

Article

Determination of Fatty Acid Profile in Processed Fish and Shellfish Foods

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Simple Summary: Fish and shellfish products are an important dietary source of polyunsaturated fatty acids, particularly n-3 polyunsaturated fatty acids (n-3 PUFA) that are known for their human health benefits. In this study, the fatty acids content in twelve types of processed fish products purchased in supermarkets and ethnic food shops in Messina (Italy) were analyzed, and AI (Atherogenicity Index), TI (Thrombogenicity Index) and FLQ (Flesh-Lipid Quality) index were determined. All samples showed abundant content of EPA (C20:5 n-3) and DHA (C22:6 n-3). AI and TI values were less than one in all samples. For this reason, these processed fish products can be considered a good source of beneficial PUFAs, and their intake can be recommended for human consumption.

Abstract: Seafood products are the main dietary source of n-3 polyunsaturated fatty acids (n-3 PUFA), which are essential for human health. However, due to the widespread use of processed fish products, the presence of these n-3 PUFA may be subject to changes related to different processing methods. The aim of this study was to determine the fatty acid composition, focusing on n-3 PUFA, in different processed fish and shellfish food of EU and non-EU origin purchased in supermarkets and ethnic food shops in Messina (Italy), using gas chromatography with flame-ionization detector (GC-FID). From the fatty acid profile, the atherogenicity index (AI), thrombogenicity index (TI) and flesh-lipid quality index (FLQ) were determined: 0.13-1.04 (AI), 0.19-0.89 (TI) and 0.41-29.90 (FLQ). The percentages of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids had the following ranges: 13.55-50.48%, 18.91-65.58%, 13.84-52.73%, respectively. All samples showed a good presence of PUFA and, in particular, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were the main n-3 polyunsaturated fatty acids.

Keywords: seafood processed foods; n-3 PUFA; fatty acids content; health lipid indices

1. Introduction

Fish and shellfish products are important for humans due to their many beneficial health effects for consumers [1,2]. They contain high-quality protein, lipids, vitamins (D, A, E and B₁₂), essential elements (e.g., selenium), and other essential nutrients [3].

According to lipids content, it is possible to classify the fish into lean fish (2-5 percent fat, e.g., cod and sole), medium-fat fish (5-6 percent fat, e.g., hake and sea bass), and fatty fish (6-25 percent fat, e.g., anchovies, herring, sardines, mackerel, tuna, and salmon) [1]. The lipid fraction is constituted by different fatty acids depending closely on various factors, such as species, age, sex, time of year, etc, as well as their percentage concentration [1].

The importance of fish and shellfish products is because these species are the only significant dietary source of polyunsaturated fatty acids, in particular of n-3 polyunsaturated fatty acids (n-3 PUFAs) and n-6 polyunsaturated fatty acids (n-6 PUFA) [4].

Among the n-3 PUFA, eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) are the most beneficial [5]. Several studies have shown a close correlation between the consumption of fish species and human health benefits. Indeed, these fatty acids, from which some eicosanoids (e.g., prostaglandins, thromboxanes, and leukotrienes) are synthesized, have important antithrombotic functions, reduce the risk of coronary and cardiovascular disease, and prevent cancer, diabetes, and other inflammatory and autoimmune disease [4,6]. Moreover, n-3 PUFA are also essential from conception through all stages of human development, contributing to normal neurological development in children [1]. Their intake also induces a decrease in dementia disorders and Alzheimer's symptoms in the elderly [5,7].

EPA and DHA are not naturally synthesized by humans. Consequently, it is necessary to introduce them through the diet and, specifically, through the consumption of fish products. In fact, there are guidelines that recommend consuming fish at least twice a week, thereby introducing about 250 mg/day of EPA + DHA [4,8]. From studies conducted by the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA EFSA Panel), it has been observed that consumption of 1-2 servings of seafood products per week, and up to 3-4 servings per week in the case of pregnant women, results in functional benefits related to neurodevelopment in children and lower risk of mortality from coronary heart disease in adults [9].

A balanced ratio of n-6/n-3 PUFA in the diet also turns out to be of fundamental importance. An unbalanced ratio, in fact, can lead to chronic diseases, due to the competition of the n-3 and n-6 PUFA towards the same enzymes [10]. From some studies [5,6], for example, a PUFA n-6/n-3 (4:1) is recommended, as it prevents the occurrence of coronary artery disease, due to the different roles played by n-3 and n-6: the former contribute to the anti-inflammatory and antithrombotic process, the latter to the inflammatory process. In this regard, the European Scientific Committee on Food has set daily intakes of n-6 PUFA and n-3 PUFA for adults of 6.4 g/day and 1.6 g/day, respectively [4,11].

Therefore, consumption of fish products is essential to increase the intake of n-3 LC PUFAs. However, due to the frenetic lifestyles of today's world, more and more processed seafood is consumed. This term refers to all foods that undergo manufacturing processes to increase their shelf life or modify their sensory characteristics [12].

Subjecting food to transformation processes can have pros and cons. On the one hand, they not only prolong the shelf life of a product and maintain its quality, but also enable the elimination of most microorganisms [12]. On the other hand, they can play key roles in nutrient retention: for example, heating treatment can cause undesirable changes, such as loss of the nutritional food value. In fact, cooking is a critical step in the retention of EPA and DHA from fish, since both are highly sensitive to oxidation. [4].

Often, to overcome the negative effects involved in processing, the incorporation of other functional food is essential to increase the amount of fatty acids in the starting samples. This choice stems from consumer research toward foods that can provide health benefits, such as improving body function or reducing the risk of some of certain diseases [13,14]. In this research, several processed fish products purchased in supermarkets and ethnic food shops in Messina (Italy) were analyzed. The aim was to determine the fatty acid composition of the samples under examination, paying particular attention to the long-chain n-3 PUFA.

2. Materials and Methods

2.1. Samples

In this study, a total of 47 samples of processed fish (tuna, mackerel, sardines, salmon, shrimps, and crabs) of different brands were purchased between September and October 2022 in supermarkets and ethnic food shops in Messina (Table 1). The processed fish products under investigation were the same as in the study previously conducted by *Nava et al.* [12].

Table 1. Information of fish and shellfish processed foods (* data not available).

Sample Code	Sample	Sample No	Species	Country or origin	Lot	Manufacture data	Processing condition	Expired data
F1	Natural shrimp	3	*	Italy	L20058	*	*	27/02/2023
F2	Canned horse mackerel	3	<i>Trachurus murphyi</i>	Chile	*	*	Canning	24/05/2025
F3	Canned sardines	4	<i>Sardina pilchardus</i>	Morocco	LKX149L/S	07/02/2019	Canning	29/05/2026
F4	Canned crab meat	3	*	Indonesia	CMCRCI064 T	*	Canning	07/02/2023
F5	Canned pink Salmon	3	*	USA	3028163	*	Canning	31/12/2022
F6	Canned tuna pate	3	<i>Euthynnus (Katsuwonus) pelamis</i>	Italy	F048-T1	*	Canning	02/2025
F7	Canned mackerel fillets	5	*	Portugal	*	*	Canning	11/11/2026
F8	Dried shrimp	3	*	Argentina	*	*	Drying	*
F9	Dried sardines	5	<i>Sardinella aurita</i>	Argentina	*	*	Drying	*
F10	Natural Alaskan salmon	5	<i>Oncorhynchus nerka</i>	Denmark	L13221-3	*	Canning	31/12/2025
F11	Grilled mackerel fillets in olive oil	5	<i>Scomber japonicus</i>	Portugal	L301U-B12	*	Grilling and Canning	10/2024
F12	Natural grilled mackerel fillets	5	<i>Scomber japonicus</i>	Portugal	L309U-B2	*	Grilling and Canning	11/2024
Tot		47						

2.2. Chemicals and Reagents

Analytical grade reagents and chemicals, purchased Merck (Darmstadt, Germany), were used. For fatty acid analysis, a mixture of fatty acid methyl esters (Supelco 37-Component FAME Mix), purchased from Sigma-Aldrich (Darmstadt, Germany), was employed. The other fatty acids, indicated in Table 2 with an asterisk (*), were identified by comparison with a well-characterized fish oil (Mehaden oil from Supelco).

2.3. Lipids extraction and preparation of FAMES

The extraction of the lipid fraction was carried out using the Folch method [4,15], subject to some modifications. Precisely, about 4 g of each sample was weighed into 50 mL tubes and homogenized with Folch’s solution (chloroform:methanol mixture, 2:1, v/v). A 0.73% NaCl solution was then added to the samples. Subsequently, the samples were

first vortexed for 1-2 min and then centrifuged at 3500 rpm for 15 minutes at 4°C. The lipid fraction (bottom layer) was recovered in a previously weighed flask and taken to dryness. This allowed the yield (%) to be determined gravimetrically.

In accordance with the ISO 5509 2000 method, fatty acid methyl esters (FAME) were obtained by transmethylation (hot esterification) of the lipid fractions of the analyzed samples, through addition of methanol:sulphuric acid mixture (9:1, v/v). The mixture was put in an oven at 100°C for one hour. After recovering the supernatant, the latter was diluted with n-hexane.

2.4. GC-FID analysis

The fatty acid composition of the investigated samples was analyzed using a gas chromatograph provided with a split/splitless injector and a flame ionization detector (GC-FID, Dani Master GC, Dani Instrument, Milan, Italy), equipped with a ZB-Wax column (Phenomenex, Torrance, California, USA) with a length of 30 m, internal diameter of 0.25 mm, film thickness of 0.25 µm.

The operating conditions were as follows: column oven temperature from 50°C (hold time 2 minute) to 240°C (holding time 15 minute) at 3°C/minute; injector and detector temperatures were set at 240°C; helium was at a linear velocity of 30 cm/s (constant). The injection volume was 1 µL, with a split ratio of 1:50.

Clarity Chromatography v4.0.2 software (DataApex, Prague, Czech Republic) was used for data acquisition and management. Each sample was analyzed in triplicate, along with the analytical blanks. By comparison with the reference retention times of the compounds present in the two mixtures, FAMES of nutritional interest were identified.

2.5. Index of Atherogenicity (AI) and index of Thrombogenicity (TI)

To assess and obtain information on the health and nutritional potential of the lipids in the analyzed samples, the atherogenicity index (AI) and the thrombogenicity index (TI) were evaluated [2,4,16,17,18].

The atherogenicity index expresses the ratio between the sum of the main saturated fatty acids and that of the main classes of unsaturated fatty acids. In fact, the former are considered to be pro-atherogenic as they promote the adhesion of lipids to the cells of the immune-circulatory system. The latter, on the other hand, are considered anti-atherogenic as they inhibit plaque aggregation and decrease levels of esterified fatty acids, cholesterol, and phospholipids, playing a preventive role about the onset of coronary micro- and macro-pathologies [2,4,18]. The following equation was used to calculate AI:

$$AI = [C12:0 + (4 \times C14:0) + C16:0] / [\Sigma n-6 \text{ PUFA} + \Sigma \text{ MUFA} + \Sigma n-3 \text{ PUFA}] \quad (1)$$

The thrombogenicity index, instead, expresses the propensity to form clots in blood vessels and is obtained as the ratio of pro-thrombogenic (saturated) and the anti-thrombogenic (MUFA, n-6 PUFA e n-3 PUFA) fatty acids [17,19]. TI was calculated with the following equation:

$$TI = [C14:0 + C16:0 + C18:0] / [0.5 \times \Sigma n-6 \text{ PUFA} + 0.5 \times \Sigma \text{ MUFA} + 3 \times \Sigma n-3 \text{ PUFA} + (n-3 \text{ PUFA}/n-6 \text{ PUFA})] \quad (2)$$

2.6. Flesh-Lipid Quality (FLQ)

This parameter expresses the percentage correlation between the main n-3 PUFAs (EPA + DHA) and total lipids. Higher values of this index are synonymous with a higher quality of the dietary lipid source [2,20]. The expression used to calculate FLQ is given below:

$$FLQ = 100 \times [EPA + DHA] / [\% \text{ of total fatty acids}] \quad (3)$$

2.7. Statistical analysis

The significance of the results was analyzed using the SPSS 13.0 software package for Windows (SPSS Inc., Chicago, IL, USA). The non-parametric Kruskal-wallis test was used to compare the means of groups of measurement data.

3. Results and discussions

The fatty acid composition of analyzed samples is shown in Table 2, where only fatty acids with a content of more than 0.1% in at least one of the products are listed.

Table 2. Fatty acid composition (% of total FA) in processed fish foods (fatty acids indicated with an asterisk are those identified with fish oil (Mehaden oil from Supelco).

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
C4:0	0.16±0.02	0.01±0.00	0.01±0.00	0.06±0.01	0.01±0.00	0.01±0.00	0.00±0.00	0.01±0.00	0.10±0.01	0.06±0.01	0.01±0.00	0.02±0.01
C10:0	0.19±0.03	0.01±0.00	0.01±0.00	0.04±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.03±0.00	0.06±0.01	0.02±0.00	0.00±0.00	0.00±0.00
C11:0	0.09±0.01	0.01±0.00	0.02±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.10±0.01	0.03±0.01	0.00±0.00	0.00±0.00
C12:0	0.26±0.05	0.09±0.01	0.03±0.00	0.15±0.02	0.09±0.01	0.01±0.00	0.03±0.00	0.23±0.03	0.13±0.02	0.05±0.01	0.01±0.00	0.07±0.02
C13:0	0.08±0.01	0.03±0.00	0.01±0.00	0.01±0.00	0.06±0.01	0.01±0.00	0.02±0.00	0.05±0.01	0.10±0.01	0.04±0.01	0.02±0.00	0.10±0.02
C14:0	2.90±0.30	6.26±0.25	1.43±0.23	1.43±0.31	4.91±0.27	0.07±0.01	3.51±0.37	4.73±0.22	3.77±0.51	3.74±0.44	0.89±0.20	4.21±0.61
C15:0	0.70±0.15	0.47±0.05	0.09±0.01	0.50±0.09	0.70±0.17	0.02±0.00	0.26±0.03	0.75±0.23	1.03±0.41	0.55±0.12	0.23±0.07	1.07±0.33
C16:0 iso*	0.10±0.02	0.10±0.01	0.01±0.00	0.02±0.00	0.11±0.01	0.01±0.00	0.02±0.00	0.12±0.01	0.15±0.02	0.10±0.02	0.02±0.00	0.02±0.00
C16:0	19.94±0.57	18.36±0.77	11.82±0.61	17.90±1.01	13.36±0.88	10.75±0.52	11.90±0.34	25.85±0.75	28.31±0.66	16.90±1.01	12.54±0.93	20.62±1.22
C17:0	0.55±0.08	0.39±0.05	0.13±0.01	1.12±0.17	0.50±0.13	0.08±0.01	0.25±0.03	1.55±0.17	1.25±0.28	0.10±0.04	0.36±0.09	1.13±0.37
C18:0	3.60±0.28	5.13±0.70	3.77±0.56	8.40±0.91	2.67±0.33	1.85±0.20	2.92±0.11	12.73±0.22	6.05±0.81	3.15±0.46	4.52±1.05	6.52±1.02
C20:0	0.26±0.04	0.18±0.03	0.38±0.07	0.43±0.07	0.14±0.02	0.37±0.07	0.29±0.05	1.01±0.37	0.21±0.07	0.12±0.03	0.38±0.06	0.39±0.10
C21:0	0.02±0.00	0.03±0.00	0.02±0.00	0.11±0.02	0.02±0.00	0.01±0.00	0.05±0.00	0.21±0.03	0.05±0.00	0.10±0.03	0.04±0.01	0.12±0.04
C22:0	0.42±0.10	0.12±0.02	0.09±0.01	0.22±0.04	0.07±0.01	0.12±0.02	0.10±0.0q	2.04±0.49	0.26±0.04	0.10±0.02	0.13±0.02	0.24±0.08
C23:0	0.05±0.01	0.02±0.00	0.02±0.00	0.08±0.01	0.02±0.00	0.02±0.00	0.03±0.00	0.25±0.03	0.07±0.01	0.02±0.00	0.02±0.00	0.06±0.01
C24:0	0.30±0.07	0.06±0.01	0.06±0.00	0.11±0.01	0.05±0.01	0.18±0.03	0.05±0.00	0.84±0.13	1.06±0.22	0.13±0.02	0.07±0.02	0.12±0.03
Σ SFA	29.66±1.33 ^{AB}	31.29±1.45 ^{AB}	17.92±1.02 ^{AB}	30.71±1.46 ^{AB}	22.77±1.27 ^{AB}	13.55±1.08 ^A	19.46±1.22 ^{AB}	50.48±1.77 ^B	42.78±1.70 ^B	25.29±1.55 ^{AB}	19.25±1.20 ^{AB}	34.70±1.78 ^{AB}
C16:1 n-9*	0.16±0.03	0.19±0.03	0.13±0.02	0.12±0.01	0.24±0.02	0.05±0.00	0.21±0.06	0.14±0.02	0.19±0.03	0.32±0.10	0.13±0.03	0.23±0.08
C16:1 n-7	3.73±0.41	7.50±1.10	2.38±0.55	3.18±0.48	3.63±0.77	0.15±0.01	2.01±0.41	8.87±1.01	1.75±0.40	2.83±0.50	1.48±0.43	3.67±0.85
C16:1 n-5*	0.20±0.04	0.14±0.02	0.04±0.00	0.04±0.00	0.31±0.05	0.01±0.00	0.18±0.03	0.10±0.01	0.16±0.02	0.22±0.06	0.03±0.01	0.09±0.02
C17:1	0.27±0.05	0.06±0.01	0.11±0.01	0.28±0.03	0.54±0.10	0.04±0.00	0.24±0.04	1.32±0.35	0.46±0.15	0.56±0.12	0.18±0.04	0.44±0.08
C18:1 n-9	21.93±0.67	15.16±1.11	60.11±2.01	20.71±0.98	14.36±0.79	29.04±1.07	40.35±1.56	12.44±0.73	11.06±0.88	14.45±0.90	57.78±3.66	16.07±1.55
C18:1 n-7*	5.68±0.38	4.25±0.91	2.15±0.51	2.84±0.40	1.89±0.29	0.58±0.13	1.71±0.22	5.18±1.01	2.55±0.44	2.30±0.44	2.15±0.77	2.82±0.68
C20:1 n-11*	0.11±0.01	0.21±0.02	0.02±0.00	0.62±0.15	5.63±0.79	0.11±0.01	0.31±0.06	0.13±0.02	0.07±0.01	4.44±1.11	0.06±0.02	0.17±0.03
C20:1 n-7*	0.04±0.00	0.43±0.08	0.02±0.00	0.42±0.09	0.68±0.22	0.22±0.07	0.06±0.01	0.12±0.02	0.03±0.00	0.22±0.08	0.08±0.02	0.24±0.07
C20:1 n-9	1.26±0.10	2.72±0.55	0.35±0.09	0.65±0.10	3.61±0.45	0.25±0.06	4.63±0.48	0.25±0.10	0.49±0.03	2.69±0.63	0.70±0.07	2.42±0.54
C22:1 n-11*	0.94±0.08	1.65±0.61	0.04±0.00	0.42±0.06	10.89±0.77	0.02±0.00	6.15±0.95	0.07±0.01	0.21±0.01	6.87±1.09	0.27±0.05	1.32±0.33
C22:1 n-7*	0.15±0.01	0.10±0.01	0.03±0.00	0.02±0.00	0.20±0.03	0.02±0.00	0.03±0.00	0.16±0.05	0.14±0.01	0.30±0.11	0.03±0.01	0.07±0.01
C22:1 n-9	0.19±0.02	0.24±0.02	0.03±0.00	0.11±0.02	1.21±0.27	0.01±0.00	0.37±0.10	0.07±0.01	0.11±0.01	0.96±0.56	0.12±0.03	0.52±0.12
C24:1 n-9	0.27±0.04	0.50±0.09	0.14±0.02	0.14±0.03	1.11±0.49	0.04±0.00	0.42±0.15	0.51±0.09	1.63±0.52	0.79±0.32	0.33±0.10	0.64±0.21
Σ MUFA	35.01±0.63 ^{AB}	33.20±1.77 ^{AB}	65.58±2.67 ^C	29.58±1.23 ^A	44.36±2.11 ^{AB}	30.56±1.62 ^A	56.71±2.25 ^B	29.40±0.33 ^A	18.91±0.86 ^A	37.08±4.02 ^{AB}	63.34±4.11 ^C	28.72±1.65 ^A
C16:2 n-4*	2.17±0.18	1.26±0.31	0.21±0.02	0.26±0.04	0.20±0.02	0.04±0.00	0.28±0.02	0.20±0.02	0.44±0.06	0.09±0.01	0.18±0.05	0.68±0.13
C16:3 n-4*	0.03±0.00	0.83±0.25	0.22±0.03	0.29±0.05	0.07±0.01	0.01±0.00	0.07±0.01	0.06±0.01	0.03±0.00	0.33±0.09	0.10±0.02	0.36±0.09
C16:4 n-4*	0.05±0.00	1.60±0.44	0.53±0.19	0.16±0.02	0.14±0.01	0.02±0.00	0.11±0.01	0.45±0.06	0.18±0.02	0.13±0.03	0.04±0.01	0.08±0.02
C18:2 n-6	4.86±0.29	1.11±0.41	5.71±0.80	6.04±0.83	2.13±0.19	50.85±3.55	5.33±0.91	1.27±0.22	1.72±0.33	1.30±0.58	3.83±0.72	1.47±0.40

C18:2 n-4*	0.12±0.01	0.28±0.07	0.07±0.01	0.35±0.12	0.19±0.03	0.02±0.00	0.06±0.00	0.29±0.09	0.06±0.01	0.15±0.05	0.06±0.02	0.21±0.07
C18:3 n-6	0.02±0.00	0.13±0.01	0.04±0.00	0.08±0.01	0.13±0.02	0.02±0.00	0.10±0.01	0.06±0.00	0.03±0.00	0.08±0.01	0.04±0.01	0.16±0.06
C18:3 n-3	0.42±0.14	0.56±0.11	0.57±0.10	0.73±0.20	1.16±0.15	0.86±0.13	1.30±0.31	0.30±0.07	0.33±0.06	1.22±0.77	0.56±0.26	0.81±0.15
C18:4 n-3*	0.31±0.11	1.71±0.22	0.51±0.13	0.36±0.11	2.48±0.33	0.02±0.00	2.94±0.49	0.10±0.01	0.31±0.07	2.07±0.32	0.21±0.10	0.96±0.11
C18:4 n-1*	0.08±0.01	0.34±0.07	0.07±0.01	0.02±0.00	0.11±0.02	0.01±0.00	0.05±0.00	0.04±0.00	0.07±0.01	0.05±0.01	0.00±0.00	0.01±0.00
C20:2 n-6	0.31±0.10	0.13±0.02	0.02±0.00	0.24±0.03	0.50±0.07	0.02±0.00	0.12±0.02	0.26±0.05	0.35±0.10	0.28±0.08	0.08±0.02	0.30±0.03
C20:3 n-6	0.07±0.01	0.11±0.02	0.03±0.00	0.08±0.01	0.14±0.02	0.01±0.00	0.05±0.00	0.05±0.00	0.04±0.00	0.12±0.03	0.05±0.01	0.17±0.06
C20:3 n-3	0.08±0.01	0.08±0.01	0.01±0.00	0.01±0.00	0.23±0.04	0.01±0.00	0.13±0.01	0.05±0.00	0.09±0.01	0.17±0.06	0.03±0.01	0.15±0.04
C20:4 n-6	1.60±0.15	1.05±0.30	0.19±0.02	2.21±0.69	0.63±0.13	0.05±0.01	0.24±0.05	1.96±0.34	1.20±0.21	0.46±0.12	0.86±0.22	2.27±0.41
C20:4 n-3*	0.20±0.02	0.72±0.19	0.16±0.02	0.37±0.15	1.42±0.22	0.02±0.00	0.64±0.19	0.72±0.12	0.17±0.02	1.37±0.56	0.11±0.03	0.51±0.19
C20:5 n-3	9.70±0.51 ^B	12.08±0.80 ^B	3.48±0.62 ^A	4.11±0.47 ^A	6.59±0.98 ^A	0.07±0.01 ^A	3.03±0.44 ^A	4.35±0.78 ^A	3.96±0.22 ^A	7.23±1.33 ^A	1.63±0.34 ^A	5.81±0.79 ^A
C21:5 n-3*	0.12±0.01	0.66±0.22	0.14±0.01	0.29±0.07	0.37±0.03	0.29±0.07	0.22±0.05	0.05±0.00	0.07±0.01	0.18±0.04	0.20±0.07	0.10±0.02
C22:2	0.92±0.12	0.05±0.01	0.10±0.01	1.12±0.28	0.11±0.01	0.02±0.00	0.02±0.00	0.38±0.09	0.04±0.00	0.04±0.01	0.00±0.00	0.04±0.01
C22:5 n-6*	0.16±0.04	0.20±0.03	0.03±0.00	0.21±0.04	0.17±0.02	0.04±0.00	0.20±0.06	0.25±0.07	0.73±0.21	0.15±0.03	0.34±0.15	0.98±0.30
C22:5 n-3*	0.81±0.16	3.72±0.67	0.40±0.13	0.59±0.11	2.26±0.34	0.02±0.00	0.56±0.20	0.25±0.05	0.55±0.12	1.81±0.66	0.62±0.21	2.08±0.55
C22:6 n-3	9.05±0.39 ^{AB}	6.48±1.00 ^{AB}	1.35±0.33 ^A	3.81±0.63 ^{AB}	11.41±1.21 ^B	0.33±0.10 ^A	6.01±1.55 ^{AB}	4.07±0.65 ^{AB}	25.09±1.55 ^C	16.39±2.11	5.34±0.70	16.04±1.88
Σ PUFA	31.08±1.50 ^{AB}	33.10±1.51 ^{AB}	13.84±0.66 ^A	21.33±1.59 ^A	30.44±2.56 ^{AB}	52.73±2.88 ^C	21.46±2.78 ^{AB}	15.16±1.21 ^A	35.46±2.10 ^B	33.62±2.54 ^B	14.28±1.87 ^A	33.19±2.02 ^B
Σ n-3	20.69±1.03 ^{AB}	26.01±1.13 ^B	6.62±0.50 ^A	10.27±1.05 ^{AB}	25.92±2.22 ^B	1.62±0.54 ^A	14.83±2.54 ^{AB}	9.89±0.92 ^{AB}	30.57±1.88 ^B	30.44±3.01 ^B	8.70±1.33 ^{AB}	26.46±2.06 ^B
Σ n-6	7.02±0.35 ^{AB}	2.73±0.53 ^A	6.02±0.76 ^{AB}	8.86±1.09 ^B	3.70±0.24 ^{AB}	50.99±2.29 ^C	6.04±0.66 ^{AB}	3.85±0.55 ^{AB}	4.07±0.58 ^{AB}	2.39±0.49 ^A	5.20±1.04 ^{AB}	5.35±0.89 ^{AB}
n-6/n-3	0.34±0.02 ^A	0.10±0.01 ^A	0.91±0.20 ^A	0.86±0.22 ^A	0.14±0.01 ^A	31.48±2.01 ^B	0.41±0.21 ^A	0.39±0.10 ^A	0.13±0.02 ^A	0.08±0.02 ^A	0.60±0.17 ^A	0.20±0.03 ^A
Undefined	4.25±0.19	2.41±0.36	2.66±0.43	18.38±1.26	2.43±0.55	3.16±0.79	2.37±0.50	4.96±0.47	2.85±0.75	4.01±0.98	3.13±0.54	3.39±0.38
PUFA/SFA	1.04±0.27 ^B	1.06±0.23 ^B	0.77±0.15 ^{AB}	0.70±0.16 ^{AB}	1.34±0.41 ^B	3.89±0.87 ^C	1.10±0.20 ^B	0.30±0.11 ^A	0.83±0.22 ^{AB}	1.33±0.40 ^B	0.74±0.21 ^{AB}	0.96±0.15 ^B
EPA+DHA	18.75±1.31 ^B	18.56±1.55 ^B	4.83±0.87 ^A	7.92±0.46 ^{AB}	18.00±1.49 ^B	0.40±0.11 ^A	9.04±1.01 ^{AB}	8.42±0.89 ^{AB}	29.05±2.56 ^C	23.62±3.22 ^{BC}	6.97±1.05 ^{AB}	21.85±2.76 ^{BC}
DHA/EPA	0.93±0.05 ^A	0.54±0.04 ^A	0.39±0.02 ^A	0.93±0.05 ^A	1.73±0.08 ^{AB}	4.71±0.11 ^B	1.98±0.09 ^{AB}	0.94±0.06 ^A	6.34±0.25 ^C	2.27±0.71 ^{AB}	3.28±0.59 ^B	2.76±0.32 ^{AB}
AI	0.51±0.03 ^{AB}	0.70±0.15 ^{AB}	0.25±0.06 ^{AB}	0.49±0.13 ^{AB}	0.45±0.09 ^{AB}	0.13±0.03 ^A	0.34±0.10 ^{AB}	1.04±0.28 ^B	0.81±0.21 ^B	0.46±0.09 ^{AB}	0.62±0.12 ^{AB}	0.21±0.04 ^A
TI	0.31±0.02 ^{AB}	0.28±0.02 ^{AB}	0.30±0.03 ^{AB}	0.54±0.02 ^B	0.19±0.01 ^A	0.28±0.02 ^{AB}	0.23±0.03 ^{AB}	0.89±0.02 ^B	0.34±0.02 ^{AB}	0.19±0.03 ^A	0.29±0.08 ^{AB}	0.31±0.05 ^{AB}
FLQ	19.58±0.55 ^B	19.02±0.79 ^B	4.96±0.38 ^{AB}	9.70±1.12 ^{AB}	18.45±1.77 ^B	0.41±0.15 ^A	9.26±1.07 ^{AB}	8.86±1.16 ^{AB}	29.90±1.87 ^C	24.61±1.74 ^B	7.20±1.01 ^{AB}	22.62±1.55 ^B

Letters A, B and C indicate homogeneous groups at $\alpha=0.05$: sample types which do not differ from each other are designated by same letter.

Generally, for all samples, the most abundant fatty acids were DHA (C22:6 n-3), EPA (C20:5 n-3), palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9) and linoleic (C18:2n-6) acids. These results are comparable with those obtained by Mesa *et al.* [9], Grazina *et al.* [21] and Maldonado-Pereira *et al.* [22]. However, the fatty acid composition proved to be highly variable and closely dependent on the different types of products investigated.

The percentage of Saturated Fatty Acids (SFA) ranged from 13.55±1.08% for canned tuna pate samples (F6) to 50.48±1.77% for dried shrimps (F8). Palmitic acid (C16:0) was the most abundant of the saturated fatty acids. Its percentages decreased in the following order: 28.31±0.66% (F9), 25.85±0.75% (F8), 20.62±1.22% (F12), 19.94±0.57% (F1), 18.36±0.77% (F2), 17.90±1.01% (F4), 16.90±1.01% (F10), 13.36±0.88% (F5), 12.54±0.93% (F11), 11.90±0.34% (F7), 11.82±0.61% (F3) and 10.75±0.52% (F6). Good percentages were also found for stearic acid (C18:0). Here, it was sample F8 that had the highest percentage 12.73±0.22%, followed by F4 (8.40±0.91%), F12 (6.52±1.02%), F9 (6.05±0.81%), F2 (5.13±0.70%), F11 (4.52±1.05%), F3 (3.77±0.56%), F1 (3.60±0.28%), F10 (3.15±0.46%) F7 (2.92±0.11%), F5 (2.67±0.33%) and F6 (1.85±0.20%). Fair percentages of myristic acid (C14:0) were also found between 0.07±0.01% (F6) and 6.26±0.25% (F2). For all other SFA, the quantities observed were lower.

Canned sardine (F3) and Grilled mackerel fillets in olive oil (F11) samples showed the significantly higher percentage of monounsaturated fatty acids (MUFA) at 65.58±2.67% and 63.34±4.11%, respectively followed by F7 (56.71±2.25%), F5 (44.36±2.11%), F10 (37.08±4.02%), F1 (35.01±0.63%), F2 (33.20±1.77%), F6 (30.56±1.62%), F4 (29.58±1.23%), F8 (29.40±0.33%), F12 (28.72±1.65%) and F9 (18.91±0.86%). Among the MUFA, the most abundant were oleic acid (C18:1 n-9, 11.06±0.88 - 60.11±2.01%), cis-vaccenic acid (C18:1 n-7, 0.58±0.13 - 5.68±0.38%), palmitoleic acid (C16:1 n-7, 0.15±0.01 - 8.87±1.01%) and cis-11-eicosenoic acid (C20:1 n-9, 0.25±0.10 - 4.63±0.48%).

The composition of polyunsaturated fatty acids (PUFA) also varied according to the type of processed product analyzed. The significantly higher percentage (52.73±2.88%) was obtained for the sample of canned tuna pate (F6), of which linoleic acid (C18:2 n-6, 50.85±3.55%) made the greatest contribution. The other samples showed a PUFA range between 13.84±0.66% and 35.46±2.10%. The most representative PUFA, apart from linoleic acid, were eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexanoic acid (DHA, C22:6 n-3). For the former, the significantly higher percentages (12.08±0.80% and 9.70±0.51%) were showed by canned horse mackerel (F2) and natural shrimp (F1), respectively; while dried sardines (F9) had the significantly higher content of DHA (25.09±1.55%).

DHA was found in higher amounts than EPA in four of the twelve fish categories analyzed (Table 2), resulting in a DHA/EPA ratio ranging from 6.34 (value significantly higher) in dried sardines (F9) to 0.39 in canned sardines (F3). Fish consumption is of paramount importance as it provides important amounts of n-3 PUFA, which are useful for different consumer groups, especially the more demanding ones (e.g., children and pregnant women). In addition to this, it is important to encourage the consumption of n-3 PUFA-rich products to balance the n-6/n-3 PUFA ratio.

In the samples analyzed, the amounts of n-3 PUFA were always higher than those of n-6 PUFA, except in the samples of canned tuna pate (F6), where $\sum$ n-6 was higher than $\sum$ n-3, considering the large contribution made by linoleic acid (50.85±3.55%). For this sample type, the n-6/n-3 PUFA ratio was significantly higher (31.48±2.01%) than others. For all other categories analyzed, low n-6/n-3 PUFA ratios were determined (between 0.08 for natural Alaskan salmon and 0.91 for canned sardines) (Table 2). This was an expected result, given the higher abundance of n-3 PUFA compared to n-6 PUFA in marine organisms, and in fact has been further confirmed in other studies in the literature [23]. It is essential to observe the n-6/n-3 ratio as an index of the nutritional quality of marine lipids. A low intake of this ratio, usually less than one, in the daily diet allows for a preventive role against certain disease [24].

Another parameter used to know the quality of dietary lipids is the ratio of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA). Given that the Ministry

of Health in 2004 recommended a minimum level for the PUFA/SFA ratio of 0.45 [24], all samples analyzed seemed to retain a PUFA/SFA ratio above the recommended level even after processing, in particular for canned tuna pate (F6) that showed the significantly higher value (3.89 ± 0.87) (Table 2).

The sum of EPA and DHA is also an important indicator of the nutritional quality of fish product lipids. In the study conducted by *Rincon-Cervera et al.* on the determination of fatty acids in South Pacific fish species, the sums of EPA and DHA in the fresh products analyzed were higher than those in our samples, further confirming the influence of processing treatments, a possible cause of the decrease in these fatty acids [23].

Concerning canned mackerel samples (F2, F7, F11 and F12), the results obtained were compared with the study conducted by *Merdzhanova et al.* [25] on the determination of fatty acid composition of raw and cooked Black Sea horse mackerel samples. From the comparison, a great difference in lipid composition was observed. Our products (F2 and F12), in fact, were characterized by a higher presence of C18:0, C18:1 n-9 and C20:5 n-3, and a lower percentage of C16:0, C18:2 n-6 and C22:6 n-3, while for F7 and F11 sample only C18:0 and C18:2 n-6 were comparable. This discordance could be due primarily to the different origin of the products analyzed, which in turn is linked to other factors, and the canning process. Furthermore, the higher oleic acid content found in our samples is explained by the presence of olive oil.

The fatty acid profile obtained for the salmon samples (F5 and F10) agreed with a previous study [26], according to which wild salmon are richer in n-3 PUFA and saturated fatty acids (SFA). However, a different contribution of linolenic acid (C18:3 n-3) was observed, for which our samples showed percentages at least five times lower than those reported in the literature. Comparable levels were, however, observed for MUFA and SFA [26]. Perfectly comparable results were observed by comparison with the study conducted by *Blanchet et al.* [27] on samples of wild and farmed Atlantic salmon.

In the canned tuna pate samples (F6), as mentioned above, the order of abundance was PUFA > MUFA > SFA. The percentage of saturated fatty acids was lower than those reported in the literature on fresh tuna. This trend, for example, was observed in a study on the change in fatty acid composition following canning [28], where C14:0, C16:0 and C18:0 was lower in canned rather than fresh tuna products. This behavior could be due to the cooking process prior to canning, which caused the SFA percentage to decrease [28]. This step, however, did not affect the unsaturated fatty acid content, which, on the contrary, was higher than in fresh tuna. In the case of our samples, for example, the high content of linoleic and oleic acids is probably due to the presence of corn seed oil, a matrix characterized by a high presence of these unsaturated fatty acids, and present at about 20% in the tuna pates under investigation. So, the incorporation of other foods added to the tuna increases the amount of MUFA and PUFA compared to fresh samples. This result agreed with other studies in literature, where the addition of olive oil influences the fatty acid composition [28]. In addition, the SFA, MUFA and PUFA contents of the investigated tuna samples was different from that reported by *Mesias et al.* [29], which determined the fatty acid profile in tuna samples following different sterilization treatments. This demonstrates, once again, how different processing methods to which products are subjected can significantly influence the fatty acid composition of a food.

About the samples of canned (F3) and dried (F9) sardines, again the different processing procedures resulted in changes in the fatty acid composition. In the literature, some studies showed higher concentrations of certain fatty acids for fresh sardines. For example, the research carried out by *Feng et al.* [30] on the analysis of the chemical composition of three fish species, including sardines, showed a higher percentage of saturated fatty acids than our samples, especially for the canned ones. Specifically, the amounts of C14:0, C15:0, C16:0 and C18:0 were markedly higher than in the canned sardines analyzed. This clear difference could be due to the different stages of the canning process, considering that the same trend was not observed for the dried sardines. For the latter, in fact, there was only a discordance in myristic acid content ($10.82 \pm 0.59\%$ vs

3.77±0.51%). Canned sardines, on the other hand, exhibited higher levels of oleic acid (60.11±2.01% vs 11.23±0.16%) and linoleic acid (5.71±0.80% vs 1.57±0.08%), due to the presence of olive oil, compared to the study by *Feng et al.*, in contrast to dried sardines with similar contents of these fatty acids [30]. Furthermore, while the EPA contents were comparable for both of our processed food categories (F3 and F9), the DHA content was significantly higher in dried sardines (F9) than in canned sardines (F3). The value reported by *Feng et al.* was higher in our canned samples, but lower in our dried samples [30].

The fatty acid composition of the crab samples (F4) showed a higher percentage of SFA (30.71±1.46%), followed by MUFA (29.58±1.23%) and PUFA (21.33±1.59%). This result was not in agreement with the study of *Latyshev et al.* [31] on the determination of lipids and fatty acids in edible crabs from the Pacific Northwest, and with that of *Cherif et al.* [32] on the determination of the fatty acid composition of green crabs from the Tunisian area, in which the order of abundance in the content of the different fatty acid classes was opposite to that of our research. Again, this difference in fatty acid content could probably be because caused by different product processing techniques, considering that it is known from the literature that marine invertebrate lipids are rich in PUFA [31].

Finally, regarding to processed shrimp products (F1 and F8), the trend differed according to the type of processing. For F1, for instance, higher percentages of PUFA (31.08±1.50%) were obtained, followed by MUFA (35.01±0.63%) and SFA (29.66±1.33%). The opposite composition, on the other hand, was shown by the F8, which were subjected to a drying process. For these, the composition followed an opposite trend to that observed for the F1. Furthermore, when comparing these results with the study by *Li et al.* [33] on the fatty acid composition in fresh Chinese shrimps, the percentages obtained for F1 were comparable, whereas those of F8 were different.

Table 2 also shows a comparison of the atherogenicity index (AI), thrombogenicity index (TI) and Flesh-Lipid Quality (FLQ) values found in the sample analyzed. In general, regarding AI and TI, values less than one are considered low and therefore beneficial to human health. High AI and TI values can lead to platelet aggregation and thrombus formation. So, lower values have beneficial effect on human health [18]. In our study, only the category of dried shrimp (F8) showed an average AI value slightly above one (1.04±0.28), whereas TI values were always lower than one.

FLQ levels ranged from 0.41 in tuna (F6) to 29.90 in dried sardines (F9) (Table 2). For some of our analyzed products (F1, F2, F4, F5, F6, F7, F8, F9, F10 and F12), these values are almost comparable with those reported by *Luczynska et al.* [2], while the remainder (F3 and F6) show significantly lower FLQ. Higher values of this index are synonymous with a higher quality of the dietary lipid source [2,20].

4. Conclusions

In this study, the fatty acids composition in processed foods was determined by GC-FID analysis. The percentage of fatty acids differed according to the type of analyzed product. The most abundant fatty acids were DHA (C22:6 n-3), EPA (C20:5 n-3), palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids in all samples. The order of abundance was as follows: oleic acid (C18:1 n-9) > palmitic acid (C16:0) > linoleic acid (C18:2 n-6) > DHA (C22:6 n-3) > EPA (C20:5 n-3) > stearic acid (C18:0). The results are comparable with other studies in the literature, although in many cases the percentage of certain fatty acids was significantly influenced by the manufacturing processes of products. The health potential of the lipids of the processed products was evaluated by considering the AI, TI and FLQ index; the results showed the good quality of lipid fraction of the analyzed samples. However, all samples tested proved to be good source of beneficial source of PUFA, especially n-3 PUFA and may be recommended for human health consumption.

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