3 PUFAs/∑SFA	1.3	0.3	1.6	5.5	2.3	0.9	14.8	4.0	1.7	21.3
UI[b]	239.8	117.0	250.0	353.0	373.0	242.6	504.0	421.3	310.4	533.0
ACL[c]	18.5	17.4	18.6	19.6	19.7	18.4	21.0	20.4	19.4	21.4

– Not detected.
[a] Sum of possible isomers.
[b] UI = Unsaturation index: ∑(mol% of each FAs) × (number of double bonds of each FAs).
[c] ACL = Average chain length: $[(\sum \%\ \text{Total}_{C14} \times 14) + \cdots + (\sum \%\ \text{Total}_{n} \times n)]/100$ (n = carbon atom number).

Table 3
Ictalurus punctatus (channel catfish): total and intrapositional FAs compositions of TAGs, PCs and PEs, mean values, mol% ± SD ($n = 3$)

FAs	TAGs				PCs			PEs		
	Total	*sn*-1-	*sn*-2-	*sn*-3-	Total	*sn*-1-	*sn*-2-	Total	*sn*-1-	*sn*-2-
C14:0	4.1 ± 0.0	6.8 ± 0.0	1.3 ± 0.1	4.1 ± 0.0	0.5 ± 0.0	0.7 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
C14:1*n*5	0.2 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.0 ± 0.1	0.1 ± 0.0	–	0.1 ± 0.0	–	–	–
C16:0	16.7 ± 0.0	28.2 ± 1.2	13.0 ± 0.9	9.0 ± 2.2	17.8 ± 0.1	32.2 ± 0.7	3.4 ± 0.5	5.1 ± 0.1	8.2 ± 0.2	2.0 ± 0.3
C16:1(*n*7 + *n*9)[a]	6.5 ± 0.0	6.9 ± 0.4	3.2 ± 0.2	9.4 ± 0.5	1.5 ± 0.2	1.4 ± 0.6	1.6 ± 0.3	0.6 ± 0.0	0.8 ± 0.0	0.5 ± 0.1
C17:0	0.3 ± 0.0	0.5 ± 0.1	1.8 ± 0.1	−0.8 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C17:1*n*7	0.7 ± 0.0	1.2 ± 0.1	1.1 ± 0.1	−0.4 ± 0.2	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C18:0	4.2 ± 0.0	6.4 ± 0.2	2.2 ± 0.4	3.7 ± 0.2	5.9 ± 0.1	11.2 ± 0.3	0.7 ± 0.0	11.5 ± 0.3	21.3 ± 0.7	1.7 ± 0.0
C18:1(*n*7 + *n*9)[a]	27.3 ± 0.1	30.2 ± 0.5	23.7 ± 2.2	27.9 ± 1.8	22.6 ± 0.2	22.6 ± 0.5	22.6 ± 0.9	14.3 ± 0.2	16.4 ± 0.2	12.3 ± 0.6
C18:2*n*6	3.8 ± 0.0	3.8 ± 0.1	6.0 ± 0.4	1.3 ± 0.4	5.3 ± 0.0	4.0 ± 0.1	6.6 ± 0.2	5.4 ± 0.1	7.0 ± 0.0	3.9 ± 0.2
C18:3*n*6	0.2 ± 0.0	0.2 ± 0.0	2.1 ± 0.1	−0.9 ± 0.1	0.1 ± 0.0	–	0.1 ± 0.0	–	–	–
C18:3*n*3	0.2 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	−0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	–	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
C18:4*n*3	2.5 ± 0.0	1.3 ± 0.0	3.3 ± 0.4	3.0 ± 0.5	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
C20:0	0.3 ± 0.0	0.4 ± 0.0	1.2 ± 0.1	−0.8 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	–	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.1
C20:1(*n*7 + *n*9 + *n*11)[a]	3.5 ± 0.0	2.1 ± 0.1	5.3 ± 0.0	3.0 ± 0.1	2.2 ± 0.0	3.3 ± 0.4	1.0 ± 0.4	2.8 ± 0.1	4.0 ± 0.2	1.6 ± 0.3
C20:2*n*6	0.3 ± 0.0	0.3 ± 0.0	1.1 ± 0.1	−0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.0
C20:3*n*6	1.4 ± 0.0	0.8 ± 0.1	1.7 ± 0.0	1.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	0.9 ± 0.1	0.3 ± 0.0
C20:4*n*6	1.0 ± 0.1	0.5 ± 0.0	1.8 ± 0.3	0.1 ± 0.5	4.7 ± 0.1	2.2 ± 0.2	7.2 ± 0.1	5.3 ± 0.1	3.2 ± 0.2	7.3 ± 0.0
C20:3*n*3	–	–	–	–	–	–	–	0.1 ± 0.0	–	0.2 ± 0.2
C22:0	0.1 ± 0.0	0.2 ± 0.0	–	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0
C22:1*n*9	0.1 ± 0.0	0.2 ± 0.0	–	0.2 ± 0.0	–	–	–	–	–	–
C20:4*n*3	0.9 ± 0.0	0.4 ± 0.0	1.1 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	0.8 ± 0.1	0.4 ± 0.1
C20:5*n*3	12.2 ± 0.0	4.2 ± 0.2	9.8 ± 0.6	22.0 ± 0.8	9.1 ± 0.1	4.2 ± 0.1	14.1 ± 0.1	6.7 ± 0.0	3.7 ± 0.5	9.6 ± 0.4
C24:0	–	–	–	–	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.0	–	–	–
C21:5*n*3	0.5 ± 0.0	0.4 ± 0.0	–	1.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	–	0.1 ± 0.0	0.2 ± 0.0	–
C22:4*n*6	0.6 ± 0.0	0.2 ± 0.0	–	1.5 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
C22:5*n*6	0.4 ± 0.0	0.1 ± 0.0	2.3 ± 0.1	−0.8 ± 0.2	0.6 ± 0.0	0.3 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	0.7 ± 0.1	1.3 ± 0.1
C22:5*n*3	1.7 ± 0.0	0.7 ± 0.0	3.6 ± 0.3	0.9 ± 0.3	2.3 ± 0.0	1.2 ± 0.2	3.4 ± 0.2	3.1 ± 0.1	2.2 ± 0.2	3.9 ± 0.0
C22:6*n*3	10.6 ± 0.0	3.3 ± 0.7	13.4 ± 0.5	14.9 ± 1.2	24.2 ± 0.1	13.0 ± 1.6	35.4 ± 1.8	40.8 ± 0.2	28.8 ± 1.6	52.6 ± 1.2
∑SFA	25.7	42.5	19.5	15.4	24.8	45.0	4.6	17.3	30.0	4.4
∑MUFA	38.2	41.1	33.6	40.2	26.5	27.5	25.5	17.8	21.2	14.7
∑PUFAs	36.1	16.4	46.9	44.9	48.7	27.6	69.8	65.0	48.9	80.9
∑*n*6 PUFAs	7.5	6.0	15.1	2.3	11.6	7.5	15.7	13.3	13.0	13.6
∑*n*3 PUFAs	28.6	10.5	31.9	42.6	37.1	20.1	54.1	51.7	36.0	67.3
*n*6/*n*3 PUFAs	0.3	0.6	0.5	0.1	0.3	0.4	0.3	0.3	0.4	0.2
C20:4*n*6/C20:5*n*3	0.1	0.1	0.2	0.0	0.5	0.5	0.5	0.8	0.9	0.8
∑(*n*6 + *n*3)/∑SFA	1.4	0.4	2.4	2.9	2.0	0.6	15.0	3.8	1.6	18.5
∑*n*6 PUFAs/∑SFA	0.3	0.1	0.8	0.2	0.5	0.2	3.4	0.8	0.4	3.1
∑*n*3 PUFAs/∑SFA	1.1	0.3	1.6	2.8	1.5	0.5	11.7	3.0	1.2	15.4
UI[b]	208.6	109.3	245.2	270.6	270.0	160.5	379.5	357.0	264.5	448.6
ACL[c]	18.2	17.3	18.8	18.6	19.0	18.0	20.0	20.0	19.4	20.7

– Not detected.
[a,b,c] See explanation in Table 2.

other chronic and inflammatory processes, and that the ratio of the eicosanoid precursor FAs (C20:4*n*6/C20:5*n*3) represent a measure of the body's eicosanoid balance (Sargent, Bell, McEvoy, Tocher, & Estevez, 1999). This ratio was generally low in TAGs and PLs fractions of the four considered freshwater fish.

Another important parameter, currently used to assess the nutritional quality of the lipid fraction of food, is the PUFAs/SFA ratio. Nutritional guidelines recommend a PUFAs/SFA ratio above 0.4–0.5 (FAO/WHO, 1994); this value was found always higher than 1 in TAGs and PLs of all freshwater fish species, with few exceptions.

The UI, which takes into account the number of double bounds of each PUFA, was the lowest for Italian catfish and the highest for trout, comparing both TAGs and PLs fractions of the four fish species. As expected, in regard to ACL index, the highest values were found in PEs fraction of all the fish species, as a consequence of the highest % content of PUFAs, in particular of DHA.

In conclusion, the findings of this research indicate that the dietary intake of cultured freshwater fish could be considered as a valid alternative to the consumption of marine fish or fish oil supplement, so as to decrease the *n*6/*n*3 ratio, that is very high in Western diet (WHO, 2003).

Table 4
Ictalurus melas (Italian catfish): total and intrapositional FAs compositions of TAGs, PCs and PEs, mean values, mol% ± SD ($n = 3$)

FAs	TAGs				PCs			PEs		
	Total	*sn*-1-	*sn*-2-	*sn*-3-	Total	*sn*-1-	*sn*-2-	Total	*sn*-1-	*sn*-2-
C14:0	3.3 ± 0.0	3.9 ± 0.2	4.3 ± 0.2	1.5 ± 0.3	0.3 ± 0.2	0.2 ± 0.6	0.3 ± 0.4	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
C14:1*n*5	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	–	–	–	–	–	–
C16:0	19.9 ± 0.1	37.0 ± 0.8	16.4 ± 0.4	6.1 ± 0.6	18.4 ± 0.2	32.5 ± 0.1	3.3 ± 2.0	6.3 ± 0.4	10.2 ± 1.6	1.9 ± 0.1
C16:1(*n*7 + *n*9)[a]	10.4 ± 0.1	14.3 ± 0.5	10.0 ± 0.3	6.8 ± 0.4	2.2 ± 0.2	1.9 ± 0.4	2.5 ± 0.5	0.8 ± 0.1	0.5 ± 0.8	1.0 ± 0.9
C17:0	1.2 ± 0.0	2.2 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	–	–	–	0.9 ± 0.0	1.4 ± 0.2	0.3 ± 0.3
C17:1*n*7	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.0	1.3 ± 0.0	0.1 ± 0.0	–	0.3 ± 0.3	0.4 ± 0.2	0.5 ± 0.4	0.2 ± 0.5
C18:0	2.7 ± 0.0	5.4 ± 0.2	1.6 ± 0.1	1.2 ± 0.0	6.0 ± 0.3	10.5 ± 0.4	0.6 ± 1.4	9.9 ± 0.5	19.0 ± 1.3	1.2 ± 0.1
C18:1(*n*7 + *n*9)[a]	25.2 ± 0.1	19.5 ± 0.3	19.8 ± 0.2	36.7 ± 0.1	16.4 ± 0.2	18.1 ± 0.9	13.3 ± 1.8	14.5 ± 1.2	18.1 ± 5.5	9.2 ± 0.8
C18:2*n*6	9.5 ± 0.0	3.8 ± 0.1	10.9 ± 0.4	14.2 ± 0.1	8.0 ± 0.4	8.5 ± 1.2	6.7 ± 1.2	9.8 ± 2.0	13.0 ± 6.2	5.1 ± 0.4
C18:3*n*6	0.3 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.1
C18:3*n*3	8.5 ± 0.0	4.0 ± 0.2	11.1 ± 0.5	11.0 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	–	0.2 ± 0.1	0.1 ± 0.2	0.2 ± 0.3
C18:4*n*3	0.6 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	0.9 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	–	0.5 ± 0.4
C20:0	0.5 ± 0.0	1.1 ± 0.1	0.2 ± 0.3	0.1 ± 0.4	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.0	0.7 ± 0.2	0.2 ± 0.1
C20:1(*n*7 + *n*9 + *n*11)[a]	1.0 ± 0.0	1.0 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	5.4 ± 0.2	6.7 ± 0.8	3.5 ± 1.2	4.4 ± 0.2	5.9 ± 1.0	2.5 ± 0.0
C20:2*n*6	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	1.2 ± 0.0	1.4 ± 0.1	1.0 ± 0.1	1.4 ± 0.0	2.0 ± 0.8	0.6 ± 0.5
C20:3*n*6	0.6 ± 0.0	0.3 ± 0.0	0.7 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	0.7 ± 0.6	2.2 ± 0.1	3.4 ± 0.6	0.8 ± 0.5
C20:4*n*6	2.4 ± 0.0	0.6 ± 0.0	3.1 ± 0.3	3.3 ± 0.1	9.8 ± 0.2	5.8 ± 2.4	15.5 ± 3.2	8.2 ± 0.7	6.8 ± 7.0	10.5 ± 6.2
C20:3*n*3	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.3 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
C22:0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	–	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C22:1*n*9	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	1.0 ± 0.0	0.7 ± 0.1	1.4 ± 0.1	1.0 ± 0.0	1.1 ± 0.5	0.5 ± 0.4
C20:4*n*3	0.8 ± 0.0	0.5 ± 0.0	0.7 ± 0.1	1.3 ± 0.2	0.7 ± 0.0	0.5 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.4 ± 0.3	0.6 ± 0.4
C20:5*n*3	4.7 ± 0.0	1.3 ± 0.1	4.8 ± 0.2	8.2 ± 0.2	10.8 ± 0.5	2.9 ± 1.1	19.4 ± 1.6	6.6 ± 0.7	2.3 ± 2.5	11.6 ± 1.3
C24:0	–	–	–	–	0.1 ± 0.0	–	0.1 ± 0.1	0.1 ± 0.0	–	0.1 ± 0.0
C21:5*n*3	0.1 ± 0.0	–	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	–	0.2 ± 0.1	1.1 ± 1.3	0.1 ± 0.0
C22:4*n*6	0.4 ± 0.0	0.2 ± 0.0	0.7 ± 0.2	0.5 ± 0.2	0.1 ± 0.0	–	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
C22:5*n*6	0.3 ± 0.0	0.1 ± 0.0	0.5 ± 0.3	–	0.9 ± 0.0	0.5 ± 0.1	1.2 ± 0.2	1.7 ± 0.1	1.7 ± 0.9	1.7 ± 1.2
C22:5*n*3	1.5 ± 0.0	0.4 ± 0.1	2.9 ± 0.2	1.4 ± 0.0	4.5 ± 0.2	1.9 ± 0.8	7.5 ± 1.1	7.0 ± 0.6	3.5 ± 3.0	11.0 ± 1.6
C22:6*n*3	2.9 ± 0.0	0.7 ± 0.2	6.0 ± 1.1	1.1 ± 0.3	12.3 ± 0.3	5.3 ± 0.6	20.6 ± 2.3	22.4 ± 0.9	7.5 ± 6.7	39.3 ± 2.3
∑SFA	27.6	49.8	23.1	9.6	25.0	43.5	4.6	17.9	31.6	4.2
∑MUFA	38.4	36.8	32.6	46.0	25.1	27.4	21.1	21.0	26.0	13.4
∑PUFAs	34.0	13.4	44.3	44.5	49.9	29.4	74.4	61.1	42.9	82.4
∑*n*6 PUFAs	14.2	5.7	17.2	19.7	20.9	17.5	25.4	23.5	27.1	19.0
∑*n*3 PUFAs	19.8	7.7	27.0	24.7	29.0	11.9	49.0	37.6	15.8	63.4
*n*6/*n*3 PUFAs	0.7	0.7	0.6	0.8	0.7	1.5	0.5	0.6	1.7	0.3
C20:4*n*6/C20:5*n*3	0.5	0.4	0.7	0.4	0.9	2.0	0.8	1.2	3.0	0.9
∑(*n*6 + *n*3)/∑SFA	1.2	0.3	1.9	4.6	2.0	0.7	16.3	3.4	1.4	19.7
∑*n*6 PUFAs/∑SFA	0.5	0.1	0.7	2.1	0.8	0.4	5.6	1.3	0.9	4.5
∑*n*3 PUFAs/∑SFA	0.7	0.2	1.2	2.6	1.2	0.3	10.7	2.1	0.5	15.2
UI[b]	156.2	79.6	193.6	193.2	245.5	138.9	371.2	300.9	187.5	433.5
ACL[c]	17.7	17.0	17.9	18.1	18.9	18.0	20.0	19.6	18.7	20.6

– Not detected.
[a,b,c] See explanation in Table 2.

Table 5
Micropterus salmoides (largemouth bass): total and intrapositional FAs compositions of TAGs, PCs and PEs, mean values, mol% ± SD ($n = 3$)

FAs	TAGs				PCs			PEs		
	Total	*sn*-1-	*sn*-2-	*sn*-3-	Total	*sn*-1-	*sn*-2-	Total	*sn*-1-	*sn*-2-
C14:0	3.0 ± 0.5	3.0 ± 0.4	4.3 ± 0.1	1.2 ± 0.1	0.3 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C14:1*n*5	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	–	–	–	–
C16:0	17.2 ± 0.9	21.3 ± 0.8	23.3 ± 0.6	6.9 ± 0.6	23.5 ± 0.4	44.0 ± 0.9	3.0 ± 0.3	7.6 ± 0.5	14.0 ± 1.1	1.2 ± 0.1
C16:1(*n*7 + *n*9)[a]	10.9 ± 0.7	13.9 ± 0.5	10.5 ± 0.1	7.9 ± 0.1	1.4 ± 0.0	1.6 ± 0.1	1.1 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.3 ± 0.0
C17:0	0.8 ± 0.0	1.4 ± 0.0	0.8 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	–	0.2 ± 0.2
C17:1*n*7	1.5 ± 0.0	2.0 ± 0.0	1.6 ± 0.1	0.9 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	–	0.2 ± 0.1
C18:0	3.4 ± 0.1	7.3 ± 0.2	1.4 ± 0.0	1.6 ± 0.0	4.6 ± 0.2	9.0 ± 0.5	0.2 ± 0.0	8.0 ± 0.1	15.3 ± 0.3	0.7 ± 0.0
C18:1(*n*7 + *n*9)[a]	25.8 ± 0.4	33.1 ± 0.9	12.7 ± 0.2	31.8 ± 0.2	13.7 ± 0.1	15.9 ± 0.7	11.4 ± 0.6	11.8 ± 0.3	14.9 ± 1.1	8.8 ± 0.6
C18:2*n*6	7.2 ± 0.1	4.5 ± 0.1	10.2 ± 0.2	6.7 ± 0.2	2.7 ± 0.0	2.5 ± 0.2	3.0 ± 0.2	3.8 ± 0.1	5.4 ± 0.2	2.1 ± 0.1
C18:3*n*6	0.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C18:3*n*3	4.4 ± 0.1	3.1 ± 0.1	5.3 ± 0.0	4.5 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	–	0.1 ± 0.0	0.2 ± 0.0	–
C18:4*n*3	0.8 ± 0.1	0.5 ± 0.1	0.9 ± 0.0	1.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
C20:0	0.3 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	–	0.2 ± 0.0	0.4 ± 0.0	0.1 ± 0.0
C20:1(*n*7 + *n*9 + *n*11)[a]	0.6 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.8 ± 0.0	1.5 ± 0.0	2.0 ± 0.1	0.9 ± 0.0	2.1 ± 0.0	3.2 ± 0.1	1.0 ± 0.0
C20:2*n*6	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
C20:3*n*6	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	1.1 ± 0.1	1.5 ± 0.3	0.7 ± 0.0
C20:4*n*6	3.9 ± 0.3	1.5 ± 0.1	2.8 ± 0.2	6.9 ± 0.2	8.4 ± 0.2	3.2 ± 0.2	13.5 ± 0.3	8.6 ± 0.1	4.8 ± 0.2	12.4 ± 0.2
C20:3*n*3	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.1 ± 0.1	0.3 ± 0.0	0.6 ± 0.0	–	–	–	–
C22:0	0.2 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	–	–	–	–	–	–
C22:1*n*9	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	–	–	–	0.4 ± 0.0	0.4 ± 0.5	–
C20:4*n*3	0.7 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	0.3 ± 0.2	0.3 ± 0.2	0.7 ± 0.1	1.2 ± 0.2	0.1 ± 0.0
C20:5*n*3	3.2 ± 0.1	1.5 ± 0.1	2.3 ± 0.1	6.1 ± 0.1	5.7 ± 0.1	2.3 ± 0.1	8.9 ± 0.2	4.4 ± 0.1	4.2 ± 0.1	4.7 ± 0.1
C24:0	–	–	–	–	–	–	–	–	–	–
C21:5*n*3	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	–	–	–	–
C22:4*n*6	1.2 ± 0.1	0.2 ± 0.0	1.2 ± 0.0	2.1 ± 0.0	1.2 ± 0.0	0.7 ± 0.1	1.6 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	–
C22:5*n*6	1.1 ± 0.1	0.2 ± 0.1	1.3 ± 0.3	1.6 ± 0.3	1.9 ± 0.0	1.1 ± 0.1	2.7 ± 0.1	2.9 ± 0.1	1.7 ± 0.2	4.1 ± 0.1
C22:5*n*3	3.9 ± 0.3	1.0 ± 0.1	5.7 ± 0.5	4.9 ± 0.5	6.2 ± 0.1	2.8 ± 0.4	9.6 ± 0.3	8.5 ± 0.3	7.0 ± 0.7	9.9 ± 0.2
C22:6*n*3	8.0 ± 0.6	1.1 ± 0.2	12.0 ± 0.3	11.6 ± 0.3	27.0 ± 0.3	11.6 ± 1.4	42.4 ± 1.3	38.0 ± 0.9	23.4 ± 2.1	52.4 ± 0.8
∑SFA	24.8	33.9	30.0	10.5	28.6	53.9	3.5	16.0	30.0	2.3
∑MUFA	39.2	50.2	25.4	41.9	16.7	19.7	13.4	14.9	19.2	10.3
∑PUFAs	35.9	15.9	44.5	47.7	54.8	26.3	83.1	69.1	50.9	87.4
∑*n*6 PUFAs	14.4	7.4	17.1	18.4	15.0	8.2	21.7	17.4	14.7	20.2
∑*n*3 PUFAs	21.5	8.5	27.4	29.3	39.8	18.1	61.4	51.6	36.3	67.2
*n*6/*n*3 PUFAs	0.7	0.9	0.6	0.6	0.4	0.5	0.4	0.4	0.4	0.3
C20:4*n*6/C20:5*n*3	1.1	1.0	1.2	1.1	1.5	1.4	1.5	2.0	1.2	2.6
∑(*n*6+*n*3)/∑SFA	1.5	0.5	1.5	4.6	1.9	0.5	23.8	4.3	1.7	38.8
∑*n*6 PUFAs/∑SFA	0.6	0.2	0.6	1.8	0.5	0.2	6.2	1.1	0.5	9.0
∑*n*3 PUFAs/∑SFA	0.9	0.3	0.9	2.8	1.4	0.3	17.6	3.2	1.2	29.8
UI[b]	186.6	104.9	208.7	249.1	296.6	147.8	444.3	372.0	268.0	476.4
ACL[c]	18.0	17.4	18.1	18.7	19.3	17.9	20.7	20.2	19.3	21.0

– Not detected.
[a,b,c] See explanation in Table 2.

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