Composition and nutritional values of fatty acids in marine organisms by one-step microwave-assisted extraction/derivatization and comprehensive two-dimensional gas chromatography -flame ionization detector

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A B S T R A C T

This work reports the characterization of the lipidic fraction of seven species of marine organisms gathered along the shoreline of the Po Delta Park of Emilia-Romagna Region (Italy) and of the north Adriatic Sea. Two species of oysters (*Crassostrea gigas* and *Ostrea edulis*), two species of clams (*Chamelea gallina* and *Ruditapes philippinarum*), one species of mussel (*Mytilus galloprovincialis*), one species of macroalgae (*Ulva rigida*), and one species of spiny dogfish (*Squalus acanthias*) were analyzed to characterize their fatty acids profile and related nutritional value. The lipid fraction was simultaneously extracted and transesterified into fatty acid methyl esters (FAMEs) by using a recently developed one-step microwave-assisted extraction/derivatization (MAED) method. The obtained FAMEs extract was analyzed by a rapid comprehensive multidimensional gas chromatography (GC × GC) method (30 min). The system was equipped with a reverse set of columns (polar × non-polar) connected through a reversed fill/flush flow modulator. The GC × GC system was coupled with a flame-ionization detector (FID) for both qualitative and quantitative purposes. The MAED- GC × GC-FID methodology was suitable in the context of samples containing high percentages of omega-3 PUFA. A total of 82 FAMEs were tentatively identified using standards, literature data, and the two-dimensional plot location. FAME profiles obtained with the proposed approach were comparable with reference methods (AOCS Ce 2b-11), showing no significant differences. Moreover, to determine the food nutritional value of the samples investigated, the most common nutritional indices (index of atherogenicity, index thrombogenicity, hypocholesterolemic/hypercholesterolemic ratio, health-promoting index, unsaturation index, and the fish lipid quality index) were calculated from FAME pro- files. Among the samples investigated, *Squalus acanthias* presented the best nutritional score, while *Ruditapes philippinarum* had the worst score in 3 out of 6 indices.

1. Introduction

The most scientific evidence of the beneficial effects on human health of marine organism lipids in the diet was proved by studies on the low incidence of heart disease and the complete absence of diabetes mellitus in the Greenlandic Eskimos population [1]. The predominant fatty acids (FAs) in fish and marine organisms are polyunsaturated fattyacids (PUFA), followed by monounsaturated (MU)FAs and saturated (S) FAs. However, the most significant contribution to the beneficial health

effect of marine organisms' intake is given by the presence of the long- chain semi-essential omega-3 FAs, *i.e.* eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3). EPA and DHA are indispensable for many metabolic processes, protecting the circulatory system, preventing the onset of many inflammatory and cardiovascular diseases, regulating the triacylglycerols level in the blood, and pro- moting the development of memory and cognitive skills [2]. However, literature data report a wide variability among the ratio of PUFA/ MUFA/SFA [3]. Moreover, this highly variable composition of the ma- rine fat depends not only on the animal species, but also on the geographical site, season, and animal's organs [4].

The common methodology to analyze FAs is based on three main steps: i) lipid extraction, ii) derivatization of FAs into FA methyl esters (FAMEs), and iii) subsequent analysis by gas chromatography (GC).

The liquid–liquid extraction (LLE) methodology is the most applied to extract the total lipid fraction. In LLE different ratios of chloroform/ methanol/water are usually applied (e.g., Folch [5], Bligh & Dyer [6]). Despite the miniaturization of these approaches over the years, they remain time-consuming [7]. After lipid extraction, acid-catalyzed derivatization transforms FAs, both non-esterified and esterified, into FAMEs. These derivatization methods require different reagents, high amounts of solvents, and a long time of reaction (in general from 25 min up to 2 h) [8]. Finally, GC is undoubtedly the technique of choice for FAs investigation. Polar stationary phases, mainly polyesters-based, are the most used thanks to their capability to resolve FAs with different numbers and positions of the double bonds within the same chain-length group. Despite an excellent resolution even for the separation of posi- tional and/or geometrical FA isomers, when dealing with highly com- plex samples (such as fish oil) the overlap of FAs having different chain lengths may occur. Moreover, a rather long analysis time is required to maximize the resolution, which shows reduced repeatability due to their limited working life [9].

Recently a one-step lipid microwave-assisted extraction/derivatiza- tion (MAED) method followed by a comprehensive two-dimensional (2D)-GC (GC × GC) coupled with a flame-ionization detector (FID) was developed for the characterization of the FAMEs profile in different food commodities. The method proved its accuracy while providing a faster and greener sample preparation step and a more powerful sepa- ration thanks to the use of GC × GC [10]. The goal of the proposed research is to verify the suitability of the MAED- GC × GC-FID methodology for the analysis of samples containing high percentages of omega-3 PUFA. Marine organisms sampled in the Italian areas of the Po Delta Park and North Adriatic Sea were analyzed for this purpose, particularly considering the importance of a detailed characterization of the PUFA's fraction as it implies important health benefits. In this context the higher separation power obtained using GC × GC compared to monodimensional GC, allowed us to report highly accurate nutritional indices based on their FA distribution in the investigated matrices [11].

2. Materials and methods

2.1. Samples and sampling area

Pacific oysters (*Crassostrea gigas*) and European oysters (*Ostrea edulis*) were collected in a farming area located in Valli di Comacchio (44.55–44.65°N; 12.10–12.25°E) which are a wide inner complex of shallow water brackish lagoons. Clams (*Chamelea gallina* and *Ruditapes philippinarum*), mussels

(*Mytilus galloprovincialis*), and macroalgae (*Ulva rigida*) were collected in the Sacca di Goro, which is the southernmost lagoon of the actual Po River Delta (44.78–44.83°N; 12.25–12.33°E). Both these lagoons belong entirely to the Po Delta Park of the Emilia- Romagna Region and are considered Sites of European Community In- terest (S.I.C.) under the "Habitat" Directive n° 43/92. Spiny dogfish (*Squalus acanthias*) was collected in the north Adriatic Sea (45.04°N; 12.33°E) Geographical Sub-Area GSA 17, according to the FAO Ge
neral Fisheries Commission for the Mediterranean (GFCM) [12]. All samples were collected in spring 2022, selected and taxonomically identified by experts in the field. Information relative to full sample names, size, number of individuals, and moisture are reported in Table S1.

1.1. Samples treatment

The entire soft tissues of the studied bivalves (*C. gigas, O.edulis, C. gallina, R. philippinarum* and *M. galloprovincialis*) were removed from the shell (by carefully cutting the adductor muscles with a scalpel), and then washed with distilled water to remove any remains of the shell or sand. The tissue was dried with filter paper and then weighed and homoge-
nized with a blender, frozen in the fridge at —20 °C and subsequently
freeze-dried with a freeze dryer (M. Christ G. GmbH - Alpha 1–2 LDplus). From the spiny dogfish (*Squalus acanthias*), fillets were taken and lyophilized, samples were individually weighed. Finally, macroalgae (*Ulva rigida*) were washed twice with artificial salt water, made at the same concentration present at the lagoon site (25 PSU), to remove sediment residues; two further thorough washes with distillate water were applied to remove salt residues; then dried at room temperature for
30 min on laboratory filter paper, weighed in freeze-dryer flasks, and stored at —20 °C until the freeze-drying step. Freeze-dried macroalgae
were homogenized with a blender. All freeze-dried samples were indi- vidually weighed again to evaluate their moisture content and ground into a homogeneous powder with a ceramic mortar and pestle.

1.2. Chemicals and reagents

Solvents (hexane, cyclohexane, methanol, chloroform, and methyl acetate), standards (Supelco C37 FAME Mix, pure standards solution of n-alkanes in a range from n-C7 to n-C30), and reactives (sodium hy- droxide, sodium sulfate, 14 % BF3/CH_3OH solution, acidic-methanolic solution) were acquired from Merck KGaA (Darmstadt, Germany).

1.3. One-step microwave-assisted extraction and derivatization (MAED)

The MAED procedure was recently described [10]. An ETHOS X MW system with an SR-12 eT TFM rotor (Milestone Srl, Bergamo, Italy) was used. Briefly, 500 mg of each sample was weighed into the microwave (MW) vessel. Ten mL of acidic-methanolic solution and 25 mL of cyclohexane were added, then, the vessel was sealed and placed inside the MW oven under continuous stirring [13]. The MW conditions were
as follows: temperature ramp reaching 120 °C in 2 min and hold for 15
min. After cooling, an aliquot of the supernatant was collected for the following GC × GC analysis. All samples were analyzed in triplicate.

1.4. *Official method Ce 2b-11 by AOCS*

The AOCS Official (Ce 2b-11 by AOCS) was applied for direct methylation of lipids in foods [11]. Briefly, samples were weighted ac- cording to the reference method [11], and 5 mL of NaOH/methanol (20 g/L) was added. The solution was heated under reflux for 15 min. Then, 5 mL of a solution of boron trifluoride-methanol was added, keeping it under reflux for 2 additional minutes before adding 5 mL of hexane and removing it from the heat source. All samples analyzed following this procedure were analyzed in triplicate.

1.5. *Comprehensive two-dimensional gas chromatography (GC × GC) instrumentation*

All the samples were analyzed in a GC × GC-FID system equipped with a medium-polar stationary phase as a first dimension (^{1}D) and a non-polar stationary phase as a second dimension (^{2}D) connected through a flow modulator. Briefly, the ^{1}D column was a SepSolve ^{1}D-FAMEs (stationary phase not disclosed) 20 m × 0.18 mm × 0.1 µm highly polar fused silica capillary column and the ^{2}D column was a SepSolve ^{2}D- FAMEs (stationary phase not disclosed) 5 m × 0.25 mm × 0.1 µm non-polar fused silica capillary column (SepSolve Analytical Ltd, UK). Details regarding parameters, columns, instrumentation, and soft- ware are reported in Table 1.

FAMEs were tentatively identified based on the retention time of the standards (Supelco C37 FAMEs Mix), their elution order on a polar column [14], and their position on the 2D plot [10,15,16], and relevant literature data [10,17,18]. Linear retention indices (LRIs) were only used for aligning FAMEs. 2.7. Statistical analysis FAME significance between MAED and AOCS methods was tested using a t-test with Holm-Bonferroni correction [19]. A significance level of $p < 0.05$ was selected.

2.8. *Nutritional evaluation*

The most common nutritional indices such as the index of athero genicity (IA) and thrombogenicity (IT) [20], hypocholesterolemic/hy percholesterolemic (HH) ratio [21,22], health-promoting index (HPI) [23], unsaturation index (UI) [24], and the fish lipid quality (FLQ) index [25,26] were calculated from identified FAs according to the equations reported in the references and below (Equations (1) to (6)):

$$IA = [C12{:}0 + (4 * C14{:}0) + C16{:}0] / \Sigma UFA \quad (1)$$

$$IT = (C14{:}0 + C16{:}0 + C18{:}0) / [(0.5 * \Sigma MUFA) + (0.5 * \Sigma n6\text{-}PUFA) + (3 * \Sigma n3\text{-}PUFA) + (n3/n6)] \quad (2)$$

$$H/H = (cis\ 18{:}1 + \Sigma PUFA) / (C12{:}0 + C14{:}0 + C16{:}0) \quad (3)$$

$$HPI = \Sigma UFA / [C12{:}0 + (4 * C14{:}0) + C16{:}0] \quad (4)$$

$$UI = 1 * (\%\ monoenoics) + 2 * (dienoics) + 3 * (trienoics) + 4 * (tetraenoics) + 5 * (pentaenoics) + 6 * (hexaenoics) \quad (5)$$

$$FLQ = 100 * (C22{:}6n3 + C20{:}5n3) / \Sigma FA \quad (6)$$

3. *Results and discussion*

3.1. *Method evaluation*

As a first step, the applicability of the MAED method on the different fish and marine organisms herein investigated was verified. The sample of Mylitus galloprovincialis, selected as the representative for the mollusks group was used for a detailed comparison with the reference method [11]. Given their different

nature to mollusks, Ulva rigida and Squalus acanthias were also analysed by comparing MAED and AOCS methods. Fig. 1 displays the GC ×GC-FID chromatogram of the total FA fraction of Mylitus galloprovincialis. The numbers in Fig. 1 correspond to the FAME listed in Table 2. The GC ×GC-FID chromatograms of the other marine organisms are reported in Fig. S1 in Supplementary Information. Except for iso/anteiso and trans/cis isomers, FAMEs 0.05) in most cases, except for a few components present in low quantities. The MAED method has shown to be suitable also for the analysis of marine organisms, significantly simplifying the overall sample preparation procedure compared to the official AOCS method, supporting the pre vious finding of Fina et al., pointing towards a broad application of the method [10]. Moreover, the use of GC ×GC-FID allowed for a detailed and sensitive characterization of the FAMEs profile without the need for long chromatographic runs and supporting the identification of the FAMEs thanks to the well-structured chromatographic separation. 3.2. Fatty acid compositions The FA profile of the seven marine organisms investigated is pre sented in Table 2. SFA ranged from 25.05 % (Squalus acanthias) to 59.78 % (Ruditapes philippinarum) of total FAMEs. Amongst SFAs, palmitic acid (C16:0) was found as the most abundant, ranging between 17.46 % (Squalus acanthias) and 39.45 % (Crassostrea gigas), followed by octa decanoic acid (C18:0) ranging between 3.70 % (Ulva rigida) and 14.87 % (Ruditapes philippinarum). Low percentages of tetradecanoic (C14:0), pentadecanoic (C15:0) and heptadecanoic (C17:0) acids were present, ranging from 0.23 % (Ulva rigida) to 4.23 % (Mytilus galloprovincial), 0.10 % (Ulva rigida) to 0.95 % (Crassostrea gigas), and 0.02 % (Ulva rigida) to 2.66 % (Chamelea gallina), respectively. Crassostrea gigas was the only sample presenting traces of short-chain SFAs in a range of C6-C13. With regards to MUFA, the highest concentration was found in Squalus acanthias (33.19 %), while the lowest was in Chamelea gallina (19.87 %). Oleic (C18:1n9) and palmitoleic (C16:1n9) were the most representative MUFAs, ranging between 5.34 % (Mytilus galloprovincia lis) and 23.86 % (Squalus acanthias), and 1.45 % (Crassostrea gigas) and 13.03 % (Mytillus galloprovincialis), respectively. Gadoleic acids (C20:1n9) and an isomeric eicosaenoic acid (C20:1) were found in a range of 0.18 % (Ulva rigida) and 6.71 % (Squalus acanthias), and 0.35 % (Ulva rigida) and 7.97 % (Ostrea edulis) but not detected in Mytilus gal loprovincialis, respectively. Trace/low concentrations (<0.01–1.36 %) of
the other MUFAs were found. Furthermore, trans-ω9-octadecanoid acid (C18:1n9t) was found in 5 out of 7 samples investigated, in a range from 0.24 % (Squalus acanthias) to 1.75 % (Crassostrea gigas). The content of PUFAs ranged between 15.23 % (Ruditapes philippinarum) and 42.38 % (Mytilus galloprovincialis). DHA (C22:6n3), present in 6 out of 7 samples (not detected in Ulva rigida), ranged from 3.29 % (Ruditapes philippinarum) to 25.56 % (Squalus acanthias). Other ω 3 PUFA such as ω α-linolenic acid (ALA, C18:3n3), EPA (C20:5n3), and docosapentaenoic acid (DPA, C22:5) were present in the sample inves tigated in different concentrations. ALA ranged between 0.09 % (Rudi tapes philippinarum) and 11.47 % (Ulva rigida), EPA was between 0.93 % (Ulva rigida) and 14.28 % (Mytilus galloprovincialis), while DPA was be tween 0.32 % (Crassostrea gigas) and 4.45 % (Squalus acanthias). Traces of 3 eptatridecenoic (C17:3n3) and eicosatrienoic acids (C20:3n3) were present, respectively, in 5 out of 7 samples investigated. Stear idonic acid (SDA, C18:4n3) was only detected in Mytilus galloprovicialis (1.11 %). Among the ω 6 PUFA, the most abundant were linoleic acid (L, C18:2n6c), followed by arachidonic acid (AA, C20:4n6c), ranging be tween 0.08 (Ruditapes philippinarum) and 5.90 % (Ulva rigida), and 0.48 (Ruditapes philippinarum) and 4.27 % (Squalus acanthias), respectively. Traces of γ-linolenic acid (GLA, C18:3n6) and homogammalinolenic acid (C20:3n6) were also present. Furthermore, traces of trans ω 6- octadecenoic acid (C18:2n6t) were detected in Crassostrea gigas (0.02 %) and Mytilus galloprovincialis (0.04 %). Other PUFAs were present in low concentrations in the sample investigated, but unfortunately, it was not always possible to determine the double bond position in the backbone of the FAs. Among them, a not well-defined docosadienoic acid (C22:2) was present in 6 out of 7 samples, ranging between 0.14 (Squalus acanthias) and 6.79 % (Ruditapes philippinarum). The composition of fats in marine organisms varies significantly in the

literature due to factors like animal species, geographical location, season, and specific organs. Indeed, reported ranges for SFAs, MUFAs, and PUFAs show considerable disparities [3,17,27–29]. 3.3. Nutritional aspects Dietary fats are generally FAs that may play positive or negative roles in the prevention and treatment of diseases. According to different food- based dietary guidelines for preventing cardiovascular diseases, an intake of FAs with ω 6/ ω 3 ratio of at least 5:1 with the diet is suggested for health promotion [30]. In Western diets, the ratio has been estimated between 15:1–16.7:1. This diet-habitude can promote the pathogenesis of many diseases, such as cardiovascular inflammatory and autoimmune diseases, whereas increased levels of ω 3 PUFAs (it means a low ω 6/ ω 3 ratio) exert suppressive effects [31]. In all the samples investigated, a greater or equal content of values of ω 6/ ω ω 3 with respect to ω 6 FAs was reported, with 3 ratios ranging from ~ 1:7 (Mytilus galloprovincialis) to ~ 1:1 (Ulva rigida). Regarding the nutritional indices, IT and IA assess the potential of a diet to promote the development of atherosclerosis and blood clot for mation. Higher values indicate a greater risk of cardiovascular disease, while lower values suggest a healthier diet in terms of reducing these risks [20]. Among the samples investigated, Crassostrea gigas had the highest values of IA (1.09) and Ruditapes philippinarum of IT (1.77), while Squalus acanthias was the sample with the lowest values in both indices (0.25 and 0.22). HH reflects the impact of a diet on cholesterol levels. A hypo cholesterolemic diet lowers cholesterol, while a hypercholesterolemic diet increases cholesterol levels. Higher HH values are desirable for cardiovascular health [21,22]. Squalus acanthias had the best HH score (3.69) while Ruditapes philippinarum was the lowest (0.68). HPI measures the overall health benefits of a diet based on multiple factors, such as nutrient composition, antioxidant content, and potential disease prevention. Higher HPI values indicate a diet that promotes better health and reduces the risk of chronic diseases [23]. HPI ranged between 3.99 (Squalus acanthias) and 0.92 (Crassostrea gigas). UI evaluates the degree of unsaturation or presence of unsaturated fats in a diet. Higher values indicate a greater proportion of unsaturated fats, such as MU and PU fats, which are considered healthier than saturated ones. A higher UI suggests a more heart-healthy diet [24]. The samples with the higher UI were Squalus acanthias (255.25) followed by Mytilus galloprovincialis (214.3), while the UI of Ruditapes philippinarum was the lowest (77.11). FLQ assesses the quality of lipids or fats derived from fish in the diet. It considers the balance of ω 3 FAs, particularly EPA and DHA (such as in the case of the samples investigated), which have numerous health benefits. A higher FLQ indicates a diet rich in beneficial ω 3 FAs, which contributes to lower cardiovascular risks and improved brain health [25,26]. FLQ ranged between 28.17 (Squalus acanthias) and 0.93 (Ulva rigida). The low value of Ulva rigida is due to the absence of DHA. These nutritional indices, reported in Table 3, provide valuable in formation about the potential health effects of different diets, guiding individuals toward making informed choices for their overall well- being. Indeed, FAs present in marine organisms play a crucial role in the human diet by providing essential/semi-essential ω3-FA, which contributes to cardiovascular health and brain function [32]. 4. Conclusion The lipid extraction/derivatization step by MAED occurred in ~ 15 min (up to 12 samples simultaneously), while the GC ×GC-FID time analysis was ~ 30 min for a single run. MAED-GC ×GC-FID method ology allowed a complete characterization of the FAs fraction present in marine organisms without the need for more powerful detectors such as mass spectrometry (MS) [33]. Indeed, the high separation capability of GC × GC alone, due to the formation of group-type patterning in the 2D space, was adequate for the tentative identification of FAs, eliminating the necessity for MS coupling, and further streamlining the method, thereby promoting a more widespread adoption of this methodology. Moreover, the detailed characterization of the FA composition in marine organisms can be crucial to i) provide valuable insights into the nutritional value of marine organisms as a food source, ii) help assess the potential health benefits associated with consuming these organisms, particularly due to the presence of essential ω 3 FAs; iii) aid in evaluating the ecological roles of marine organisms and their impact on marine ecosystems, possibly supporting sustainable fisheries management and conservation efforts by providing information on

the lipid profiles of marine species. Among the samples investigated, Squalus acanthias always presented the best nutritional score, while Ruditapes philippinarum had the worst scores in 3 out of 6 indices. However, even if no experiments were done regarding geographical, seasonal, and environmental factors, it is worth reporting that the specific composition of the surrounding water, which varies by location, can affect the availability of nutrients for marine organisms. Seasonal changes can impact factors like water temperature and food availability, influencing the growth rates and nutritional content of marine organisms. Environmental factors, including pollution and changes in habitat, can introduce contaminants and/or alter nutrient sources, further influencing the overall nutritional profile of marine organisms. Considering all these reasons, the nutritional index cannot be the only parameter to take into account to assess food quality. Therefore, further dedicated studies would be needed to increase knowledge about the nutritional quality of a much larger set of marine organisms collected in different geographical areas, seasons, and environmental conditions.

Table 1 Instrumental details.

Table 1
Instrumental details.

GC × GC-FID PARAMETERS		
Carrier gas	helium	
^{1}D flow-rate	0.5 mL/min	
^{2}D flow-rate	20 mL/min	
Modulation period	3 s	
Reinjection time	100 ms	
Oven temperature program	3 min at 40 °C; from 40 to 260 °C at 9 °C/min and hold it for 2 min	
Injection mode	split mode (1:10)	
Injection volume	0.5 μL	
Injector temperature	250 °C	
FID temperature	270 °C	
FID data acquisition frequency	100 Hz	
FID parameters	air flow: 350 mL/min, H2: 35 mL/min; make-up gas: 20 mL/min	
Instrumentation		
Autosampler	AOC −30i Autoinjector	Shimadzu
GC	Nexis GC-2030	Shimadzu
Detector	FID	Shimadzu
^{1}D column	^{1}D-FAMEs 20 m × 0.18 mm × 0.1 μm polar fused silica capillary column	SepSolve
^{2}D column	^{2}D-FAMEs 5 m × 0.25 mm × 0.1 μm non-polar fused silica capillary column	SepSolve
Bleedline	4.20 m × 0.1 mm uncoated capillary segment	SepSolve
Modulator	reversed fill/flush INSIGHT flow modulator	SepSolve
Software		
Data acquisition	LabSolution Version 5.111	Shimadzu
Data processing	ChromSpace Version 1.5.1	Markes International Limited

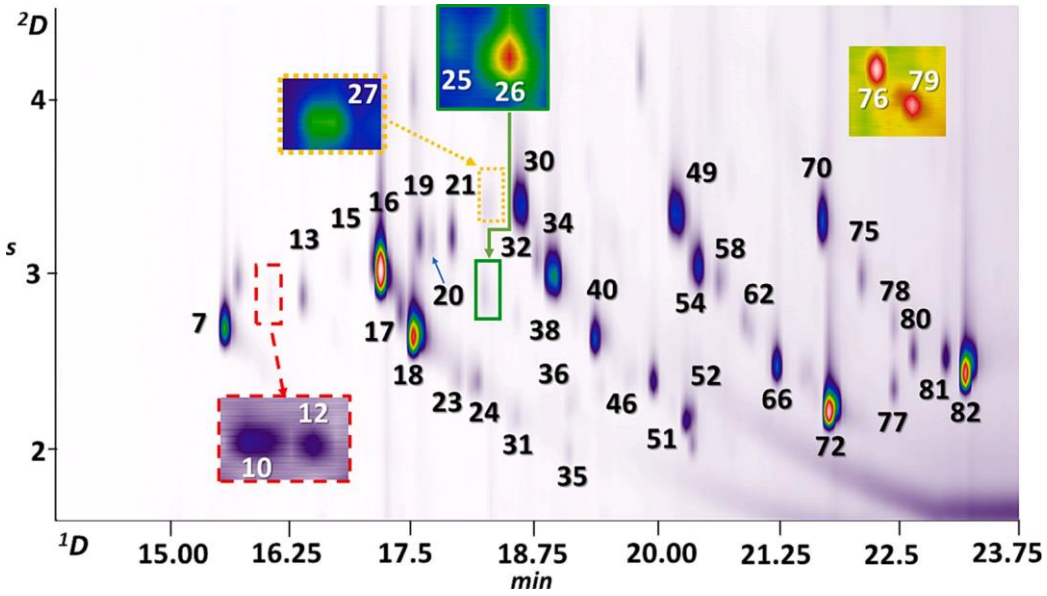

Fig. 1. GC × GC-FID chromatogram of the total FA fraction of *Mylitus galloprovincialis*. Each number in Figure 1 corresponds to a different FAME, also listed in Table 2. Except for iso/anteiso and trans/cis isomers, FAMEs < 0.1% are not reported.

Table 2
FAME profile (% ± standard deviation of three replicates) of the marine organisms investigated by MAED-GC × GC-FID. RT: retention time in first (^{1}D) and second (^{2}D) dimension. RT: retention time in first (1D) and second (2D) dimension.

N.	FAME	^{1}D-RT	^{2}D-RT	*Crassostrea gigas*	*Ostrea edulis*	*Chamelea gallina*	*Ruditapes philippinarum*	*Squalus acanthias*	*Ulva rigida*	*Mytilus galloprovincialis*
1	**C6:0***	7.60	1.80	0.04 ± 0.006						
2	**C8:0***	10.07	1.95	0.02 ± 0.005						
3	**C10:0***	12.24	2.15	tr						
4	**C11:0***	13.24	2.27	0.01 ± 0.001						
5	**C12:0***	14.16	2.38	0.02 ± 0.004	0.03 ± 0.003		0.03 ± 0.001		0.02 ± 0.001	0.03 ± 0.008
6	**C13:0***	15.06	2.52	0.02 ± 0.004	0.01 ± 0.00 3	tr	0.03 ± 0.003			tr
7	**C14:0***	15.89	2.63	1.98 ± 0.242	1.99 ± 0.111	1.98 ± 0.205	1.76 ± 0.011	0.34 ± 0.006	0.23 ± 0.038	4.23 ± 0.499
8	**C14:1**	16.14	2.46	0.02 ± 0.001	tr		tr	tr	tr	0.06 ± 0.011
9	**C14:1**	16.30	2.41	tr	tr	0.01 ± 0.002	0.03 ± 0.002	tr	0.02 ± 0.002	
10	**C15:0iso**	16.38	2.72	0.16 ± 0.023	0.18 ± 0.007	0.05 ± 0.007	0.17 ± 0.003		tr	0.04 ± 0.004
11	**C14:1**	16.41	2.37	0.01 ± 0.001	tr	0.01 ± 0.001	tr	0.01 ± 0.001		0.02 ± 0.003
12	**C15:0anteiso**	16.49	2.78	0.01 ± 0.013	0.02 ± 0.000	0.02 ± 0.002	0.03 ± 0.001	0.01 ± 0.004		0.02 ± 0.006
13	**C15:0***	16.75	2.78	0.95 ± 0.107	0.67 ± 0.033	0.55 ± 0.050	0.82 ± 0.010	0.15 ± 0.004	0.10 ± 0.026	0.48 ± 0.041
14	**C15:1**	17.00	2.61					0.13 ± 0.017		
15	**C16:0iso/ anteiso**	17.16	2.92	0.12 ± 0.015	0.15 ± 0.025	0.52 ± 0.042	0.76 ± 0.010	0.09 ± 0.017	tr	0.08 ± 0.016
16	**C16:0***	17.47	2.96	39.45 ± 3.606	35.37 ± 1.417	23.94 ± 2.826	32.97 ± 0.489	17.46 ± 0.935	32.46 ± 1.050	22.68 ± 1.546
17	**C16:1n7**	17.71	2.71	0.57 ± 0.069	0.56 ± 0.046	0.54 ± 0.092	0.45 ± 0.013	0.22 ± 0.001		0.82 ± 0.097
18	**C16:1n9**	17.84	2.62	1.45 ± 0.161	1.70 ± 0.246	3.90 ± 0.681	3.89 ± 0.223	2.70 ± 0.133	6.34 ± 0.254	13.03 ± 0.828
19	**C17:0iso**	17.90	3.09	0.59 ± 0.080	0.60 ± 0.010	2.91 ± 0.169	3.39 ± 0.404	0.34 ± 0.037		0.76 ± 0.055
20	**C17:0anteiso**	18.05	3.12	0.03 ± 0.043	0.14 ± 0.012	0.92 ± 0.047	0.62 ± 0.057	0.28 ± 0.022		0.14 ± 0.033
21	**C17:0***	18.23	3.12	2.37 ± 0.212	2.20 ± 0.210	2.66 ± 0.118	2.49 ± 0.095	0.53 ± 0.050	0.02 ± 0.005	0.69 ± 0.028
22	**C17:1**	18.32	2.83		0.01 ± 0.001	0.73 ± 0.031	0.62 ± 0.074	0.29 ± 0.009		0.04 ± 0.009
23	**C16:2**	18.32	2.42	tr		0.05 ± 0.004	tr	0.02 ± 0.002	0.82 ± 0.090	0.22 ± 0.005
24	**C16:2**	18.48	2.39	0.01 ± 0.001		0.04 ± 0.006	0.02 ± 0.004	0.02 ± 0.003		0.49 ± 0.032
25	**C17:1n7t**	18.50	2.99							0.02 ± 0.006
26	**C17:1n7c**	18.56	2.81	0.46 ± 0.049	0.14 ± 0.017	0.32 ± 0.059	0.23 ± 0.005	0.54 ± 0.032	0.86 ± 0.012	0.15 ± 0.021
27	**C18:0iso**	18.58	3.33	0.19 ± 0.025	0.17 ± 0.024	0.45 ± 0.022	0.03 ± 0.010	0.42 ± 0.012		0.12 ± 0.012
28	**C18:0anteiso**	18.74	3.23			0.03 ± 0.004	0.01 ± 0.000	0.01 ± 0.003		tr
29	**C17:2**	18.91	2.69	0.07 ± 0.017	0.11 ± 0.001	0.08 ± 0.002	0.12 ± 0.007			
30	**C18:0***	18.93	3.29	8.07 ± 0.597	12.06 ± 0.767	10.37 ± 0.141	14.87 ± 0.990	5.11 ± 0.217	0.27 ± 0.010	3.75 ± 0.042
31	**C16:3n3**	18.94	2.19	0.01 ± 0.001	0.01 ± 0.001	0.02 ± 0.003		0.01 ± 0.001	0.53 ± 0.020	0.17 ± 0.019
32	**C18:1n9t**	19.12	2.99	1.75 ± 0.175	0.27 ± 0.001	0.34 ± 0.046		0.24 ± 0.046		0.45 ± 0.016
33	**C16:3**	19.20	2.04	tr	0.01 ± 0.001	0.01 ± 0.004			4.40 ± 0.151	tr
34	**C18:1n9c**	19.27	2.91	10.81 ± 0.911	9.27 ± 0.352	5.82 ± 0.908	8.36 ± 0.504	23.86 ± 0.310	18.73 ± 0.215	5.34 ± 0.044
35	**C16:4**	19.44	2.01		0.01 ± 0.001	0.01 ± 0.001				0.07 ± 0.012
36	**C17:3n3**	19.45	2.24	0.15 ± 0.085	0.06 ± 0.009	0.09 ± 0.011	0.30 ± 0.041			0.11 ± 0.013
37	**C19:0iso/ anteiso**	19.44	3.42	0.04 ± 0.006	0.03 ± 0.002	0.05 ± 0.009	0.01 ± 0.001	0.05 ± 0.003		
38	**C18:2n6t**	19.49	2.69	0.02 ± 0.010		tr		0.05 ± 0.004		0.04 ± 0.006
39	**C19:0**	19.62	3.47	0.06 ± 0.006	0.12 ± 0.029	0.13 ± 0.024	0.14 ± 0.010	0.10 ± 0.003		0.02 ± 0.007
40	**C18:2n6c**	19.71	2.61	1.29 ± 0.077	1.61 ± 0.053	1.24 ± 0.009	0.08 ± 0.015	0.76 ± 0.045	5.90 ± 0.160	2.06 ± 0.016
41	**C20:0iso**	19.87	3.67	0.09 ± 0.019	0.04 ± 0.002					

(*continued on next page*)

Table 2 (*continued*)

N.	FAME	^{1}D-RT	^{2}D-RT	*Crassostrea gigas*	*Ostrea edulis*	*Chamelea gallina*	*Ruditapes philippinarum*	*Squalus acanthias*	*Ulva rigida*	*Mytilus galloprovincialis*
42	**C19:1**	19.92	3.03	0.17 ± 0.046	0.21 ± 0.031			0.34 ± 0.020		0.03 ± 0.003
43	**C18:2**	19.49	2.76							0.05 ± 0.014
44	**C18:3n6**	20.05	2.40	0.02 ± 0.006		0.89 ± 0.077		0.10 ± 0.012	0.75 ± 0.1058	0.09 ± 0.015
45	**C20:0anteiso**	20.05	3.56	0.03 ± 0.004	0.07 ± 0.010					
46	**C20:0***	20.29	3.66	0.08 ± 0.002	0.15 ± 0.018	0.49 ± 0.071	0.75 ± 0.045	0.09 ± 0.015	0.34 ± 0.005	tr
47	**C18:3n3**	20.31	2.37	1.45 ± 0.068	1.94 ± 0.287	1.41 ± 0.264	0.09 ± 0.007	0.28 ± 0.001	11.47 ± 0.457	1.05 ± 0.021
48	**C18:4n3**	20.47	2.12							1.11 ± 0.073
49	**C20:1n9**	20.48	3.29	5.79 ± 0.364	4.22 ± 0.014	2.40 ± 0.047	6.71 ± 0.505	2.61 ± 0.006	0.18 ± 0.025	4.18 ± 0.243
50	**C21:0iso/ anteiso**	20.51	3.69				0.27 ± 0.036			
51	**C20:1**	20.59	3.22	2.76 ± 0.158	7.97 ± 1.118	5.30 ± 0.111	3.12 ± 0.180	1.50 ± 0.117	0.35 ± 0.005	
52	**C18:4n6**	20.66	2.19	0.75 ± 0.133	0.57 ± 0.053	2.37 ± 0.204	0.15 ± 0.012	0.13 ± 0.001	7.46 ± 0.314	0.03 ± 0.002
53	**C19:3**	20.76	2.50	0.25 ± 0.010	0.26 ± 0.034					
54	**C20:2**	20.78	2.98	0.31 ± 0.053	0.46 ± 0.070	0.61 ± 0.054	0.38 ± 0.043	0.09 ± 0.071		2.48 ± 0.153
55	**C21:1**	20.84	3.43			0.09 ± 0.017	0.05 ± 0.006			
56	**C21:0***	20.91	3.84	0.06 ± 0.008	0.06 ± 0.003	0.03 ± 0.001	0.04 ± 0.008			
57	**C21:1**	20.94	3.42			0.17 ± 0.001	0.03 ± 0.006			
58	**C20:2**	21.01	2.90	0.19 ± 0.153	0.53 ± 0.061	0.51 ± 0.054	0.48 ± 0.076	0.28 ± 0.020		0.46 ± 0.025
59	**C20:3**	21.09	2.80							0.25 ± 0.012
60	**C19:4**	21.11	2.33	0.27 ± 0.061	0.30 ± 0.043	0.33 ± 0.046				
61	**C21:1**	21.12	3.44		0.13 ± 0.011	0.15 ± 0.023	0.03 ± 0.006			tr
62	**C20:3n6**	21.25	2.68	0.09 ± 0.023	0.07 ± 0.013			0.05 ± 0.015		0.13 ± 0.021
63	**C20:3n3**	21.39	2.62	0.07 ± 0.008	0.10 ± 0.013	0.08 ± 0.012		0.10 ± 0.002	0.38 ± 0.013	
64	**C21:2**	21.48	3.12	0.03 ± 0.003	0.03 ± 0.004		0.03 ± 0.005			
65	**C22:0***	21.50	4.04	0.05 ± 0.013	0.07 ± 0.007	0.26 ± 0.032	0.26 ± 0.020	0.05 ± 0.010	2.42 ± 0.356	0.01 ± 0.001
66	**C20:4n6**	21.55	2.45	0.80 ± 0.032	1.09 ± 0.165	2.45 ± 0.243	0.48 ± 0.049	4.27 ± 0.094	0.63 ± 0.014	1.86 ± 0.115
67	**C22:1n9**	21.73	3.62	0.19 ± 0.021	0.18 ± 0.030	0.08 ± 0.010	1.36 ± 0.032	0.48 ± 0.033	0.31 ± 0.042	0.01 ± 0.001
68	**C20:4**	21.87	2.42	0.15 ± 0.007	0.24 ± 0.017	0.27 ± 0.032	0.19 ± 0.033	0.30 ± 0.011	0.96 ± 0.097	0.16 ± 0.010
69	**C20:5**	21.96	2.24	0.17 ± 0.027	0.19 ± 0.030	0.20 ± 0.028	0.24 ± 0.005	tr		
70	**C22:2**	22.04	3.21	4.06 ± 0.276	5.25 ± 0.620	0.78 ± 0.064	6.79 ± 0.110	0.14 ± 0.020		2.69 ± 0.262
71	**C23:0***	22.09	4.21	4.31 ± 0.290	0.28 ± 0.072	0.14 ± 0.016	0.02 ± 0.002	tr		0.04 ± 0.005
72	**C20:5n3**	22.11	2.25	1.96 ± 0.141	2.42 ± 0.446	2.64 ± 0.330	1.33 ± 0.048	2.61 ± 0.078	0.93 ± 0.033	14.28 ± 0.849
73	**C21:3**	22.17	2.85	0.14 ± 0.014	0.12 ± 0.004					
74	**C20:5**	22.41	2.19	0.08 ± 0.005	0.17 ± 0.013				0.43 ± 0.059	
75	**C22:3**	22.47	2.92	0.29 ± 0.002	0.54 ± 0.073	0.29 ± 0.024	0.20 ± 0.041	0.06 ± 0.017	tr	0.49 ± 0.060
76	**C24:0***	22.69	4.34	0.17 ± 0.040	0.16 ± 0.029	0.23 ± 0.033	0.14 ± 0.002	tr	0.01 ± 0.001	0.17 ± 0.015
77	**C21:5**	22.77	2.34	0.08 ± 0.006	0.07 ± 0.013	0.50 ± 0.006	0.13 ± 0.018	0.10 ± 0.007		0.29 ± 0.024
78	**C22:4**	22.79	2.69	0.08 ± 0.013	0.16 ± 0.039	2.05 ± 0.133	0.16 ± 0.020	1.30 ± 0.008		0.17 ± 0.038
79	**C24:1n9**	22.94	3.87	tr	0.01 ± 0.001		0.02 ± 0.005	0.26 ± 0.022	tr	0.12 ± 0.003
80	**C22:4**	22.98	2.55	0.42 ± 0.011	0.69 ± 0.005	1.61 ± 0.121	0.19 ± 0.011	1.12 ± 0.022	0.13 ± 0.014	0.53 ± 0.044
81	**C22:5**	23.36	2.63	0.32 ± 0.004	0.40 ± 0.017	2.33 ± 0.243	0.58 ± 0.051	4.45 ± 0.019	2.53 ± 0.167	0.95 ± 0.093

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Table 2 (*continued*)

N.	FAME	^{1}D-RT	^{2}D-RT	*Crassostrea gigas*	*Ostrea edulis*	*Chamelea gallina*	*Ruditapes philippinarum*	*Squalus acanthias*	*Ulva rigida*	*Mytilus galloprovincialis*
82	**C22:6n3c***	23.54	2.58	3.47 ± 0.183	3.33 ± 0.325	14.05 ± 0.343	3.29 ± 0.030	25.56 ± 0.384		12.11 ± 1.413

FAME* identified by standard; tr: FAME present in trace (<0.01 %).

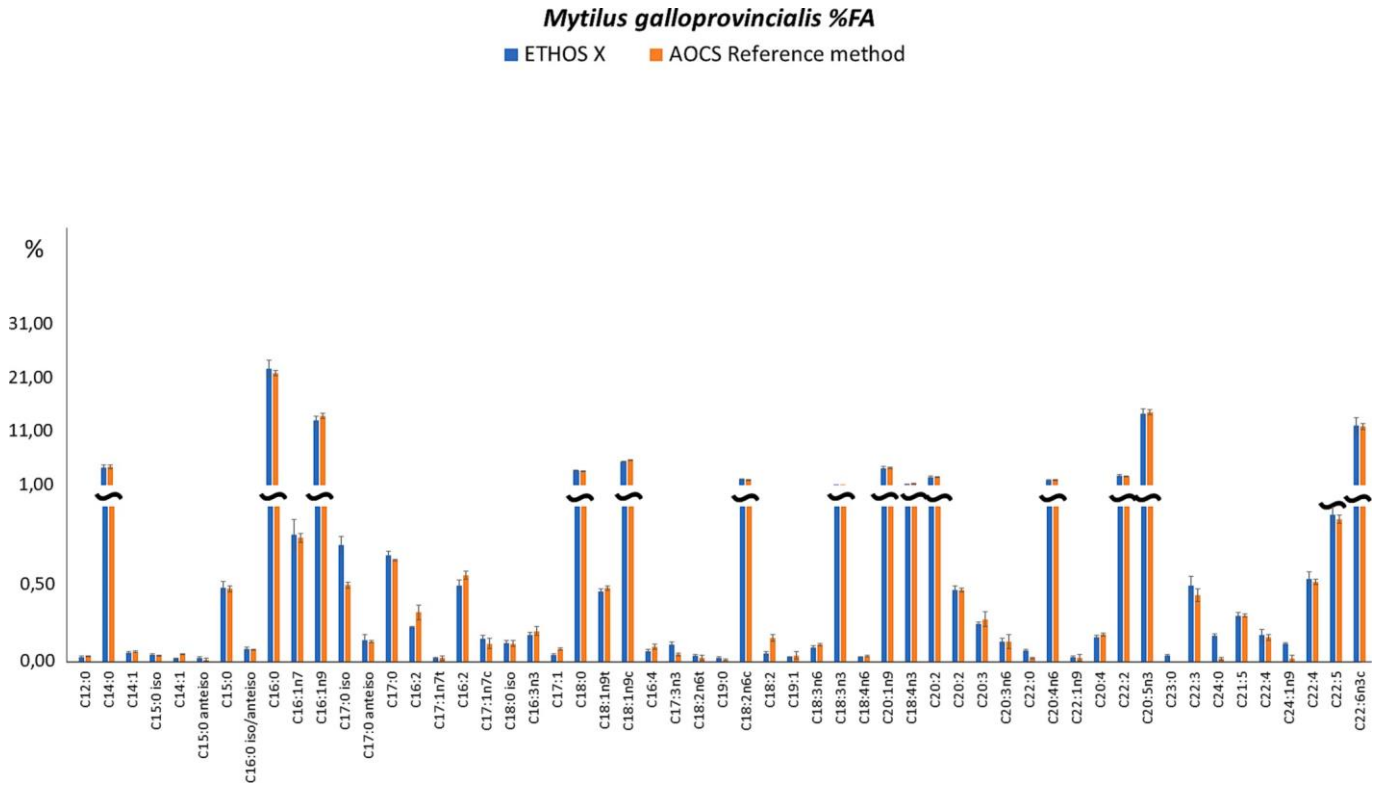

Fig. 2. Comparison of the FAMEs percentage profile of Mylitus galloprovinvialis using the reference method (orange) [11] and the MAED method (blue). Error bars indicate the standard deviation of three replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Nutritional indices of FAs present in marine organisms(IA: index of atherogenicity; IT: index of thrombogenicity; HH: hypocholesterolemic/hypercholesterolemic ratio; HPI: health-promoting index; FLQ: fish lipid quality index; UI: unsaturation index). SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; UFA: unsaturated fatty acid. The values reported are the average of 3 replicates ± standard deviation.

	Crassostrea gigas	*Ostrea edulis*	*Chamelea gallina*	*Ruditapes philippinarum*	*Squalusacanthias*	*Ulva rigida*	*Mytilusgalloprovincialis*
Total SFA	58.01 ± 1.063	54.44 ± 0.100	45.7 ± 3.170	59.78 ± 0.360	25.05 ± 1.300	35.59 ± 1.720	33.29 ± 2.180
Total MUFA	24.11 ± 0.169	24.68 ± 2.970	19.87 ± 2.540	24.91 ± 0.150	33.19 ± 1.250	26.78 ± 0.240	24.32 ± 0.630
Total PUFA	19.03 ± 2.880	20.73 ± 3.060	34.4 ± 5.720	15.23 ± 0.510	41.75 ± 0.050	37.32 ± 1.890	42.38 ± 2.910
TOTAL UFA	43.15 ± 3.048	45.42 ± 0.090	54.27 ± 3.180	40.14 ± 0.360	74.94 ± 1.300	64.10 ± 2.120	66.70 ± 2.280
PUFA ω3	6.85 ± 0.379	7.85 ± 1.060	18.25 ± 3.830	5.01 ± 0.030	28.56 ± 0.310	13.31 ± 0.520	28.84 ± 2.170
PUFA ω6	3.47 ± 0.712	3.34 ± 0.560	6.50 ± 0.460	0.72 ± 0.000	5.33 ± 0.150	14.74 ± 0.620	4.21 ± 0.100
ω6/ω3	0.51 ± 0.132	0.42 ± 0.010	0.36 ± 0.050	0.14 ± 0.000	0.19 ± 0.010	1.11 ± 0.000	0.15 ± 0.010
PUFA/SFA	0.26 ± 0.038	0.38 ± 0.060	0.76 ± 0.180	0.25 ± 0.010	1.67 ± 0.090	1.05 ± 0.100	1.28 ± 0.180
IA	1.09 ± 0.091	0.95 ± 0.040	0.59 ± 0.130	1.00 ± 0.010	0.25 ± 0.020	0.52 ± 0.040	0.60 ± 0.070
IT	1.40 ± 0.004	1.30 ± 0.090	0.54 ± 0.130	1.77 ± 0.000	0.22 ± 0.010	0.53 ± 0.030	0.31 ± 0.040
HH	0.73 ± 0.082	0.80 ± 0.110	1.58 ± 0.390	0.68 ± 0.020	3.69 ± 0.180	1.72 ± 0.110	1.78 ± 0.250
HPI	0.92 ± 0.076	1.05 ± 0.050	1.74 ± 0.390	1.00 ± 0.020	3.99 ± 0.270	1.92 ± 0.130	1.70 ± 0.220
FLQ	5.35 ± 0.144	5.76 ± 0.770	16.69 ± 4.070	4.63 ± 0.030	28.17 ± 0.310	0.93 ± 0.030	26.40 ± 2.220
UI	88.74 ± 3.527	97.45 ± 7.940	181.33 ± 23.602	77.11 ± 0.600	255.25 ± 2.350	148.99 ± 6.910	214.3 ± 13.940

CRediT authorship contribution statement

Donatella Ferrara: Writing – review & editing, Software, Formal analysis, Data curation. **Mirco**

Cescon: Writing – review & editing, Investigation, Formal analysis. **Giulia Giacoppo:** Software, Formal analysis, Data curation. **Valentina Costa:** Writing – review & editing, Investigation, Formal analysis. **Giorgia Purcaro:** Writing – review & editing, Supervision, Software, Resources, Methodology, Funding acquisition, Conceptualization. **Natasha Damiana Spadafora:** Writing – review & editing, Software, Data curation. **Chiara Cordero:** Writing – review & editing, Supervision. **Luisa Pasti:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Alberto Cavazzini:** Writing – review & editing, Supervision. **Marco Beccaria:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

[1] H.O. Bang, J. Dyerberg, A.B. Nielsen, Plasma lipid and lipoprotein in greenlandic west-coast eskimos, Lancet 297 (1971) 1143–1146, https://doi.org/10.1016/ S0140-6736(71)91658-8.

[2] D. Swanson, R. Block, S.A. Mousa, Omega-3 fatty acids EPA and DHA: health benefits throughout life, Adv. Nutr. 3 (2012) 1–7, https://doi.org/10.3945/ an.111.000893.

[3] R. Costa, M. Beccaria, E. Grasso, A. Albergamo, M. Oteri, P. Dugo, S. Fasulo, L. Mondello, Sample preparation techniques coupled to advanced chromatographic methods for marine organisms investigation, Anal. Chim. Acta 875 (2015) 41–53, https://doi.org/10.1016/j.aca.2015.03.032.

[4] V.J. Sinanoglou, C. Proestos, D.Z. Lantzouraki, A.C. Calokerinos, S. Miniadis- Meimaroglou, Lipid evaluation of farmed and wild meagre (Argyrosomus regius), Eur. J. Lipid Sci. Technol. 116 (2014) 134–143, https://doi.org/10.1002/ ejlt.201300346.

[5] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509, https://doi.org/10.1016/s0021-9258(18)64849-5.

[6] E.G. Blingh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 912–917, https://doi.org/10.1139/o59-099.

[7] R.K. Saini, P. Prasad, X. Shang, Y.S. Keum, Advances in lipid extraction methods—a review, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms222413643.
[8] W.W. Christie, X. Han, Preparation of derivatives of fatty acids, in: Lipid Analysis, Woodhead Publishing, 2012: pp. 145–158. https://doi.org/10.1533/9780857097866.145.
[9] W.W. Christie, X. Han, Gas chromatographic analysis of fatty acid derivatives, in: Lipid Analysis, Woodhead Publishing, 2012: pp. 159–180. https://doi.org/10.1533/9780857097866.159.
[10] A. Fina, S. Mascrez, M. Beccaria, C. De Luca, J. Aspromonte, C. Cordero, G. Purcaro, A high throughput method for fatty acid profiling using simultaneous microwave-assisted extraction and derivatization followed by reversed fill/flush flow modulation comprehensive multidimensional gas chromatography, Adv Sampl Prep 4 (2022) 100039, https://doi.org/10.1016/j.sampre.2022.100039.
[11] AOCS, Official Method Ce 2b-11. Direct Methylation of LIpids in Foods by Alkali Hydrolysis, Urbana,USA, 2017.
[12] General Fisheries Commission for the Mediterranean - GFCM, (n.d.). https://www.fao.org/gfcm/data/maps/gsas/en/ (accessed August 30, 2023).
[13] D. Ferrara, M. Beccaria, C.E. Cordero, G. Purcaro, Microwave-assisted extraction in closed vessel in food analysis, J. Sep. Sci. (2023), https://doi.org/10.1002/ jssc.202300390.
[14] K. Eder, Gas chromatographic analysis of fatty acid methyl esters, J Chrom B 671 (1995) 113–131, https://doi.org/10.1016/0378-4347(95)00142-6.
[15] P.Q. Tranchida, A. Giannino, M. Mondello, D. Sciarrone, P. Dugo, G. Dugo, L. Mondello, Elucidation of fatty acid profiles in vegetable oils exploiting group- type patterning and enhanced sensitivity of comprehensive two-dimensional gas chromatography, J. Sep. Sci. 31 (2008) 1797–1802, https://doi.org/10.1002/ jssc.200800002.
[16] S. Salivo, M. Beccaria, G. Sullini, P.Q. Tranchida, P. Dugo, L. Mondello, Analysis of human plasma lipids by using comprehensive two-dimensional gas chromatography with dual detection and with the support of high-resolution time- of-flight mass spectrometry for structural elucidation, J. Sep. Sci. 38 (2015) 267–275, https://doi.org/10.1002/jssc.201400844.
[17] C. Ragonese, L. Tedone, M. Beccaria, G. Torre, F. Cichello, F. Cacciola, P. Dugo, L. Mondello, Characterisation of lipid fraction of marine macroalgae by means of chromatography techniques coupled to mass spectrometry, Food Chem. 145 (2014) 932–940, https://doi.org/10.1016/j.foodchem.2013.08.130.
[18] M. Beccaria, R. Costa, G. Sullini, E. Grasso, F. Cacciola, P. Dugo, L. Mondello, Determination of the triacylglycerol fraction in fish oil by comprehensive liquid chromatography techniques with the support of gas chromatography and mass spectrometry data, Anal. Bioanal. Chem. 407 (2015) 5211–5225, https://doi.org/ 10.1007/s00216-015-8718-y.
[19] G. Hommel, A stagewise rejective multiple test procedure based on a modified Bonferroni test, 1988. http://biomet.oxfordjournals.org/.
[20] A.M. Fehily, J.E. Pickering, J.W.G. Yarnell, P.C. Elwood, Dietary indices of atherogenicity and thrombogenicity and ischaemic heart disease risk: the Caerphilly prospective study, Br. J. Nutr. 71 (1994) 249–257, https://doi.org/ 10.1079/bjn19940131.
[21] A. Bonanno, A. Di Grigoli, F. Vitale, M. Alabiso, C. Giosu`e, F. Mazza, M. Todaro, Legume grain-based supplements in dairy sheep diet: effects on milk yield, composition and fatty acid profile, Anim. Prod. Sci. 56 (2016) 130–140, https:// doi.org/10.1071/AN14019.
[22] J. Santos-Silva, R. Bessa, F. Santos-Silv˜a, S. Silva˜, Effect of genotype, feeding system and slaughter weight on the quality of light lambs II. Fatty Acid Composition of Meat, Livest. Prod. Sci. 77 (2002) 187–194, https://doi.org/ 10.1016/S0301-6226(02)00059-3.

[23] S. Chen, G. Bobe, S. Zimmerman, E.G. Hammond, C.M. Luhman, T.D. Boylston, A. E. Freeman, D.C. Beitz, Physical and sensory properties of dairy products from cows with various milk fatty acid compositions, J. Agric. Food Chem. 52 (2004) 3422–3428, https://doi.org/10.1021/jf035193z.

[24] J.A. Logue, A.L. De Vries, E. Fodor, A.R. Cossins, Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure, J. Exp. Biol. 203 (2000) 2105–2115, https://doi.org/10.1242/jeb.203.14.2105.

[25] G. Abrami, F. Natiello, P. Bronzi, D. McKenzie, L. Bolis, E. Agradi, A comparison of highly unsaturated fatty acid levels in wild and farmed eels (Anguilla Anguilla), Comp. Biochem. Physiol. 101 (1992) 79–81, https://doi.org/10.1016/0305-0491(92)90161.

[27] M. Krajnovic-ozretic, M. Najdek, B. Ozretic, Fatty acids in liver and muscle of farmed and wild sea bass (Dicentrarchus labrax L.), Comp. Biochem. Physiol 109 (1994) 611–617, https://doi.org/10.1016/0300-9629(94)90200-3.

[28] E. Prato, A. Danieli, M. Maffia, F. Biandolino, Lipid and Fatty Acid Compositions of Mytilus galloprovincialis Cultured in the Mar Grande of Taranto (Southern Italy): Feeding Strategies and Trophic Relationships, n.d.

[29] V.I. Kharlamenko, L. Würzberg, J. Peters, E.E. Borisovets, Fatty acid compositions and trophic relationships of shelled molluscs from the Kuril-Kamchatka Trench and the adjacent abyssal plain, Deep Sea Res 2 Top Stud Oceanogr 111 (2015) 389–398. https://doi.org/10.1016/j.dsr2.2014.09.002.

[30] V.J. Sinanoglou, D. Meimaroglou, S. Miniadis-Meimaroglou, Triacylglycerols and their fatty acid composition in edible Mediterranean molluscs and crustacean, Food Chem. 110 (2008) 406–413, https://doi.org/10.1016/j.foodchem.2008.02.035.

[31] A. Eilander, R.K. Harika, P.L. Zock, Intake and sources of dietary fatty acids in Europe: are current population intakes of fats aligned with dietary recommendations? Eur J Lipid Sci and Tech 117 (2015) 1370–1377, https://doi. org/10.1002/ejlt.201400513.

[32] A.P. Simopoulos, The importance of the ratio of omega-6/omega-3 essential fatty acids, Biomed. Pharmacother. 56 (2002) 365–379, https://doi.org/10.1016/ s0753-3322(02)00253-6.

[33] D.R. Tocher, Metabolism and functions of lipids and fatty acids in teleost fish, Rev. Fish. Sci. 11 (2003) 107–184, https://doi.org/10.1080/713610925.

[34] M. Beccaria, F.A. Franchina, M. Nasir, T. Mellors, J.E. Hill, G. Purcaro, Investigation of mycobacteria fatty acid profile using different ionization energies in GC–MS, Anal. Bioanal. Chem. 410 (2018) 7987–7996, https://doi.org/10.1007/ s00216-018-1421-z