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FATTY ACIDS PROFILE IN FISH PLASMA

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Abstract
Eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) are the major fatty acids found in fish. These fatty acids are produced by unicellular algae and phytoplankton which are consumed and then accumulate in fish. [1-3]. The aim of this work was to establish the extraction procedure, the derivatization method, the separation temperature program and the identification of the fatty acids from fish plasma. The extraction of the fatty acids was performed by mixing plasma and chloroform:methanol (2:1) during 30 seconds, at room temperature. The identification of fatty acids was obtained by comparison of FAME mass spectra with the mass spectra of fatty acids methyl esters (FAME) kits and of NIST library.
Keywords: fatty acids, GC-MS, derivatization.

1. Introduction

The importance of the (n-3) highly unsaturated fatty acids (HUFA) [2] in human nutrition has led to considerable research effort in the intervening years. Subsequently, the efficacy of (n-3) HUFA in the prevention or attenuation of many of the inflammatory conditions that are prevalent in the developed world, including rheumatoid arthritis, atopic illness, inflammatory bowel disease and various neurological conditions, has been established. Fish, and particularly those with oily flesh, such as herring, mackerel and salmon, represent a rich and almost unique source of the (n-3) HUFA, especially eicosapentaenoic acid [20:5(n-3), EPA] and docosahexaenoic acid [22:6(n-3), DHA]. Demand for fish products is increasing, yet the traditional capture fisheries are in decline worldwide such that the potential shortfall in fish products must be met by aquaculture production. Aquaculture production currently uses 60% of global fish oil production and by 2010, 85% will be consumed in aquaculture feeds. Future expansion of aquaculture, and in particular salmon

production, can continue only if suitable and sustainable alternatives to fish oil (FO) are developed and introduced [1].

A gas chromatography-mass spectrometry (GC/MS) technique was used for qualitative and quantitative analysis of fatty acids in fish [1, 2, 4]. The aim of the paper was to develop an analytical method for determination of fatty acids in biological samples. The method involves extraction procedure, derivatization and gas chromatography/mass spectrometric analysis (GC/MS). Fatty acids from plasma, were derivatized as methyl ester derivatives [1, 2, 4]. Finally, the extracted analytes were detected by GC/MS in the electron impact (EI) mode [3-6]. The method was validated by using fatty acid standard samples. The method was applied for fatty acids analysis in fish plasma.

2. Method and samples

Undecaenoic acid (C11:1) was used as internal standard. A Trace DSQ ThermoFinnigan quadrupole mass spectrometer coupled with a Trace GC was used. The extracts were separated on a Rtx-5MS capillary column, 30m x 0.25mm, 0.25µm film thickness, using a temperature program from 50^{o}C, 1 min, 8^{o}C/min at 300^{o}C, the flow rate 1ml/min, with helium 5.5 as carrier gas. The following conditions were followed: transfer line temperature 250^{o}C, injector temperature 250^{o}C; ion source temperature 250^{o}C; Splitter: 10:1. Electron energy was 70eV and the emission current, 100µA. Figure 1 presents the separation chromatogram of fatty acids methyl esters (FAME) in a plasma fish sample.

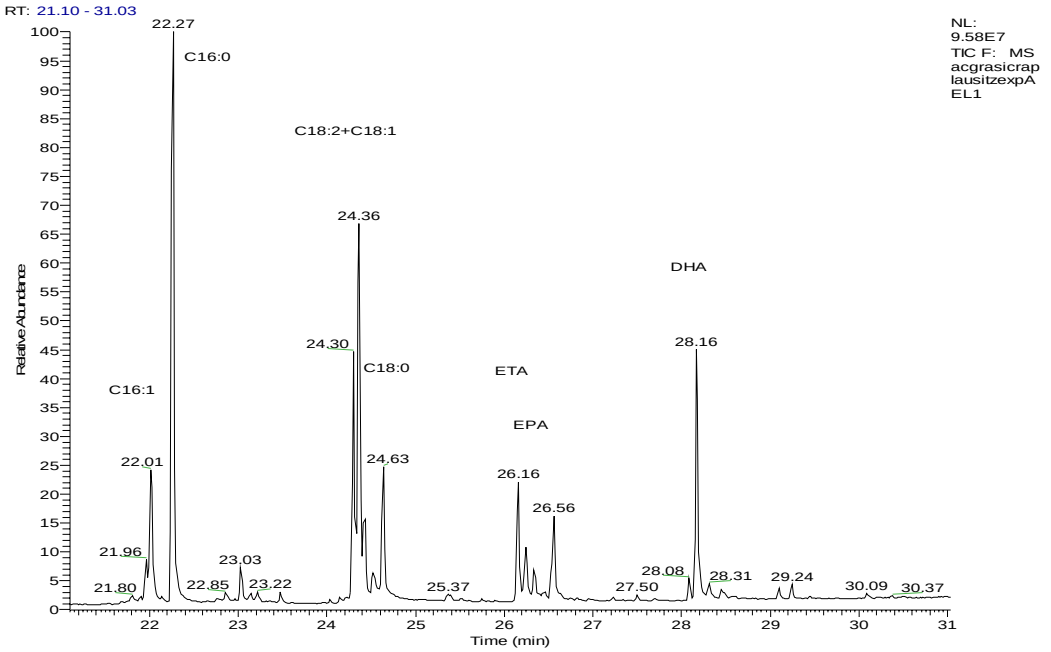

FIGURE 1. The separation chromatogram of the important fatty acids identified in fish plasma.

*Fatty acid extraction and derivatization***:** The fatty acids were extracted from the samples in different ways. From plasma they were extracted from 2ml water passed over a Dowex 50W-W8 exchange resin, where amino acids were collected. The extraction was performed in 0,5 ml chloroform:methanol (2:1), by mixing 30 sec and then centrifugation 5 min. Derivatization procedure was applied for esterification with methanol-acetyl chloride (4:1 v/v) for 20 min at 80^{o}C [1,2].

Method Validation: The method was validated and some validation parameters, precision and sensitivity were tested. GC/MS analyses were performed for the determination of fatty acids in some biological samples.

Fatty acid quantitation: 20µg of C11:1 internal standard was added after extraction and derivatization to each sample. The fatty acids were calculated according with the internal standard quantity and by using the response factors (for detector response correction) obtained by repetitive injections into GC/MS of the standard mixture containing known quantity of each fatty acid. The fatty acids calculations in biological samples were performed following the formulas:

$$F_i = \frac{\frac{A_i}{A_j}}{\frac{m_i}{m_j}} \qquad m_i(\mu g) = m_j(\mu g)\frac{A_i}{F_i \cdot A_j}$$

where C_i (or m_i) is the quantity corresponding to the compound i; m_j is the internal standard quantity added before sample preparation; A_i and A_j are the peak areas of the compounds i and respectively j; F_i and F_j are the response factors for compound i and respectively j (the internal standard) calculated by using standards.

3. Results and discussion

The developed method is selective and specific. The mass spectra recorded on each chromatographic peak permit the precise identification of the fatty acids, by using NIST library of mass spectra. Also, the overlapping of the compound is easily discovered. The method was validated by using fatty acid standards. Precision gave lower values than 10% for R.S.D. and the sensitivity values were lower than 10 ng of fatty acid injected. All the samples followed the same extraction and derivatization steps.

The comparison of the separated FAME from different carp fish extracts, showed very similar profiles, as could be observed in Figure 2.

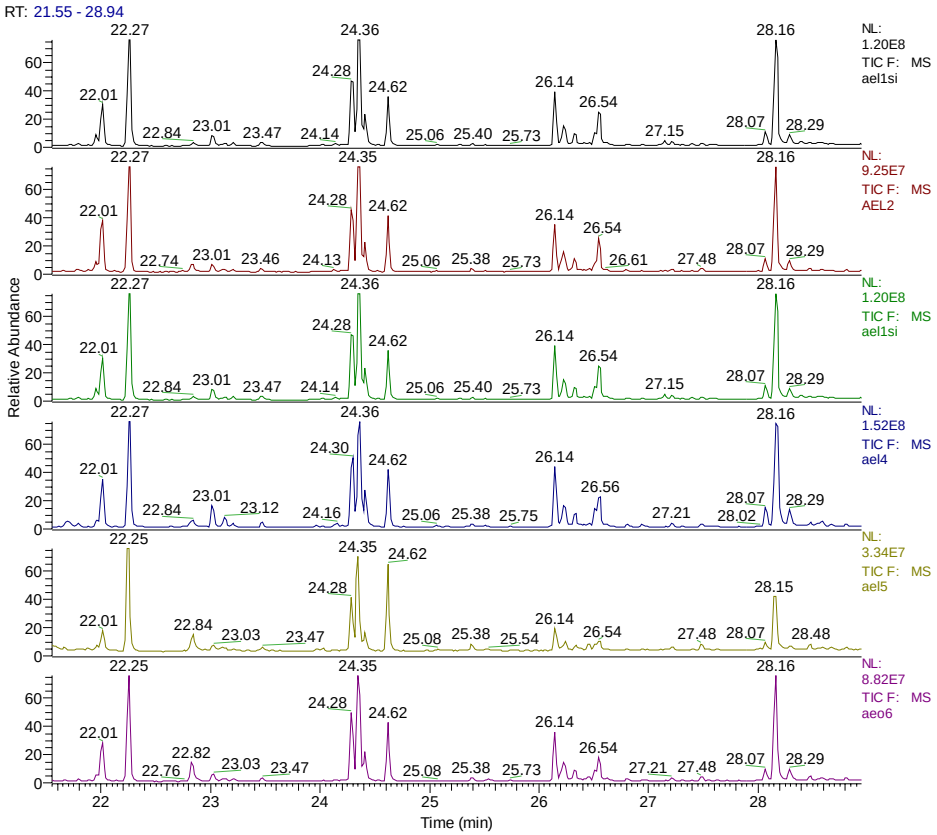

FIGURE 2. Comparative chromatogram profiles of plasma fatty acids methyl esters from different carp fish extracts.

TABLE 1. Fatty acids composition in carp fish plasma; Values are expressed as percent of total fatty acids, n=8

FAME	T_R	Mean±DS
hexadecenoic acid (C16:1)	22.01	6.84±1.21
hexadecanoic acid (C16:0)	22.27	18.61±4.53
9,12octadecadienoic acid (C18:2)	24.28	8.05±2.04
9-octadecenoic acid (C18:1)	24.36	17.59±2.20
octadecenoic acid (C18:1)	24.41	3.85±0.48
octadecanoic acid (C18:0)	24.62	6.28±0.62
5,8,11,14eicosatetraenoic acid (C20:4)ETA	26.14	7.68±1.20
5,8,11,14,17eicosapentaenoic acid (C20:5)ETA	26.22	3.76±1.03
ETA	28.07	2.85±1.36
4,7,10,13,16,19docosahexenoic acid(C22:6)DHA	28.16	14.85±6.46
ETA	28.29	1.18±0.58
Σ SFA		23.53
Σ MUFA		21.42
Σ PUFA		53.17
ΣUSFA/ ΣSFA		3.2
DHA /EPA		4.3

Plasma concentrations of fatty acids were measured in carp fish plasma.

The fatty acids identified in the plasma samples of the carp fish are shown in Table 1 as a mean value of 8 control carps. The analysis of the fatty acid composition of revealed that monounsaturated fatty acids (MUFA) were the most representative (*ca.*21%), followed by saturated fatty acids (SFA) (*ca.* 24%) and finally by polyunsaturated fatty acids (PUFA) (*ca.* 53%). Among MUFA, monoenic 16:1(ranging from 5% to 8%) and 18:1ω9 (ranging from 14% to 21%) formed a considerable percentage of the total fatty acids. The dominant species of SFA were 16:0 (ranging from 15% to 30%) and 18:0 (ranging from 6% to 9%). EPA (20:5ω3) and DHA (22:6ω3) were the dominant PUFA and percentages were 30% (Table 1). No significant differences were found for each fatty acid between varieties of carp.

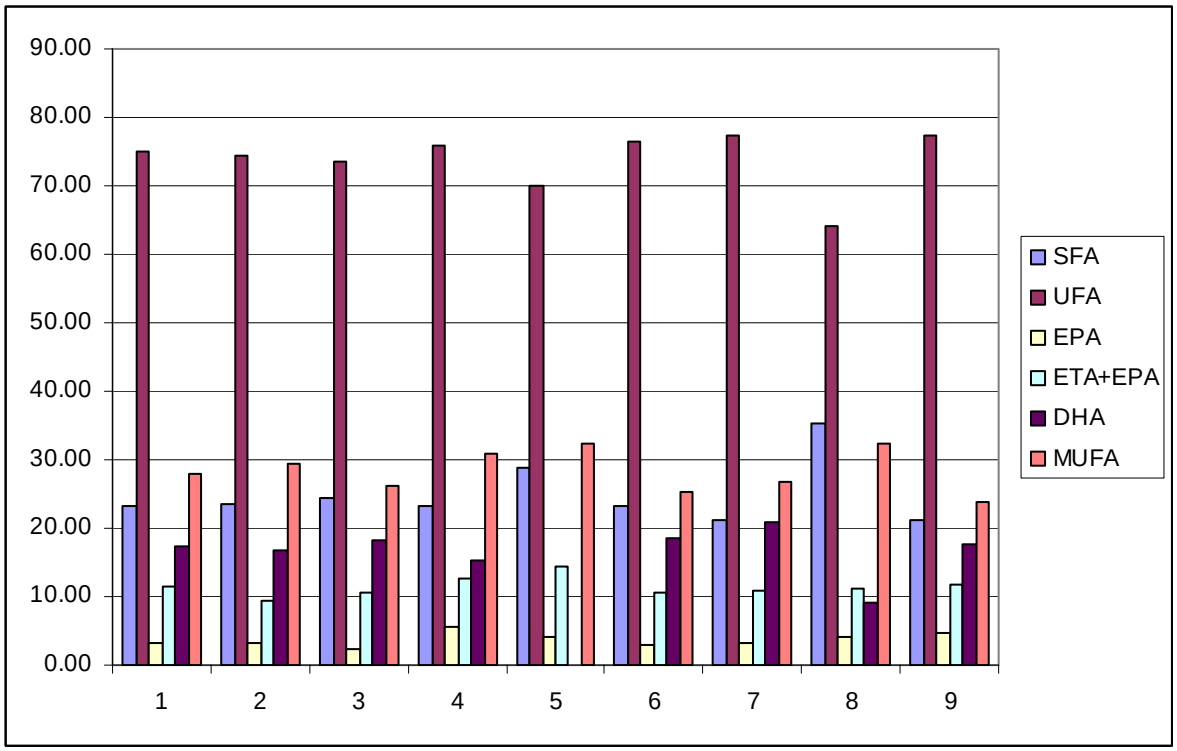

FIGURE 3. Comparison among SFA, UFA, ETA + EPA, DHA and MUFA in experimental fish**.**

In comparison with fish fatty acids, the vegetal oils (Table 2, Figure 4) show higher value for palmitoleic acid in seabuckthorn fruits, the oil being very useful in cosmetics for skin protection and lower values for HUFA.

TABLE 2. Vitamin F determination in seabuckthorn dry fruits.

FAME	Mean mg/g	S.D.	R.S.D. %
methyl undecenoate (C11:1)IS	12.38	0.25	2.02
methyl palmitoleate(C16:1)	10.74	1.30	12.11
methyl palmitate(C16:0)	16.36	1.65	10.06
methyl linoleate (C18:2)(9,12)	3.26	0.38	11.63
methyl oleate (C18:1)(9)	13.58	1.28	9.42
methyl stearate (C18:0)	4.16	0.39	9.30
total	48.11	4.79	9.96
Vitamin F	28.04	0.20	0.70

The fatty acids methyl esters separated in seabuckthorn are presented in Table 2. Figure 4 presents the differences of the FAME profiles in the case of different vegetal oils.

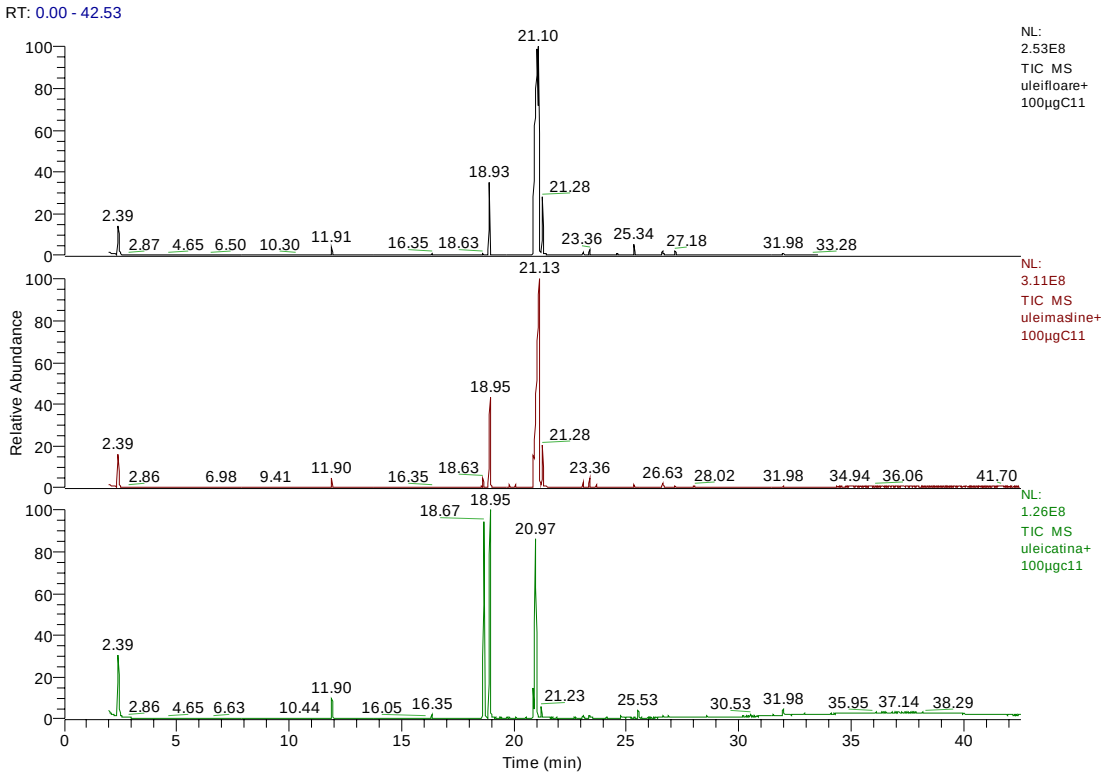

FIGURE 4. Chromatograms of some vegetal oils (sunflower, olive and seebuckthorn).

Conclusions

The method developed showed good precision in the analysis of fatty acids from different biological media. The high value of palmitoleic acid in seabuckthorn fruits makes the oil very useful in cosmetics for skin protection. The high values of HUFA in fish plasma prove the high nutritious of fish.

References

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