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Characterization of the Fatty Acid Composition in Cultivated Atlantic Bluefin Tuna (*Thunnus thynnus* L.) Muscle by Gas Chromatography-Mass Spectrometry

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ABSTRACT

A previously established gas chromatography-mass spectrometry method was applied for the determination of the fatty acid composition in the muscles of Atlantic bluefin tuna (*Thunnus thynnus* L). The fish were collected in the Mediterranean Sea and cultivated for several months. The goal of the study was to demonstrate the feasibility of these measurements and to improve knowledge about the influence of size and gender on the fatty acid composition. Although no significant differences were found with respect to gender, males and females showed different correlations of fatty acid composition with weight. In the males, a statistically significant linear correlation was found for 14:0, 16:0, 18:1n9, 20:1n9, 22:1n9 (positive correlation), and for 20:4n6, 20:5n3 22:6n3 (negative correlation). In the females, a statistically significant polynomial correlation for specific fatty acids was highlighted: specimens with medium size showed the lowest content of 20:1n9 and 22:1n9 and the highest content of 20:4n6, 20:5n3, and 22:6n3. Therefore, males smaller than 150 kg and females from 150 to 250 kg appeared to have the highest greater nutritional value. This study demonstrated the practicality of the analytical methodology and enhanced the knowledge of the influence of size on the fatty acid composition of Atlantic bluefin tuna muscle, reporting a newly established gender difference and representing a starting point to produce the best nutritional characteristics for farmed tuna.

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Atlantic bluefin tuna (*Thunnus thynnus* L.); fatty acids; gas chromatography-mass spectrometry (GC-MS); microwave lipid extraction

Introduction

Tuna is highly valued as a food around the world. Farmed tuna meat is higher in fat content than wild tuna (Topic Popovic et al. 2012), an excellent quality that makes it favored in the seafood market. Bred fishes are fed continuously and excess calories are accumulated in the form of triglycerides, rich in poly-unsaturated fatty acids (PUFAs), particularly in omega-3 fatty acids, such as eicosa-5,8,11,14,17-pentaenoic acid (EPA), and docosa-4,7,10,3,16,19-hexaenoic acid (DHA) (Wheeler and Morrissey 2003; Topic

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Popovic et al. 2012), that are very important from a nutritional point of view (Albert et al. 2002; Sidhu 2003; Simopoulos 2008; Benjamin 2017). Moreover, tuna muscle shows a high ratio of omega-3/omega-6 that is beneficial for human health (Økland et al. 2005).

It is generally accepted that FA composition of lipids is species specific. However, the composition may vary greatly within a species. This variation is caused by both external factors, including habitat, temperature, and salinity (Farkas et al. 1980; Saito et al. 1997), season capture (Saito et al. 1997; Jensen et al. 2007; Ould Ahmed Louly et al. 2011), diet (Saito et al. 1996; Özogul and Özogul 2007; Celik 2008; Morais et al. 2015), and internal factors such as physiological conditions (migration and reproduction) and age (Ishihara and Saito 1996; Kiessling et al. 2001; Nakamura et al. 2007; Mourente et al. 2015). In this regard, very few studies have been performed until now to relate lipid content of muscle of Atlantic Bluefin tuna to age and sex: total lipid content seems to be influenced by size (Ishihara and Saito 1996; Wheeler and Morrissey 2003), whereas DHA content does not seem to be affected by maturity (Ishihara and Saito 1996). Other studies mainly regard variation of lipid content during oocytes maturation (Knapp et al. 2014), or during juvenile growth and the calorie needs (Goñi and Arrizabalaga 2010; Logan et al. 2015). However, until now no evidence on the lipid fatty acid composition of Atlantic bluefin tuna muscle in relation to size and sex is available to date.

To elucidate the influence of size and sex on the FA composition, it is necessary to keep constant other variables such as diet and environmental conditions. For this purpose, farmed tuna, living in the same habitat at the same environmental conditions and diet for several months, satisfy all these required conditions and allowed the comparison of FA composition to sex or size.

Generally, classical lipid extraction (Folch et al. 1957; Bligh and Dyer 1959) and gas chromatography coupled with flame ionization detection has been used for the analysis of FAs in tuna meat (Mourente et al. 2002; Nakamura et al. 2007; Al-Busaidi et al. 2015, Hernández-Martínez et al. 2016). These results have been reported as the percentage of each FA compared to the total fatty acids. Recently, we optimized an analytical method for the determination of FAs composition in fish muscle, that involves a microwave-assisted extraction (MAE) of lipids from a lyophilized sample, a derivatization of lipid extract to fatty acid methyl esters (FAMEs), and their separation and identification by gas chromatography-mass spectrometry (GC-MS) (Truzzi et al. 2017). This method allows the identification of the absolute variation of every single fatty acid. We demonstrated that the microwave-assisted lipid extraction requires a minimum sample handling and provides fast and reliable extraction at low temperatures that minimize fatty acid peroxidation to which monounsaturated fatty acid (MUFA) and PUFA are particularly sensitive.

We applied this methodology for the first time to the determination of the fatty acids in the muscle of Atlantic bluefin tuna (*Thunnus thynnus* L.), to demonstrate the feasibility and the practicality of this methodology for the determination of fatty acid composition in tuna muscle. In addition, this study will improve knowledge on the influence of size and sex on the fatty acid composition of muscle of farmed Atlantic bluefin tuna. The characterization of the lipid composition with sex and size may be useful for the

farmers to produce tuna with the best FA composition and excellent quality meat, satisfying the market preferences and increasing the value of the product.

Material and methods

Experimental design and animal sampling

Atlantic bluefin tuna (*Thunnus thynnus* L.) specimens were caught by purse seine from spawning grounds around the Mediterranean Sea during May–June 2015. Fishes were immediately moved into towing cages and transported over a period of several months to the tuna fish farm Fish and Fish (southeast of Malta). Fishes were fed with defrosted raw fish, as Pacific mackerel *Scomber japonicus*, Atlantic mackerel *Scomber scombrus*, and Atlantic herring *Clupea harengus*.

A total of 40 bluefin tuna specimens (20 males and 20 females) were obtained from the tuna farm in November 2015 during the post-spawning period. Curved fork length (from the tip of the upper jaw to the fork of caudal fin) and total body weight were measured for all tuna sampled. The overall mean length was 228 ± 33 cm, (males 240 ± 31 cm, females 216 ± 31 cm). The overall mean body weight was 238 ± 93 kg, (males 279 ± 86 kg, females 198 ± 83 kg). The sex of the fish was determined by examining gonads under a dissecting microscope. All fish were in the adult stage. Muscle samples were obtained from the upper part of the back, frozen immediately on dry ice, and then stored at −80 °C until analysis. For each specimen, three independent samples of muscle (about 10 g each) were collected.

The animals were sampled under the guidelines Art 36, par.1 REg (EU) N°508/2014. The procedures did not include animal experimentation, so ethics approval is not necessary in accordance with the Italian legislation.

Fatty acid analysis

All solvents and reagents were of HPLC grade (Illuminati et al., 2010; Truzzi et al. 2018). Each fish fillet was minced, homogenized (homogenizer MZ 4110, DCG Eltronic), and divided into aliquots of ∼1 g each. Analyses were carried out on three aliquots per fish. Tissues were accurately weighed and freeze-dried (Edwards EF4, Crawley, Sussex, England, UK) until constant weight. Lipid extraction was carried out on lyophilized powders following MAE according to the procedure of Truzzi et al. (2017).

Fatty acids were converted to their FAMEs and determined on an Agilent-6890 GC System coupled to an Agilent-5973N quadrupole Mass Selective Detector following the method optimized by Truzzi et al. (2017). The response factor for each FA was calculated against nonadecanoic acid methyl ester used as internal standard (19:0, 99.6%, Dr. Ehrenstorfer Gmbh, Germany). Fatty acid quantification was carried out by calculating both the percentage of each FA vs. total FAs and the mass fraction of fatty acids in g kg^{-1} of edible portion (fillet). For each sample, at least three measurements were performed by GC-MS.

The estimated limits of detection and quantification, calculated as in Truzzi et al. (2014a, b), ranged from ∼4 to ∼22 µg mL^{-1}, and from ∼13 to ∼66 µg mL^{-1}, respectively (Truzzi et al. 2017).

Nutritional indices

From the fatty acid profile (as percentage of each fatty acid vs. total fatty acids), three nutritional indices were calculated, which attributed different weights to fatty acids depending on the different contribution of these to the promotion or prevention of cardiovascular disorders: atherogenicity index (AI) and thrombogenicity (TI) indices (Ulbricht and Southgate 1991), and the hypocholesterolemic to hypercholesterolemic fatty acid ratios (HH) (Santos-Silva et al. 2002):

$$AI = [12{:}0 + (14{:}0 \times 4) + 16{:}0]/(\Sigma MUFAs + \Sigma PUFA - n6 + \Sigma PUFA - n3) \quad (1)$$

$$TI = \Sigma(14{:}0 + 16{:}0 + 18{:}0)/[0.5 \times \Sigma MUFAs + 0.5 \times \Sigma(n6) + 3 \times \Sigma(n3) + (n3/n6)] \quad (2)$$

$$HH = (18{:}1n9 + 18{:}2n6 + 20{:}4n6 + 18{:}3n3 + 20{:}5n3 + 22{:}5n3 + 22{:}6n3)/(14{:}0 + 16{:}0) \quad (3)$$

where MUFAs are the monounsaturated fatty acids, PUFAs are polyunsaturated fatty acids, distinguished in PUFA-n6 (the sum of the omega-6 PUFAs) and PUFA-n3 (the sum of omega-3 PUFAs). Other nutritional indices such as n3/n6, PUFAs/SFAs, unsaturated fatty acids/saturated fatty acids (UFA/SFAs), EPA(20:5n3)/DHA(22:6n3) ratios, and the sum EPA + DHA, were calculated from the fatty acid profiles.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) of 20 specimens *per* group. For each variable, Student's *t* test was applied to find significant differences between groups at the 95% confidence level. Relationships between variables were assessed using Pearson's correlation coefficient *r* (Steel et al. 1996). All statistical tests were performed with the statistical software Statgraphics 18 Centurion (2017).

Results and discussion

Lipid content

The tuna showed a water content of $600 \pm 40\ g\ kg^{-1}$, with a mean of $610 \pm 20\ g\ kg^{-1}$ for males and $600 \pm 60\ g\ kg^{-1}$ for females (Figure 1). Lipid content was $120 \pm 40\ g\ kg^{-1}$, with a mean of $110 \pm 20\ g\ kg^{-1}$ for males, and $130 \pm 50\ g\ kg^{-1}$ for females. Therefore, all of the analyzed tuna specimens have a high-fat content according to Olagunju et al. (2012). No significant differences were noted with regard to water and lipid content in

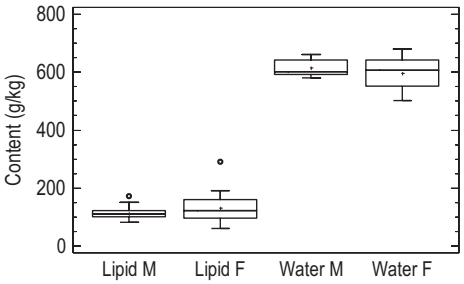

Figure 1. Box-and-whisker plot of lipid and water content in male (M) and female (F) Atlantic bluefin tuna.

relation to size and sex. Our results were consistent with the lipid percentage of bluefin tuna captured in the mid-Adriatic Sea (Topic Popovic et al. 2012) and of albacore tuna (*Thunnus alalunga*) found in the Pacific Ocean (Wheeler and Morrissey 2003).

Fatty acid composition

Figure 2 shows an example of a chromatogram obtained from a muscle sample of Atlantic bluefin tuna: no overlappings between peaks were noted. Table 1 shows the fatty acid composition as the percentage of total FAs and as g kg^{-1} fillet of specimens grouped by sex. For greater clarity, only fatty acids with a content >0.1% vs. total FAs were reported. No significant differences between males and females were found in the percentage of each FA vs. total FAs. For the SFAs, the main component was 16:0 (palmitic acid, 14%–15%) as reported in literature (Özogul et al. 2007; Ould Ahmed Louly et al. 2011; Topic Popovic et al. 2012), followed by 14:0 (myristic acid, ∼6%) and 18:0 (stearic acid, 4%–5%), both for males and female. Within the MUFAs, the most abundant were 18:1n9 (oleic acid, ∼12%), 22:1n9 (erucic acid, ∼11%), 20:1n9 (eicosenoic acid, ∼8%), and 16:1n7 (palmitoleic acid, ∼7%).

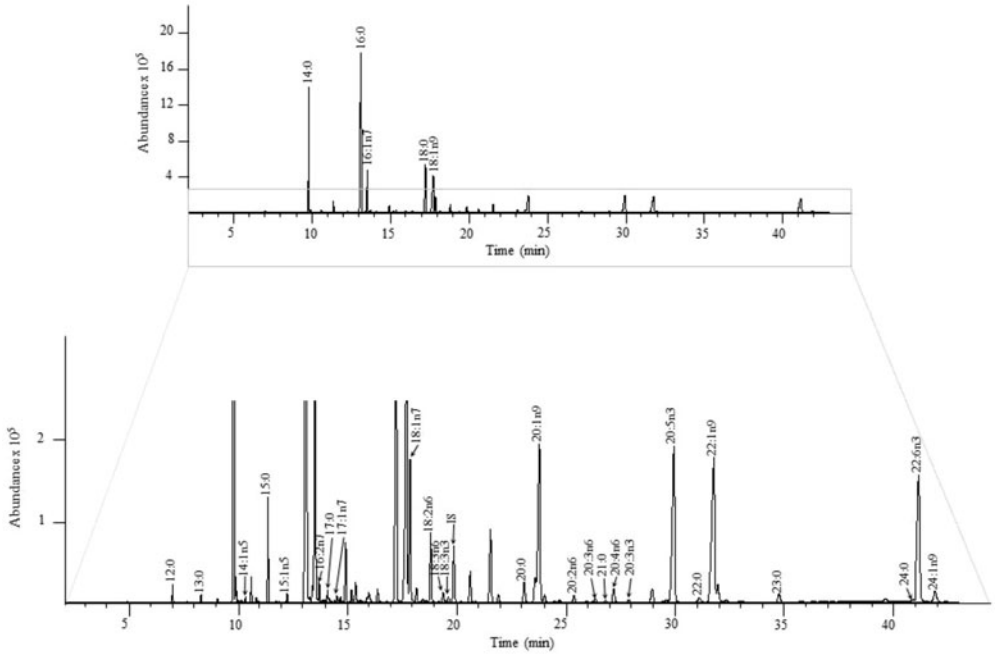

Figure 2. Example chromatogram of the fatty acid composition from a muscle sample of Atlantic bluefin tuna. Analytical conditions for FAME separation: an Agilent-6890 GC System was coupled to an Agilent-5973N quadrupole Mass Selective Detector with a CPS Analitica CC-wax-MS capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness). The injection volume was 1 μL, the split ratio 1:5 or 1:10; and the inlet temperature 250 °C. The helium carrier gas was 1 mL min^{-1} and 8.0 psi. The oven temperature was 100 °C, 1 min, 25 °C min^{-1} to 150 °C, 5 °C min^{-1} to 200 °C, and 1 °C min^{-1} to 230 °C. The total analysis time was 43 min. The transfer line was 280 °C; quadrupole 150 °C, mass source 230 °C, and solvent delay 2 min.

Table 1. Fatty acid composition as the percentage of total fatty acids and as g kg^{-1} fillet of Atlantic bluefin tuna muscle specimens by gender.

Fatty acid	Male (n = 20)		Female (n = 20)	
	Percentage of total fatty acids	g kg^{-1} fillet	Percentage of total fatty acids	g kg^{-1} fillet
14:0	$6.3^{a} \pm 0.5$	$5.7^{a} \pm 0.7$	$6.1^{a} \pm 0.7$	$6.2^{a} \pm 1.5$
15:0	$0.53^{a} \pm 0.04$	$0.48^{a} \pm 0.05$	$0.53^{a} \pm 0.05$	$0.54^{a} \pm 0.13$
16:0	$15^{a} \pm 1$	$13^{a} \pm 2$	$14^{a} \pm 1$	$15^{a} \pm 4$
17:0	$0.45^{a} \pm 0.04$	$0.40^{a} \pm 0.04$	$0.47^{a} \pm 0.07$	$0.47^{b} \pm 0.10$
18:0	$4.5^{a} \pm 0.4$	$4.1^{a} \pm 0.4$	$4.7^{a} \pm 0.5$	$4.8^{a} \pm 1.1$
20:0	$0.35^{a} \pm 0.03$	$0.31^{a} \pm 0.04$	$0.36^{a} \pm 0.06$	$0.37^{b} \pm 0.09$
22:0	$0.17^{a} \pm 0.02$	$0.15^{a} \pm 0.02$	$0.18^{a} \pm 0.03$	$0.18^{b} \pm 0.04$
Total SFAs[+]	$28^{a} \pm 1$	$25^{a} \pm 2$	$27^{a} \pm 1$	$27^{a} \pm 4$
16:1n7	$6.7^{a} \pm 0.4$	$6.0^{a} \pm 0.7$	$6.6^{a} \pm 0.6$	$6.7^{a} \pm 1.7$
17:1n7	$0.44^{a} \pm 0.02$	$0.39^{a} \pm 0.04$	$0.45^{a} \pm 0.04$	$0.46^{b} \pm 0.12$
18:1n9	$12^{a} \pm 0.8$	$11^{a} \pm 1$	$13^{a} \pm 1$	$13^{a} \pm 4$
18:1n7	$2.4^{a} \pm 0.2$	$2.2^{a} \pm 0.3$	$2.5^{a} \pm 0.2$	$2.5^{a} \pm 0.6$
20:1n9	$7.7^{a} \pm 0.8$	$7.0^{a} \pm 1.2$	$7.7^{a} \pm 1.0$	$7.9^{a} \pm 2.4$
22:1n9	$12^{a} \pm 1$	$10^{a} \pm 2$	$11^{a} \pm 2$	$12^{a} \pm 3$
24:1n9	$1.1^{a} \pm 0.1$	$1.0^{a} \pm 0.2$	$1.2^{a} \pm 0.2$	$1.2^{a} \pm 0.3$
Total MUFAs[*]	$42^{a} \pm 2$	$38^{a} \pm 3$	$43^{a} \pm 3$	$43^{a} \pm 6$
18:3n3	$1.0^{a} \pm 0.1$	$0.92^{a} \pm 0.16$	$1.0^{a} \pm 0.1$	$1.1^{a} \pm 0.33$
20:3n3	$0.13^{a} \pm 0.01$	$0.12^{a} \pm 0.02$	$0.14^{a} \pm 0.01$	$0.14^{a} \pm 0.04$
20:5n3	$14^{a} \pm 2$	$12^{a} \pm 2$	$13^{a} \pm 2$	$14^{a} \pm 5$
22:6n3	$12^{a} \pm 2$	$11^{a} \pm 3$	$13^{a} \pm 3$	$13^{a} \pm 5$
Total n-3 PUFAs	$27^{a} \pm 3$	$25^{a} \pm 4$	$26^{a} \pm 4$	$29^{a} \pm 7$
18:2n6	$1.5^{a} \pm 0.1$	$1.3^{a} \pm 0.2$	$1.5^{a} \pm 0.1$	$1.5^{a} \pm 0.4$
18:3n6	$0.20^{a} \pm 0.02$	$0.18^{a} \pm 0.03$	$0.21^{a} \pm 0.02$	$0.21^{a} \pm 0.06$
20:2n6	$0.24^{a} \pm 0.01$	$0.22^{a} \pm 0.03$	$0.25^{a} \pm 0.02$	$0.25^{b} \pm 0.08$
20:3n6	$0.16^{a} \pm 0.02$	$0.15^{a} \pm 0.03$	$0.17^{a} \pm 0.02$	$0.17^{b} \pm 0.05$
20:4n6	$0.91^{a} \pm 0.2$	$0.83^{a} \pm 0.21$	$0.94^{a} \pm 0.2$	$0.96^{a} \pm 0.32$
Total n-6 PUFAs	$3.0^{a} \pm 0.2$	$2.7^{a} \pm 0.3$	$3.1^{a} \pm 0.2$	$3.1^{a} \pm 0.5$
16:2n7	$0.18^{a} \pm 0.02$	$0.16^{a} \pm 0.02$	$0.18^{a} \pm 0.02$	$0.18^{a} \pm 0.04$
Total PUFAs	$30^{a} \pm 3$	$28^{a} \pm 4$	$30^{a} \pm 4$	$32^{a} \pm 7$
EPA + DHA	$26^{a} \pm 3$	$24^{a} \pm 4$	$26^{a} \pm 4$	$27^{a} \pm 7$

Means within rows of the same parameter bearing different letters are significantly different ($p < 0.05$).
[+]includes 12:0, 13:0, 21:0, 23:0, 24:0.
[*]includes 14:1n5, 15:1n5.

As well as palmitic acid, the higher concentration of oleic acid found in the farmed fishes was attributed to the feed mainly composed of herring and mackerel (Jensen et al. 2007; Özogul et al. 2007; Celik 2008). Moreover, all of the MUFAs cited above are biosynthesized from palmitic acid; consequently, a high palmitic acid percentage could determine a high content of its metabolites. The most abundant PUFAs in both Atlantic bluefin tuna males and females were 20:5n3 (EPA, 13%–14%), and 22:6n3 (DHA, 12%–13%): their sum (~26%) was higher than the sum in the mid-Adriatic bluefin tuna, i.e., ~19% and ~23% for farmed and wild tuna, respectively (Topic Popovic et al. 2012).

The very low concentration of arachidonic acid (20:4n6) found in the specimens (~1%) may be related to the low percentage of its precursor, linoleic acid (18:2n6). The fatty acid class that accounted for the most important percentage was MUFAs (~42%), followed by PUFAs (30%–31%), and SFAs (~27%) for both males and females. A high percentage of MUFAs is characteristic of fish from warm or temperate regions (Dey et al. 1993). For the PUFAs, the most represented were the omega-3 fatty acids (about 90% of total PUFAs) in agreement with the literature (Mourente et al. 2015).

In terms of absolute content (g kg^{-1} fillet) (Table 1), females showed a statistically significant higher values of 17:0, 20:0, 22:0, 17:1n7, 20:2n6, and 20:3n6 than males (Table 1), but these changes did not produce significant differences in total SFAs,

MUFAs, and PUFAs since the minor FAs were each less than or equal to 1%. The omega-3 showed an overall mean of ~27 g kg^{-1} fillet (no significant differences were noted between males and females), comparable to the omega-3 content of bluefin tuna from mid-Adriatic, 26 g kg^{-1} fillet (Topic Popovic et al. 2012), and of albacore tuna *Thunnus alalunga* from the west coast, 21–35 g kg^{-1} fillet (Wheeler and Morrissey 2003). In particular, the EPA + DHA sum ranged from ~21 to 32 g kg^{-1} fillet in males, and from ~17 to 32 g kg^{-1} fillet in females, with an overall mean of ~24 and ~27 g kg^{-1} fillet, respectively (Table 1). No statistically significant differences were found between males and females.

Considering the overall results, the MUFAs, PUFAs, and SFAs contributed ~41, ~30, and ~26 g kg^{-1} fillet, respectively. Our measurements are consistent with those in Atlantic bluefin tuna from the mid-Adriatic Sea (MUFAs 44.8 g kg^{-1} fillet, PUFAs 29.5 g kg^{-1} fillet), except for SFAs (34.9 g kg^{-1} fillet, Topic Popovic et al. 2012).

In addition to sex, the fatty acid composition was also analyzed in relation to the tuna size. Individual correlations between FA and fish weight were studied. The results of the correlation coefficients at the 95% confidence intervals are shown in Figure 3. Only FAs that significantly correlated to the fish weight are reported. For males, eight out of the total FAs with a content >1% were found to have a statistically significant linear correlation with fish weight: 14:0, 16:0, 18:1n9, 20:1n9, and 22:1n9 (positive correlation), and 20:4n6, 22:6n3, and 20:5n3 (negative correlation). Overall, in males both total SFAs and total MUFAs showed a statistically significant positive linear correlation with weight, from ~24% to ~32%, and from ~38% to ~46% with increasing size, respectively. The total PUFAs showed a statistically significant negative linear correlation with weight from ~38% to ~23% with increasing size.

We can conclude that with increasing weight, male specimens accumulate in fat saturated fatty acids, reducing significantly the content of PUFAs, very important from a nutritional point of view. For females, only five out of the total FAs with a content >1% were found to have a statistically significant correlation with fish weight. The 20:1n9 and 22:1n9 (as well as total MUFAs) showed a polynomial positive correlation with weight: the lowest percentage was observed in female with medium size (150–250 kg). 20:4n6, 20:5n3 (EPA), 22:6n3 (DHA) (as well as total PUFAs), showed a statistically significant negative polynomial correlation with weight. Therefore, medium-sized females showed the highest percentages of EPA and DHA. These results underline that in females, weight seems to have a different influence on fatty acid composition compared to males, showing clear sex differences in the composition with respect to size.

Nutritional characteristics

Based on the chemical differences of the fatty acid composition with specimen size, the nutritional indices were also analyzed in relation to weight. The results are shown in Table 2 and in Figure 4, where only indices that were significantly correlated to fish weight are reported.

Generally, as seen for the FAs, males and females showed different correlation values of nutritional indices with weight: a significant linear correlation for males, and a significant polynomial correlation for females. In the analyzed tuna, a very high n3/n6 ratio was found (~9) and no significant differences were noted in relation to sex, but

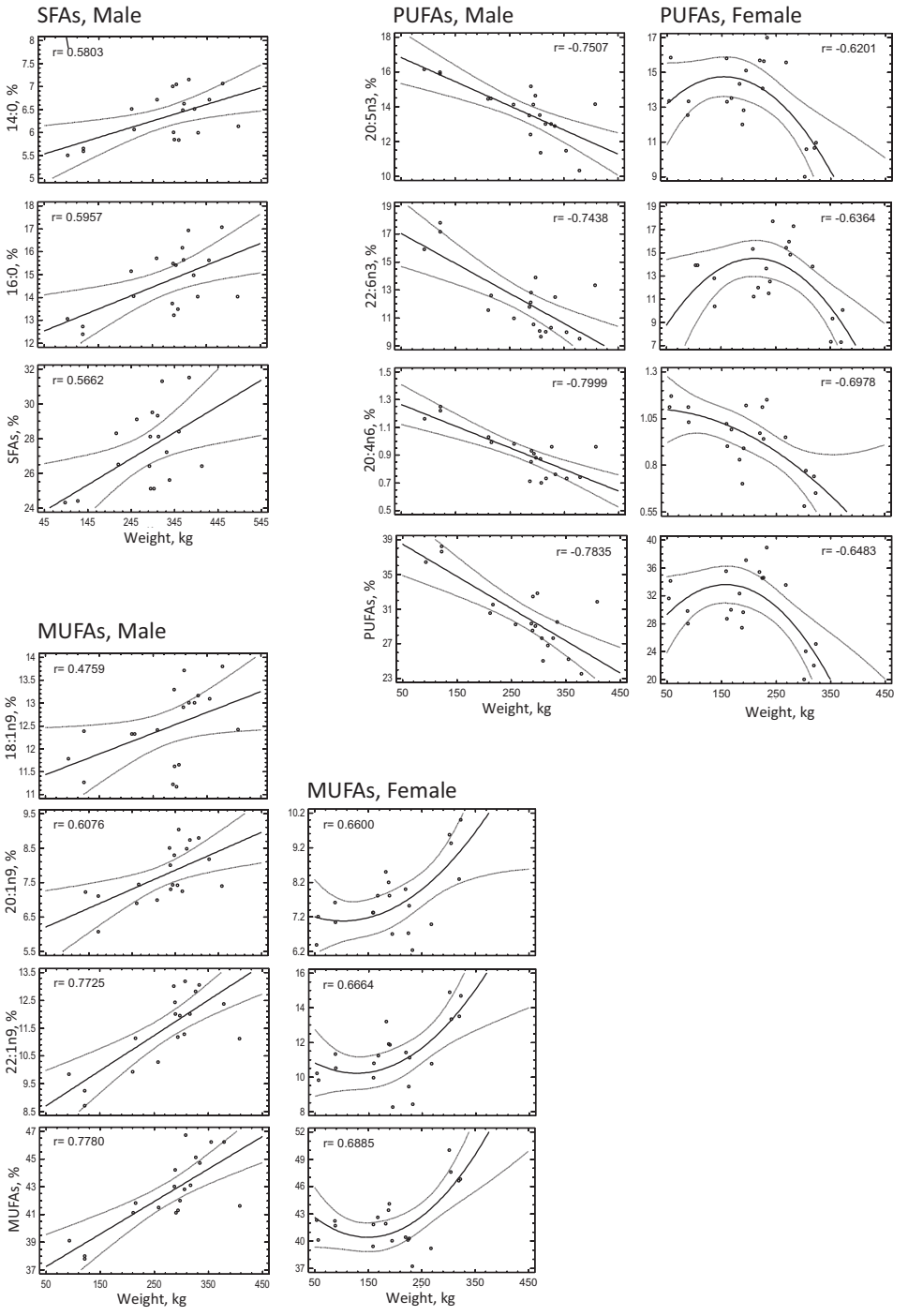

Figure 3. Correlation at 95% confidence interval between the fatty acid content as the percentage as a function of total fatty acids and the body weight of male and female Atlantic Bluefin tuna. The correlation coefficients *r* characterize the significance of the fatty acid percentage vs. total fatty acids and the tuna weight ($p < 0.05$).

Table 2. Nutritional indices of lipid fractions in Atlantic bluefin tuna muscle.

Nutritional index	Male	Female
n3/n6	9.1 ± 1.1 (7.8–10.3)	8.9 ± 1.3 (6.7–10.8)
DHA/EPA	0.86 ± 0.2 (0.74–1.1)	1.0 ± 0.3 (0.68–1.2)
PUFAs/SFAs	1.1 ± 0.2 (0.75–1.62)	1.1 ± 0.2 (0.64-1.64)
UFAs/SFAs	2.6 ± 0.1 (2.2–3.2)	2.7 ± 0.2 (2.1–3.3)
Atherogenicity Index	0.57 ± 0.04 (0.47–0.68)	0.55 ± 0.05 (0.44–0.72)
Thrombogenicity Index	0.23 ± 0.02 (0.16–0.31)	0.22 ± 0.02 (0.16–0.34)
Hypo-/Hypercholesterolemic Fatty Acid ratio	1.9 ± 0.2 (1.5–2.7)	2.1 ± 0.2 (1.4–2.9)

the highest n3/n6 ratio (∼10, Figure 4) was found in males smaller than 150 kg and in females of medium size from 150 to 250 kg. Nutritionists have established a desirable ratio n3/n6 $\geq$ 5 in fish to improve nutritional value and to reduce disease (Moreira et al. 2001). The high value of n3/n6 ratio in farmed tuna in the Mediterranean Sea suggests good meat quality. The sum of the percentages of EPA + DHA ranged from 20% to 33%, both in males and females, and the highest values were found in males with size smaller than 150 kg and in females of medium size from 150 to 250 kg.

Generally, wild bluefin tuna are characterized by higher DHA/EPA ratios ($\geq$2), characteristic of a carnivore diet (Topic Popovic et al. 2012; Mourente et al. 2015). In this study, this ratio was about 1 both for males and females (Table 2). This composition is consistent with results obtained by Topic Popovic et al. (2012), where the DHA/EPA ratio was 1.2 in farmed tuna. Moreover, it can be noted that wild bluefin tuna use preferentially, as source of metabolic energy, MUFAs, such as 18:1n9, 20:1n9, and even EPA, but not DHA. This phenomenon is probably one of the causes of the high DHA/EPA ratio (i.e., values of 2–3) (Mourente et al. 2015). In farmed tuna, the chronic motion insufficiency probably determines the lower energy requirements, which reduces the metabolism of fatty acids, improving the meat characteristics of cultured fish, such as the major percentage of the essential omega-3 EPA.

The PUFA/SFA ratio (∼1.1 for the whole set) was similar to the results found in Atlantic bluefin tuna from the Gulf of Mexico (Hernández-Martínez et al. 2016). All specimens showed a ratio >0.45, i.e., the minimum recommended value to avoid the potential to raise blood cholesterol levels (Department of Health and Social Security 1984).

Concerning the AI, TI, and HH indices, the results of the present study agreed with the literature values (Al-Busaidi et al. 2015; Hernández-Martínez et al. 2016). Low values of AI ($\leq$0.51) and TI ($\leq$0.30) are beneficial to health (Ulbricht and Southgate 1991). The TI values of the analyzed Atlantic bluefin tuna were below the limit for all specimens (range ∼0.16 to ∼0.34, Table 2), whereas the AI values (range ∼0.45 to ∼0.70) were below the limit only for males with less than 150 kg and medium size females from 150 to 250 kg. The HH index was high, from ∼1.5 to ∼2.8, both for males and females (Table 2), indicating that a regular intake of this fish may produce hypocholesterolemic effects (Santos-Silva et al. 2002).

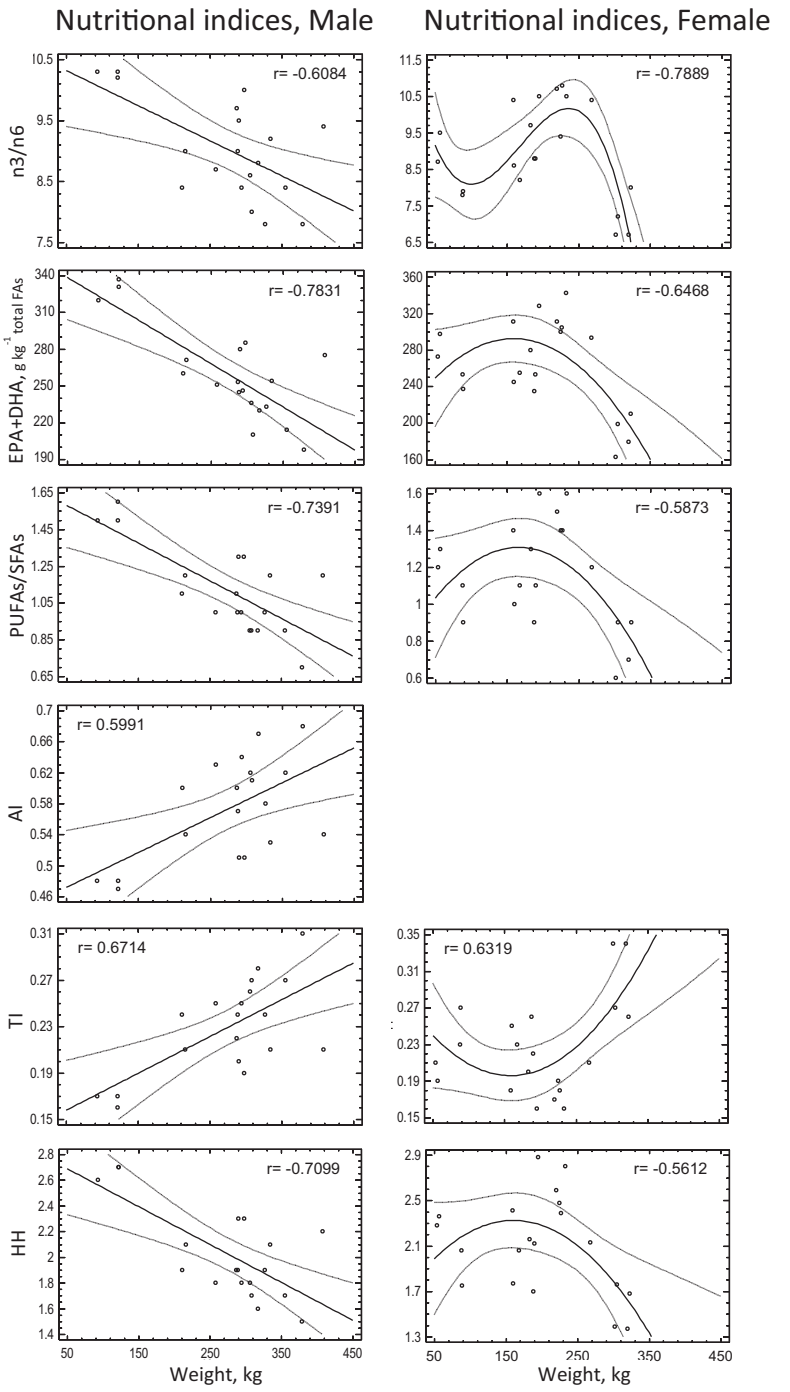

Figure 4. Correlation of nutritional markers with tuna body weight of male and female Atlantic bluefin tuna at the 95% confidence interval. Nutritional indices were obtained from the fatty acid profile (each fatty acid as percentage of the total). The correlation coefficients *r* characterize the significance between the nutritional markers and the tuna weight ($p < 0.05$).

Conclusions

An analytical method based on GC-MS was applied for the determination of fatty acids in the muscle of Atlantic bluefin tuna to improve knowledge on the influence of size and sex on the fatty acid composition. This study demonstrated the feasibility and the practicality of the analytical methodology applied and improved the knowledge about the influence of specimen size on the fatty acid composition of muscle of *Thunnus thynnus* L. In particular, we found that the muscle of these tuna farmed in the Mediterranean Sea did not show substantial differences in lipid profile in relation to sex. However, surprisingly, males and females showed different correlations of FAs composition with size, highlighting a never reported clear gender difference. Therefore, although in general Atlantic bluefin tuna have very high value from a nutritional point of view, males smaller than 150 kg and females from 150 to 250 kg appear to be of greater nutritional value. These results represent a starting point to characterize the best nutritional characteristics for both sexes in farmed tuna and may support the farmers to produce the best FA composition and high quality meat, increasing the value of this product.

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