Original Research Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Nutritional quality traits of Mediterranean mussels (*Mytilus galloprovincialis*): A sustainable aquatic food product available on Italian market all year round

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Abstract

In this study, Mediterranean mussels (*Mytilus galloprovincialis*) coming from Italian production were purchased in the most important Italian wholesale fish market in different seasons. Biometrical parameters and chemical composition were investigated, with a particular focus on lipid quality and fatty acids (FAs) composition. Results showed a valuable nutritional profile independently by the season of production, represented by high protein and low-fat content, with the lipid portion represented by high amounts of beneficial FAs, particularly the long chain of the n-3 series. Some differences ($p < 0.05$) were found in carbohydrates and fat content of mussels edible tissues and in FAs profile of specimens collected in different seasons. The most favourable composition in terms of lipid quality was found in mussels collected during spring, corresponding to the moment of the year when mussels store energy reserves in the form of carbohydrates and fat (preparing for the spawning events) and when seawater is enriched in phytoplankton. The lipid health indices calculated (n6/n3, AI, TI) showed optimal values independently by the season of production. The outcomes obtained in this study could help supporting the appreciation of Mediterranean mussels as nutritional valuable seafood product, thus helping encouraging their consumption and promoting the appraisal of this farming sector essential for Italian aquaculture and related to low environmental impact.

Keywords

Fatty acids, omega-3 fatty acids, lipids, fish and fish products

Date received: 22 March 2022; accepted: 2 June 2022

INTRODUCTION

Fatty acids (FAs) profile of food products is a topic of great interest in human nutrition. The quality of dietary lipids affects both positively and negatively the health of consumers, regulating fundamental metabolic functions (Zárate et al., 2017). An appropriate consumption of long chain n-3 FAs has been demonstrated to have potential health benefits in human nutrition toward immune and inflammation regulation (Gil and Gil, 2015; Patterson et al., 2012). Last decades have been characterised by huge nutritional changes in fat quality of Western diets, leading to an overall decrease of n-3 intakes and a huge increase of n-6 intakes in diets characterised by 30–40% of total energy coming from FAs (Patterson et al., 2012; Strobel et al., 2012). The unbalanced n-6 to n-3 ratio (n-6/n-3) that characterises modern Western diets (reaching values of 15–20/1), has been indicated to shift the physiological state of consumers toward proinflammatory and prothrombotic conditions (Leclercq et al., 2011; Patterson et al., 2012;

Food Science and Technology International 0(0) 1–11

DOI: 10.1177/10820132221109582
journals.sagepub.com/home/fst
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Simopoulos, 2010; Zárate et al., 2017). On the contrary, optimal dietary n-6/n-3 ratios, ranging from 1/1 to 4/1, have been associated protective effects toward many pathological condition (Simopoulos, 2010).

Seafood are considered the primary source of beneficial FAs, particularly the long chain FAs of the n-3 series eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid, due to a natural enrichment of the marine food chain (Strobel et al., 2012; Wall et al., 2010). Despite the existence of some risks related to the consumption of seafood (toxins, allergens, chemical pollutants, etc.) scientific evidences have reported that the benefits of seafood products consumption exceed the potential risks (Gil and Gil, 2015). Among seafood, mussels are considered an important source of good nutrients in the human diet, being characterised by the presence of high-quality proteins, low fat and highly available n-3 FAs, together with other nutrients (such as iron, vitamins and others) (Yaghubi et al., 2021). Recent research showed that including mussels as protein component of meals, n-3 status in consumers can be significantly improved (Carboni et al., 2019). In addition, it has been evidenced that mussels farming is responsible for lower greenhouse gas emissions compared to the production chain of other animal origin protein sources and, thus, it can considered a more sustainable production segment (Carboni et al., 2019; Yaghubi et al., 2021). Actually, the impact of mussels production on the environment, in terms of greenhouse gas emissions, freshwater and land use and eutrophication potential could be considered as negligible if compared to finfish aquaculture (Tamburini et al., 2020). Hilborn et al. (2018) assessed 148 types of animal source food measuring four metrics of their environmental impacts (energy use, greenhouse gas emissions, release of nutrients and acidifying compounds). Interestingly, among all foods analysed, molluscs aquaculture showed overall the lowest impact in these categories. Furthermore, it is worth mentioning that mussels have positive credits due to the removal of nutrients from the ecosystem and to the possible valorisation of the shell waste (Vélez-Henao et al., 2021). Encouraging the population to increase consumption of mussels may be a viable strategy to improve the intake of long chain n-3 FAs with reduced concerns about toxins and environmental damage compared to fish farming (Carboni et al., 2019).

According to the latest statistics, bivalve molluscs have experienced an increase in global annual production of over 100% from 8.2 to 17.5 million tons between 1995 and 2018, with aquaculture production accounting for 94% of the total yield (EUMOFA, 2019; FAO, 2020). In Europe the first producer is Spain (287,000 tons), followed by France and Italy (145 and 93 thousand tons, respectively) (EUMOFA, 2019). The species most commonly farmed in the EU is represented by the Mediterranean mussel (*Mytilus galloprovincialis*), accounting for the 61% of the total production (EUMOFA, 2019). Farming of *M. galloprovincialis* represents one of the main segment of the Italian aquaculture. Mediterranean mussel is the only species cultured along the Italian coasts by means of floating rafts and long-line systems on the Ligurian, Ionian, whole Adriatic and central Tyrrhenian Seas, due to favourable natural condition for production and growth. The national sector is composed of ~245 production sites, with a production of 62,000 tons of mussels for 2018 (12% of EU), with the entire production coming from aquaculture (EUMOFA, 2019). Mediterranean mussel is largely appreciated by Italian consumers for its organoleptic properties and for its competitive price, especially if compared with other seafood products (Orban et al., 2002). The apparent consumption in Italy amounted to 120,257 tons live weight equivalent in 2016, with a high level of local consumption mainly concentrated in touristic areas. Mussel consumption in Italy is highly subjected to seasonal peaks, with the higher amounts (4000–5000 tons per month) consumed during summer, when the demand grows due to tourism and the national production is highly available and a second peak (4000 tons) recorded in December, when a lack of national production occurs and, consequently, the consumption is strictly dependent on imports (particularly, from Spain) (EUMOFA, 2019).

Mussels feed mainly on phytoplankton and organic matter by constantly filtering seawater. Several studies have shown that the quality of mussels is influenced by different environmental factors such as climatic conditions and, particularly, by seasonality, due to natural changes of water temperature and food availability (Azpeitia et al., 2016; Bongiorno et al., 2015; Cherifi et al., 2018; Khan et al., 2006; Orban et al., 2002; Prato et al., 2010). Seasonal fluctuations of FAs profile are not uncommon among bivalve molluscs, as the blooming of different planktonic populations during the year may affect lipid profile of the organisms relying on plankton as the main food source (Orban et al., 2002). Furthermore, fluctuations in meat yield (MY) and quality in mussels is related to the gametogenic cycle of animals, involving the consumption of lipid and carbohydrates reserves because of the spawning efforts (Azpeitia et al., 2016; Gabbot, 1983; Gallardi et al., 2014).

Because of suchconsiderations, the awareness of encouraging the consumption of Mediterranean mussel as nutritional valuable food associated to reduced negative implications is considerable, in order to promote the appraisal of this farming sector essential for Italian aquaculture. Thus, the aim of the present study was to characterise the nutritional quality traits of Mediterranean mussels specimens obtained on the most important Italian wholesale fish market, in Milan, in different seasons, with a particular focus on FAs profile and lipid quality.

MATERIALS AND METHODS

Sampling and measurement of biometric parameters

Samples of Mediterranean mussels (*Mytilus galloprovincialis*) analysed in this study were purchased from

wholesale fish market of Milan (Italy). A total of 28 net bags (1 kg of mussels each, about 50 individuals) were collected in in three subsequent seasons, as follows: 8 net bag in S1 (March-May), 15 net bag in S2 (June-September) and 5 net bag in S3 (November-January). In order to simulate the buying process of an average consumer, samples were purchased based on their availability on the market, collecting samples coming from the mayor Italian producing sites (La Spezia, Olbia, Goro, Gabicce, Taranto) in each season. After collection, samples were transported under refrigeration (+4–6°C) to the University laboratory. Upon arrivals, mussels were inspected and animals with open valves were discarded. All mussels were manually shucked by cutting the adductor muscle with a knife. Forty individuals were taken at random from each net bag for measurement and biometric registration: total length, whole weight, shell weight and fresh meat weight. Intervalvular fluid was determined per difference. The MY and condition index (CI) were calculated according to Okumuş and Stirling (1998) as follows:

$$\mathrm{MY} = \frac{\text{wet meat weight}}{\text{total weight}} \times 100$$

and

$$\mathrm{CI} = \frac{\text{wet meat weight}}{\text{total weight-shell weight}} \times 100$$

Chemical and fatty acid composition

The forty fresh individuals from each net bag mentioned above were divided in two casual pools of twenty specimens (total sample size N = 56) and stored in vacuum-sealed bags at −20°C for proximate and FAs analysis. Before the analysis, the edible part of each pool was homogenised in a laboratory blender (Ultra Turrax, T25, IKA-Labortechnik, Germany). Dry matter, crude protein and ash content of each pooled sample were determined according to AOAC methods (AOAC, 1996). Extraction and quantification of total lipids was performed according to the method of Folch et al. (1957), using CHCl3:MeOH (2:1, v/v). Carbohydrates were calculated by difference. The energy content of 100 g of edible portion was calculated multiplying the g of lipid, protein and carbohydrate by energy per g (4.27 kcal/g for protein, 4.11 kcal/g for carbohydrate and 9.02 kcal/g for lipid). Fatty acid methyl esters (FAMEs) were prepared following the method described by Christie (2003) on an aliquot of 20 mg of total lipid. FAMEs were analysed by gas chromatography (Agilent 6890 GC) and flame ionization detection (FID). The column was an HP-Innowax fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) by Agilent Technologies (Santa Clara, CA, USA). The oven temperature programme was: from 100°C to 180°C at 3° C/min; 2.5°C/min up to 250°C and held for 10 min. FAMEs were identified using external standards purchased by Supelco (Bellafonte, PA, USA). Chromatographic peak areas were corrected according to the theoretical relative response correction factors published by Ackman (2002). Results were expressed as g/100 g of total FAs. All chemical analyses were performed in duplicate.

Data analysis

Statistical analysis were performed by JMP 16 from SAS Inc. (Cary, NC, USA) and Unscramble X 10.1.1 (Camo Software AS, Oslo, Norway). Comparison among the three groups (S1, S2, S3) was performed by van der Waerden test, considering the test significant when $p\text{-value} < 0.05$. Steel-Dwass was performed as post-Hoc test for multiple comparisons. Data obtained by biometric measurements and chemical analysis were fused in a data matrix and submitted to auto-scaling, in order to avoid the bias due to the different measure units of each variable. Principal Component Analysis was employed as unsupervised multivariate method in order to detect the presence of trend among samples according to the season of sampling. The score plot and the loading matrix were used for data interpretation.

RESULTS AND DISCUSSION

Biometric measurements and proximate composition

The results of the biometric measurements and the proximate composition of mussels analysed in this study are presented in Table 1.

Among the biometric parameters measured, meat weight, MY and CI showed significant differences among seasons. Interestingly, MY was higher in S1 than in S2 and S3; CI was higher in S1 than in S2, whilst intermediate values were found in S3. MY and CI represent the most practical and simplest methods of monitoring commercial quality of bivalve molluscs, related to their gametogenic activity and consequent seasonal variations in tissue storage cycle (Orban et al., 2002). These parameters vary seasonally depending on latitude, water temperature and salinity, food availability and even in relation to the gametogenic cycle of animals. Actually, changes in biometric parameters of mussels have been associated to the alternation of phases of energy accumulation and depletion, related to the animal physiological cycle, occurring during the year (Bongiorno et al., 2015). Several authors observed significant fluctuations in MY and CI of mussels collected in different seasons (Çelik et al., 2012; Cherifi et al., 2018; Fernández-Reiriz et al., 2015; Gallardi et al., 2014). It has been suggested that lower values for the MY recorded in mussels produced during the cold season could be related

Table 1. Biometric parameters and proximate composition of Mediterranean mussels collected on the Italian market. Data are presented as mean ± standard deviation.

Season	S1		S2		S3	
Biometric measurements						
Total length (cm)	6.31 ± 0.34		6.20 ± 0.49		5.92 ± 0.25	
Total weight (g)	19.91 ± 2.45		19.44 ± 4.75		16.39 ± 2.79	
Meat weight (g)	9.20 ± 1.79	a	7.73 ± 2.59	ab	6.30 ± 1.06	b
Meat yield, MI (%)	46.45 ± 6.99	a	39.52 ± 5.58	b	38.97 ± 4.00	b
Condition Index, CI	72.51 ± 11.43	a	64.11 ± 9.26	b	62.53 ± 11.27	ab
Proximate composition (g per 100 g of edible portion)						
Moisture	78.57 ± 3.41	b	79.19 ± 3.30	b	84.99 ± 1.56	a
Protein	9.45 ± 1.22		10.01 ± 1.46		8.93 ± 1.17	
Lipid	2.17 ± 0.99	a	1.50 ± 0.33	a	0.93 ± 0.25	b
Ash	2.29 ± 0.33		2.41 ± 0.72		2.76 ± 0.45	
Carbohydrates	7.52 ± 2.59	a	6.89 ± 2.28	a	2.38 ± 0.58	b
Energy (kJ per 100 g of edible portion)	379.98 ± 82.83	a	353.92 ± 61.18	a	235.54 ± 37.00	b

a, b = mean values associated to different letters on the same row were significantly different ($p < 0.05$).

to a higher energetic expense that animals have to deal with during a poorer environmental seasons to maintain or produce gametes (Azpeitia et al., 2016). According to this, we found the highest values for the MY and the CI during spring (S1), when the environmental conditions are favourable for mussels growth and when mussels are probably in a stage of energy accumulation, before spawning events.

Significant differences were detected in different seasons for the proximate composition of Mediterranean mussels. Overall, mussels specimens were characterised by 8.93–10.01% protein, 0.93–2.17% fat, 2.29–2.76% ash and 2.38–7.52% carbohydrates, with values comparable to those previously published for Mediterranean mussels coming from different producing sites (Azpeitia et al., 2016; Bongiorno et al., 2015; Dernekbaşı, 2015; Orban et al., 2002; Stratev et al., 2017). No differences were detected among S1, S2 and S3 for protein content. This outcome is very appealing, suggesting that Mediterranean mussels delivered to the Italian wholesale market can be considered as an optimal source of animal origin proteins independently by the season of purchasing. Actually, mussels have been indicated as a considerable source of high quality proteins characterised by an amino acid score comparable to those of the best animal origin proteins (Venugopal and Gopakumar, 2017; Yaghubi et al., 2021), thus advisable for an higher inclusion in human diet. Approximately, per 100 kcal of cooked product mussels provide as much protein as other animal source foods (Soren and Biswas, 2019).

Significant differences ($p < 0.05$) were detected for lipid and carbohydrates content of mussels collected in different seasons. Lipid and carbohydrates content was higher in S2 and S1 and then decreased in S3. Similar fluctuations, (minimum lipid content in winter and maximum in summer) have been reported in literature studies performed on the nutritional quality of Mediterranean mussels (Bongiorno et al., 2015; Cherifi et al., 2018; Orban et al., 2002; Prato et al., 2010). Carbohydrates and lipids represent the energetic reserves in molluscs. The accumulation or the depletion of the stored reserves can be affected by several factors such as reproductive cycle, food availability, water temperature and salinity (Fernández-Reiriz et al., 2015; Gallardi et al., 2014). Particularly, the growth phase of molluscs coincides with the storage of lipids, while during the maturation phase lipids stores are mobilised (Brousseau, 1983; Newell et al., 1982) and a decrement of lipid content related to the spawning period is observable (Dernekbaşı, 2015; Narváez et al., 2008). Gametogenesis in mussels represents an energy demanding process that requires the mobilisation of lipid reserves, which are stored when food supplies are abundant and gonad activity is minimal. On such considerations, the different lipid and carbohydrates contents detected in mussels in this study might be attributed to different growth phases of specimens and to the process of storage and depletion of the body energy reserves. However, the average values detected (2.17% fat as maximum) suggested Mediterranean mussels as lean or low-fat seafood product, according to the classification system proposed by Ackman (1990). Experimental trials evidenced that including lean seafood (in substitution of meat from lean terrestrial animals) in mice diets may positively influence healthy status (Liisberg et al., 2016). Thus, the promotion of Mediterranean mussels from Italian farming as a lean seafood product could help expanding its appreciation on the market given the renewed interest of consumers toward low-fat foods, generally considered to improve the healthiness of the diet.

Fatty acid composition and lipid quality indices

Results obtained for FAs profile of Mediterranean mussels are reported in Table 2 and in Figure 1.

The relative proportion for the classes of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) was similar in all the samples of mussels analysed, independently by the season. According to previously published data (Azpeitia et al., 2016; Bongiorno et al., 2015; Cherifi et al., 2018; Dernekbaşı, 2015; Ventrella et al., 2008), the class of PUFAs was found to be prevalent during the entire sampling period, representing 43–50% of total FAs, followed by SFAs and MUFAs. However, PUFAs content showed significant differences ($p < 0.05$) according to the season, reaching the highest value in S3 and the lowest in S1. This outcome could be ascribed to physiological factors associated to environmental conditions. Actually, an inverse relationship between the content of PUFAs in marine organisms and the water temperature has been reported (Mortensen et al., 1988; Thompson et al., 1992), being this class of FAs highly employed to maintain cell membrane fluidity in molluscs at cold environmental temperature (Beninger and Stephan, 1985). Similar seasonal trends for PUFAs content were found by Bongiorno et al. (2015) in Mediterranean mussels collected in the Adriatic Sea, with higher content evidenced in winter and then decreasing during summer.

Among PUFAs, the long chain FAs of the n-3 series presented a higher content than FAs of the n-6 series (Figure 1). Two FAs accounted for the most of n-3 PUFAs, namely EPA (20:5n-3) and DHA (22:6n-3) (Table 1). Elevated levels of long chain PUFAs of the n-3 series, with a high contribution of EPA and DHA, were previously reported in Mediterranean mussels (Azpeitia et al., 2016; Bongiorno et al., 2015; Cherifi et al., 2018; Dernekbaşı, 2015; Freites et al., 2002; Orban et al., 2002; Ventrella et al., 2008). The high content and the variety of PUFAs found in mussels in this study might be explained

Table 2. Fatty acid profile (g/100 g of total fatty acids) of Mediterranean mussels collected on the Italian market. Data are presented as mean ± standard deviation.

	S1		S2		S3	
14:0	4.86 ± 1.62	a	3.52 ± 1.10	b	2.90 ± 0.81	b
16:0	17.81 ± 1.37	c	22.38 ± 1.17	a	20.81 ± 1.40	b
16:1n-7	19.05 ± 5.17	a	8.77 ± 3.42	a	5.21 ± 1.09	c
16:4n-1	4.55 ± 2.00	c	7.28 ± 1.59	b	9.88 ± 2.39	a
18:0	3.60 ± 0.72	b	4.72 ± 0.70	a	4.74 ± 0.56	a
18:1n-9	1.82 ± 0.38	b	2.22 ± 0.58	a	1.67 ± 0.19	b
18:1n-7	3.66 ± 0.46	a	3.16 ± 0.54	a	2.30 ± 0.34	b
18:2n-6	1.30 ± 0.89	b	2.34 ± 0.49	a	1.50 ± 0.33	b
18:3n-6	0.24 ± 0.09	a	0.15 ± 0.05	b	0.10 ± 0.12	b
18:3n-3	0.97 ± 0.28	b	1.66 ± 0.38	a	1.50 ± 1.00	b
18:4n-3	4.36 ± 1.62	a	2.01 ± 0.52	b	2.24 ± 1.67	b
20:1n-9	1.57 ± 0.36	b	2.49 ± 0.56	a	2.68 ± 0.32	a
20:2n-6	0.37 ± 0.09	b	0.67 ± 0.12	a	0.63 ± 0.07	a
20:3n-6	0.23 ± 0.08		0.21 ± 0.06		0.23 ± 0.13	
20:4n-6	1.69 ± 0.90	c	3.51 ± 1.20	b	4.90 ± 0.94	a
20:5n-3	20.68 ± 1.60	a	15.77 ± 2.67	b	19.72 ± 2.28	a
22:4n-6	0.14 ± 0.15	c	0.36 ± 0.13	b	0.59 ± 0.09	a
22:5n-3	0.94 ± 0.22	c	1.26 ± 0.20	b	1.63 ± 0.15	a
22:6n-3	12.16 ± 3.57	b	17.54 ± 3.63	a	16.77 ± 2.07	a

a, b, c = values associated with different letters on the same row were significantly different ($p < 0.05$).

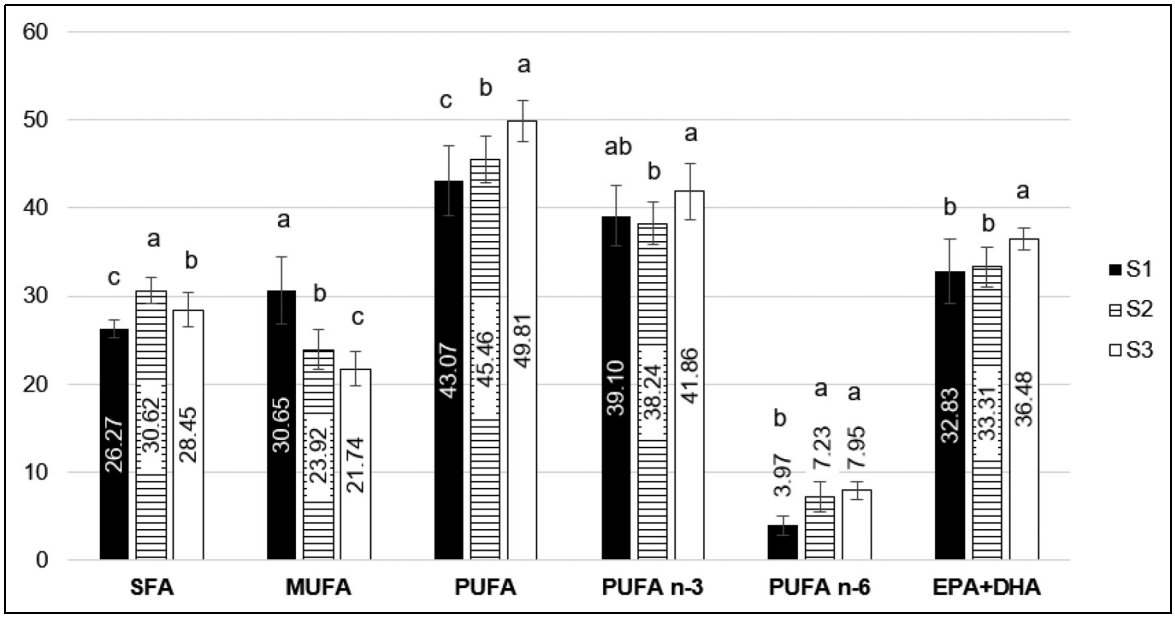

Figure 1. FAs grouped as follows: saturated FAs (SFA), monounsaturated FAs (MUFA), polyunsaturated FAs (PUFA), PUFA n-3, PUFA n-6, EPA + DHA. Data are expressed as g/100 of total FAs, mean and standard deviation bars. a, b = values associated to different letters for each group of FAs were significantly different ($p < 0.05$).

by the typical FAs profile of the most common food source for bivalve molluscs. Actually, a high content of DHA in mussels is related to the presence of dinoflagellates and zooplankton species in the marine environment, especially enriched in DHA, while the main source of EPA are diatoms species (Ezgeta-Balić et al., 2012). Mussels have been suggested as an excellent dietary source of EPA and DHA, for human consumers (Carboni et al., 2019; Yaghubi et al., 2021). Due to their function to promote adequate growth, development and body function, these FAs acquire a primary nutritional importance and are considered essential nutrients for humans (Gil and Gil, 2015). Since a large proportion of the FAs in lean seafood is bonded in the phospholipids molecules, the bioavailability and the biological activity of the beneficial FAs in this kind of products is improved (Liisberg et al., 2016). Several Health Organisations have released their own recommendations of EPA and DHA requirements based on available research and various other criteria. Most of them recommend a weekly intake of 1.75–3.5 g EPA + DHA for healthy adults (Siscovick et al., 2017). Based on our study, 100 g of raw mussel meat contain ≈1–2 g lipid, accounting for 32–36% EPA + DHA. This means that the consumption of a serving of 100 g of raw mussels provides around 0.35 g EPA + DHA, contributing to 10–20% of the recommended weekly intake of EPA and DHA. Thus, the high proportion of EPA and DHA detected in the edible tissue of Mediterranean mussels in this study could represent a valuable information to highlight with the aim to enhance further the appreciation of this product on the market.

FAs of the n-6 series own opposite functional properties compared to their n-3 counterparts, representing the precursors of metabolites associated with inflammatory functions (Simopoulos, 2010; Zárate et al., 2017). Among the n-6 PUFAs detected in mussels in this study, the highest contribution was due to linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6). The latter was the most abundant n-6 FAs, with significant differences ($p < 0.05$) detected in different months, reaching the highest content in S3 and the lowest in S1. Similar results were reported by Ventrella et al. (2008) who detected high levels of arachidonic acid in mussels in the cold season, suggesting different explanations, such as a natural seasonal enrichment in the aquatic food chain during autumn or a specific animal requirement for arachidonic acid in this season. Actually, arachidonic acid has a huge importance in the reproductive processes and as structural membrane lipid of marine organism, thus it is subjected to selective deposition in tissues (Ezgeta-Balić et al., 2012; Wacker and Von Elert, 2003). Other authors (Ezgeta-Balić et al., 2012) observed higher proportion of arachidonic acid in meat of bivalves during the cold months due to higher accumulation of ingested zooplankton in this season. Hence, the fluctuations observed in this work for the concentration of this FA in mussels meat during the year could be associated to changes mainly related to environmental factors.

Regarding the other classes of FAs, the sum of SFAs varied from 26.27% (S1) to 30.62% (S2), with palmitic acid (16:0) representing the most abundant ($p < 0.05$). SFAs are among the main nutrient present in particulate matter (detritus, terrestrial plant) and natural microzooplankton assemblages in marine environment (Dalsgaard et al., 2003; Fahl and Kattner, 1993). MUFAs content ranged from 21.74% (S3) to 30.65% (S1), withe the values are comparable to previous findings for the same species of mussels collected in the Adriatic Sea (Bongiorno et al., 2015; Ventrella et al., 2008). Palmitoleic acid represented the most abundant MUFAs, with highly variable values, ranging from 5.21% in S3 to 19.05% in S1 ($p < 0.05$). The same trend was detected by the above-mentioned studies, highlighting the highest presence of palmitoleic acid in spring and the lowest during late autumn (Bongiorno et al., 2015; Ventrella et al., 2008). The primary source of MUFAs, mainly palmitoleic acid (16:1n-7), in the marine food web is represented by phytoplankton biomasses (Dalsgaard et al., 2003; Ezgeta-Balić et al., 2012). Interestingly, even 20:1n-9, which is considered as marker of zooplankton consumption in *M. galloprovincialis* (Dernekbaşı, 2015), was found at different concentration ($p < 0.05$) during different seasons. Since the prevalence of different species (zooplankton, phytoplankton, bacterial particulates) as main food source for mussels in different seasons has been evidenced (Ventrella et al., 2008), we can suggest that the differences observed in this study for the proportion of SFAs and MUFAs could be imputed to the different incidence of the food sources, characterised by different and specific FAs profile, in the natural feeding environment were mussels were farmed.

Due to the distinctive functional roles of many FAs introduced above, many lipid indices have been formulated and widely used with the aim to evaluate the healthiness of the lipid portion of food products. The results obtained calculating the lipid quality indices for Mediterranean mussels analysed in this study are reported in Figure 2.

The n-6/n-3 ratio is one of the most used index considered as reliable indicator of lipid quality of foodstuffs and, more generally, of diets (Simopoulos, 2010). Actually, it has been estimated that the n-6/n-3 ratio in diets should vary from 1/1 to 4/1 in order to be considered optimal, whilst modern diets have been evidenced to reach higher, unfavourable values (Leclercq et al., 2011; Patterson et al., 2012; Simopoulos, 2010; Strobel et al., 2012). The values for the n-6/n-3 ratio detected in this study showed a significant difference ($p < 0.05$) between S1 (1/10, best balance) and the other seasons (1/5, worst balance) (Figure 2). However, these values can be considered favourable (<1) independently by the sampling season, supporting the consideration of mussels as seafood products characterised by optimal n-6/n-3 ratio compared to most of food products of terrestrial origin (Van Hecke et al., 2019).

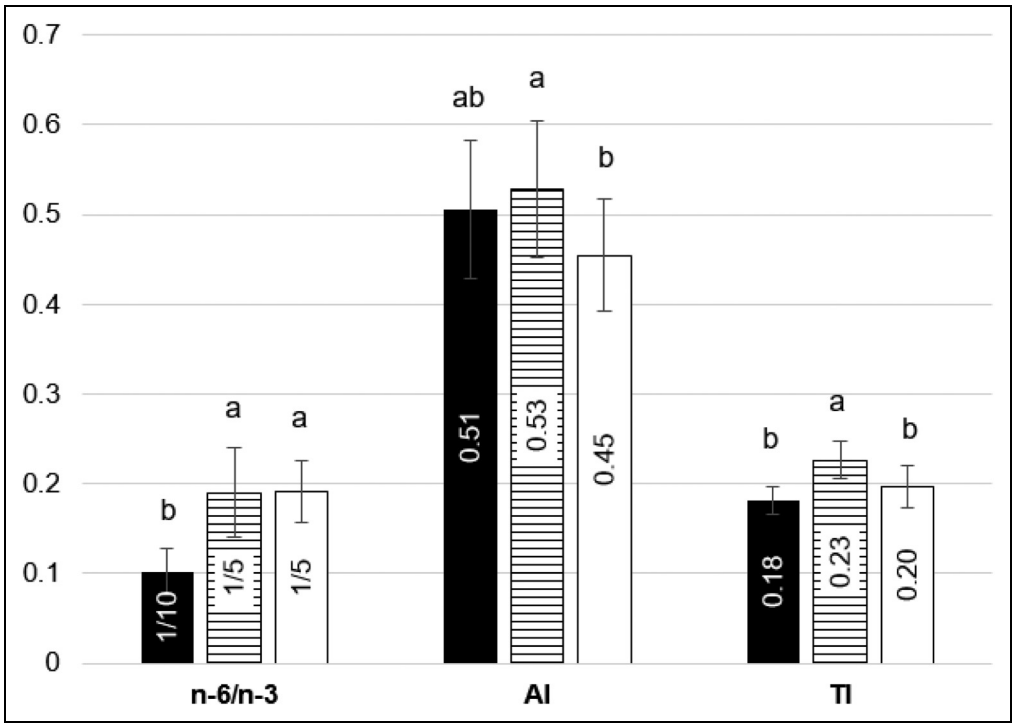

Figure 2. Lipid quality indices (n-6/n-3 ratio, atherogenic index AI and thrombogenic index TI) calculated in Mediterranean mussels collected on the Italian market. Data are reported as means plus standard deviation bars. a, b = means associated to different letters for each index were significantly different ($p < 0.05$).

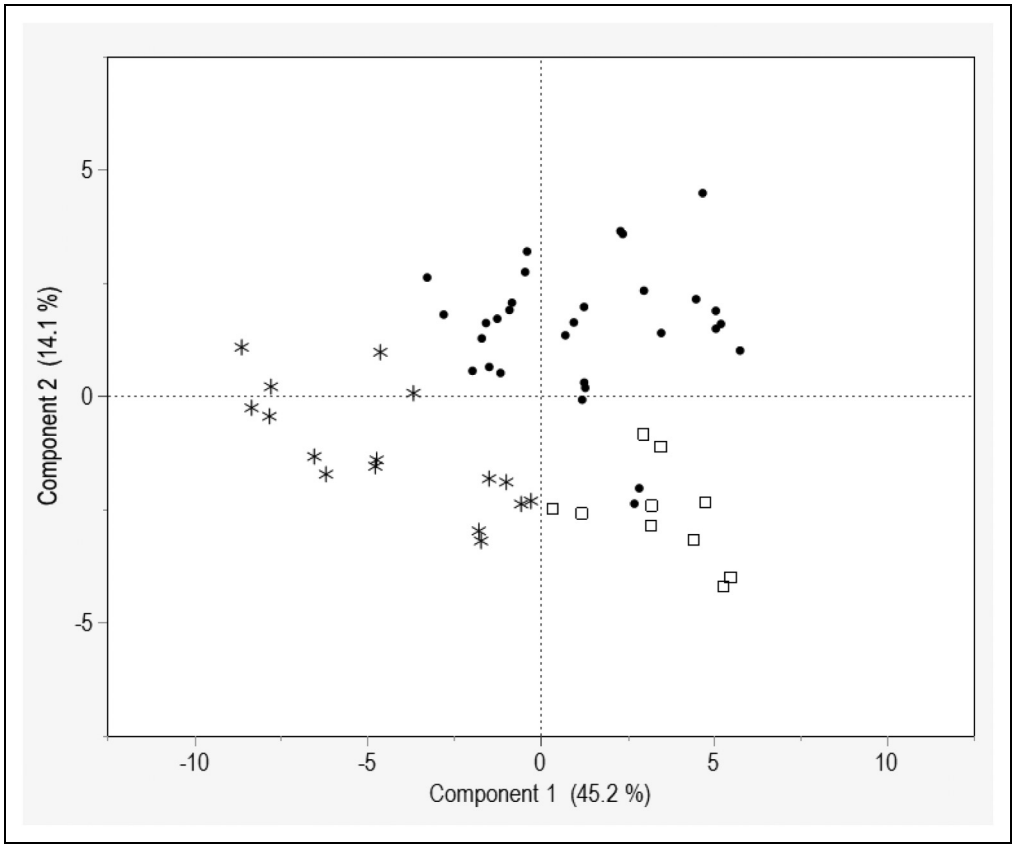

Figure 3. PC-1 vs PC-2 scores plot.

Finally, atherogenity (AI) and thrombogenity (TI) indices, suggested as powerful indicators of lipid quality in food products, were calculated based on different ratios among FAs associated to pro/anti atherogenic and thrombogenic functions on human metabolism (Chen and Liu, 2020; Ulbricht and Southgate, 1991). AlI Mediterranean mussels analysed in this study ranged from 0.45 to 0.53, with a significant difference ($p < 0.05$) detected between S2 and S3. At the same time, TI ranged from 0.18 to 0.23, with a significant difference ($p < 0.05$) between S2 and the other seasons (Figure 2). These results are comparable to those previously published for different seafood products (Chen and Liu, 2020), including mussels (Neri et al., 2021). Food products characterised by low AI and TI can be considered nutritionally valuable and it is though that their consumption may have positive effects on consumers health (Chen and Liu, 2020). Particularly, it has been suggested that AI and TI should be <1 and that higher values could negatively affect consumers health (Ouraji et al., 2009).

Multivariate analysis

The PCA model built using a combination of collected biometrical measurements and chemical data explained 69.3% of the total variance in original data matrix by a combination of the first two PCs. The PCA scores plot, with PC-1 and PC-2 on the x- and y- axis respectively, is reported in Figure 3.

In the scores plot, a trend to sample grouping according to the season was observable, with the exception of two samples from S2 that clustered together with S3 samples. Observing PC-1 direction, samples mainly turned from negative to positive scores during sampling time, with a separation of S1 (negative scores) and S3 (positive scores), while S2 was in an intermediate position (both negative and positive scores). Observing PC-2 direction, a separation of S2 (positive scores) and S3 (negative scores) was observable, while S1 was in an intermediate position, but mostly associated with negative scores. The loadings of the original variables over the first two PCs are reported in Table 3.

According to observation made by interpretation of individual FAs amounts and relative sums, the trend noticed in the multivariate plot mirrored an influence of both intrinsic and extrinsic factors, such as physiological stage and seasonality, on energy and macronutrient accumulation in mussels tissue and, further, in FAs distribution. Particularly, the group of specimens collected in spring (S1) was positively associated to higher values for MY and CI and for lipids and carbohydrates accumulation in the edible tissue of mussels. This outcome supported the hypothesis of a higher storage of energy reserves occurring in gonads and tissues during spring, probably ahead the beginning of the reproductive activity and the consequent mobilisation for spawning. At the same time, specimens collected in this season were characterised by high levels of FAs representative of phytoplankton species consumption, mainly 16:1n-7 and 20:5n-3, in the lipid fraction of meat, suggesting a strong influence of the feeding habits of mussels produced in this season. Mussels collected during summer (S2) were still associated to high amounts of carbohydrates stored in tissues. Contemporarily, these samples were positively associated to the amount of many FAs characteristic of a high presence of particulate matter and microzooplankton assemblages in marine food environment, suggesting a partial changing on the feeding pattern from spring to season. Finally, mussels samples collected in autumn and winter (S3) were positively associated to high moisture content in tissues and, consequently, negatively associated to lipid and carbohydrates content. At the same time, this group of samples was positively associated to the presence of long chain PUFAs of the n-9 and n-6 series, considered markers of zooplankton consumption, and to higher n-6/n-3 ratios. By a nutritional point of view, these outcomes suggested that, when compared to specimens available on the market in spring and summer, mussels purchased in autumn and winter represented a poorer source of lipid and carbohydrates for consumers and, contemporary, characterised by a lipid fraction with the most unfavourable n-6/n-3 balance.

Table 3. PC-1 and PC-2 loadings matrix. Only variables that accounted for >50% of the explained variance in the two-dimensional space defined by PC-1 and PC-2 were retained.

	Prin1	Prin2
20:1n-9	0.94	0.11
22:4n-6	0.90	−0.23
20:2n-6	0.87	0.39
20:4n-6	0.84	−0.14
22:6n-3	0.80	0.24
16:4n-1	0.80	−0.20
n-6/n-3	0.79	0.23
22:5n-3	0.76	−0.32
18:0	0.74	0.14
Moisture	0.61	−0.65
18:2n-6	0.57	0.74
16:0	0.55	0.62
Energy	−0.72	0.60
Carbohydrates	−0.70	0.57
Lipid	−0.82	0.28
18:3n-6	−0.75	−0.02
20:5n-3	−0.56	−0.69
Meat weight	−0.72	0.06
CI	−0.74	0.17
18:4n-3	−0.70	−0.17
MY	−0.85	0.07
16:1n-7	−0.95	−0.06

CONCLUSIONS

In conclusion, the results obtained allowed supporting the evaluation of Mediterranean mussels as a seafood product of valuable nutritional quality, characterised by high protein and low fat content, independently by the season of production. Seasonal differences were detected, mainly regarding the amounts of energy reserves stored in tissues by mussels (lipid and carbohydrates), probably depending on physiological factors, such as the reproductive cycle. Furthermore, differences in lipid quality were detected among the season, most probably due to factors associated to environmental conditions (such as water temperature and food sources availability). However, a valuable lipid composition was evidenced in specimens collected in all the seasons, characterised by optimal lipid indices (n-6/n-3 < 1/5, AI and TI < 1), conferring to Mediterranean mussels potential valuable effects as dietary food source. These results can be appealing with the aim to promote and enhance consumers appreciation toward Mediterranean mussels as a valuable seafood product, characterised by favourable nutritional quality and available on the market all over the year.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ETHIC STATEMENT

Not applicable.

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