

## Nutritional Quality of Edible Marine Bivalves from the Southern Coast of Italy, Mediterranean Sea

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Nutritional quality parameters of eight commercially important bivalve species (*Arca noae*, *Flexopecten glaber*, *Limaria tuberculata*, *Mimachlamys varia*, *Modiolus barbatus*, *Mytilus galloprovincialis*, *Ostrea edulis* and *Solen marginatus*) from the Ionian Sea (Southern Italy, Mediterranean Sea), were determined. The meat yield and lipid nutritional quality indices (atherogenic index, thrombogenicity index and hypocholesterolaemic/hypercholesterolaemic fatty acid ratio) have been also evaluated.

Meat yield values ranged from 31.4% in *F. glaber* to 44.5% in *M. varia*. The results showed that all species might be considered as food items with interesting dietetic properties due to high contents of proteins, minerals (Ca, K, Na, Fe, Zn, Cu), essential polyunsaturated fatty acids (PUFAs), and to low cholesterol content. Among PUFAs, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) exhibited the highest levels in *M. galloprovincialis* (11.74%) and in *M. varia* (14.41%), respectively. Elevated *n-3/n-6* ratio characterized the fatty acids profile of all species ranging from 2.65 in *F. glaber* to 7.19 in *M. galloprovincialis*. The lipid nutritional quality indices showed that *M. varia*, *M. galloprovincialis*, *O. edulis*, *S. marginatus*, and *L. tuberculata* might have beneficial effects on the consumer's health. This paper will be of practical value from a health perspective for populations who consume shellfish and a powerful marketing tool for farmers of the bivalves.

### INTRODUCTION

The marine molluscs are important for marine ecology and play an important role in human's diet, since they are a good source of nutrients. High quality of protein, minerals, low lipid content, and especially high proportion of polyunsaturated fatty acids (PUFAs) characterize the mollusc flesh, contributing to their nutritional value and organoleptic characteristics [Orban *et al.*, 2007].

PUFAs are considered as essential fatty acids that humans cannot synthesize and must be provided with food. Recent studies have clearly shown the importance of PUFAs and their nutritional value for human health. A particular emphasis is placed on the *n-3* PUFAs, eicosapentaenoic (EPA) acid and docosahexaenoic (DHA) acid that can be associated with several health benefits. DHA plays a role in the development and function of the brain, the photoreception, and the reproductive system [Kris-Etherton *et al.*, 2003; Sidhu, 2003]. EPA is the precursor of a family of prostaglandins, which control blood clotting and other arterial functions. This may be important in reducing the risk of cardiovascular disease, decrease in mild hypertension, prevention of certain cardiac arrhythmias and sudden death [Kris-Etherton *et al.*, 2003]. EPA and DHA also lower blood triglyceride concentrations and are substrates

for the synthesis of resolvins, which are believed to play a key role in terminating inflammatory processes [Kohli & Levy, 2009]; moreover, they have beneficial effects on other diseases, namely skin disease, asthma, arthritis, nephritis, lupus erythematosus, multiple sclerosis, and certain types of cancers [Harris, 2010; Massaro *et al.*, 2010]. PUFAs are influenced by taxonomic relations and environmental conditions and depend on species, nutrient habits, food availability and physiological conditions, so they differ for molluscs species that come from different geographical areas [Orban *et al.*, 2007].

Minerals such as sodium, potassium, magnesium, calcium, iron, zinc and copper, are essential substances for organisms and for their vital functions and growth. In fact, minerals are important components of hormones and enzymes activated in human nutrition [Khan, 1992], and are involved in physiological processes in the body, the most important of which are pH maintenance, osmotic pressure, nerve conductance, muscle contraction and energy production. Zinc, for example, has different important biochemical functions, in particular as cofactor to more than 300 enzymes involved in DNA and RNA metabolism and stabilization of cell membranes [Beyersmann, 2002; Rink & Gabriel, 2000].

Unsuited dietary intakes of the minerals Ca, Mg, Na and K, either insufficient or excessive, can cause serious health issues such as cardiovascular disease, osteoporosis, and hypertension. In general, the seafoods are excellent sources of Ca, K, Na, Fe, Zn, Cu and in particular, oysters are good sources of Fe, Zn, and Cu.

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Bivalve molluscs represent a significant proportion of the world's fishery and aquaculture. In Italy, the harvesting and commercialization of bivalves represents an important productive sector in the national economy (~111.000 t of marine molluscs), after Spain (~223.000 t) and France (~155.000 t). About 70% of the Italian shellfish production is consumed domestically, while the remaining 30% is exported to European countries, first among them Spain [FAO, 2016].

Despite the presence of a rich diversity of edible and commercial seafood species along the Mediterranean coast [Coll *et al.*, 2010], little information is available with regard to the nutritional quality for most parts of the edible marine bivalves from this important coastal region.

In recent years, consumers have become more health conscious and interested in maintaining or improving their health through the diet [Hasler, 2002]. Thus, the knowledge on biochemical composition of any edible organisms is extremely important because it reflects their nutritional value [Periyasamy *et al.*, 2011].

The ability to identify healthy components enable consumers to make healthy food choices. There is a strong connection between foods and health, therefore making healthier food choices can prevent diseases with simple changes that can contribute to your overall health and well-being. In addition, this information can be useful to producers as a powerful marketing tool, in the promotion of food of high economic and nutritional values.

For these reasons, the present work aimed to investigate the nutritional quality of eight commercially important bivalve species from Ionian Sea (Southern Italy, Mediterranean Sea), well appreciated in the Mediterranean diet. For some of them it is the first time that such information is given. Therefore, the proximate, essential minerals and fatty acids compositions, as well as meat yield and lipids nutritional quality indices (LNQI) were determined.

## MATERIALS AND METHODS

### Collection, samples preparation and meat yield of bivalves

The bivalves *Arca noae*, *Flexopecten glaber*, *Limaria tuberculata*, *Mimachlamys varia*, *Modiolus barbatus*, *Mytilus galloprovincialis*, *Ostrea edulis*, and *Solen marginatus* from the Gulf of Taranto (Southern Italy, Mediterranean sea), were purchased from a local market in January-February 2014. Samples (maximum 24–48 h after harvesting) were immediately iced and transported to the laboratory within 1 h to be brushed, washed, and processed. Upon arrival to the laboratory, each sample of about 30 individuals was split up in two sub-groups: one for biometric measurements and determination of meat yield (MY) and one for biochemical determinations. Adult specimens of commercial size of each species with homogenous shell length were selected to ensure that any biochemical differences were not size dependent. Length (maximum measure along the anterior-posterior axis) and width (maximum lateral axis), were measured using a 0.1mm precision calliper. The bivalves were weighed, opened by cutting the adductor muscle with a scalpel, and the wet meat

and shells were weighed. MY was calculated as follows: (wet meat weight/whole mussel weight) × 100 [Okumus & Stirling, 1998]. The biometric measurements are shown in Table 1.

A minimum of ten individuals (ranging 40–200 g of wet meat weight) from each species was minced for biochemical analyses. The specimens were manually shucked by cutting the adductor muscle with a knife. The bivalves juice was removed and the edible portion was collected. The soft body was not separated into organs or body parts to avoid leakage of intracellular fluids. Three replicates of ten individuals each were obtained. Each sample was stored at -20°C (for a maximum 7 days) in polyethylene bags.

### Proximate analysis

Moisture content was determined by drying the sample in an oven at 105°C overnight until a constant weight was obtained. Ash content was determined by burning the samples in the furnace at 550°C overnight. The crude protein content was measured by the Kjeldahl procedure (nitrogen to protein conversion factor = 6.25). The methods of the Association of Official Analytical Chemists [AOAC, 1995] were used. Carbohydrates were quantified according to the phenol-sulphuric acid method [Dubois *et al.*, 1956], using glucose as the standard. Total lipid (TL) content was determined gravimetrically after chloroform-methanol extraction according to Folch *et al.* [1957].

All analyses were conducted in triplicate and results were expressed on wet weight basis.

### Lipid classes

Triacylglycerols (TAGs), total cholesterol (CHL), and phospholipids (PLs) were measured by the colorimetric enzymatic method using a commercial kit (SGM, Rome, Italy) as reported in Prato *et al.* [2010]. The values are expressed as percentage (mean ± SD) of total lipids. All analyses were performed in triplicate.

### Fatty acid analysis

Fatty acids of total lipids were transesterified to methyl ester (FAMES) according to the procedure described by Prato *et al.* [2018].

Analysis of FAMES was performed by gas chromatography (GC) using an HP 6890 series GC (Hewlett Packard, Wilmington, DE, USA), equipped with a flame ionization detector. FAMES were separated with an Omegawax 250 capillary column (Supelco, Bellafonte, PA, USA) (30 m long, 0.25-mm internal diameter, and 0.25-mm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The column temperature program was as follows: 150 to 250°C at 4°C/min and then held at 250°C. Peaks were identified by their comparison to the relative retention times of standards (Supelco 37 component FAME Mix), and the results were expressed as the percentages of peak areas.

### Mineral composition

Mineral analysis was carried out on a lyophilized sample with 9 mL of concentrated HNO<sub>3</sub> (70 % v/v) and 1 mL H<sub>2</sub>O<sub>2</sub> (30 % v/v) [US EPA, 1996] using a microwave diges-

tion system (MARSX CEM Corporation, Matthews, NC). After mineralization, the digested samples were diluted to 50 mL with Milli-Q® water. A blank digest was performed in the same way.

Contents of K, Na, Mg, Ca, Zn, Cu, and Fe were determined by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry), using a Perkin Elmer model Elan 6100 DRC Plus (PerkinElmer, Norwalk, CT, USA). Each sample was analyzed in three replicates and the relative standard deviation (RSD) was <5%. Accuracy was verified using the Community Bureau of Reference (BCR) Certified Reference Material CRM 278R (Trace elements in mussel tissue) produced by Joint Research Centre, Geel, Belgium.

The recovery of CRM 278R ranged between 95% (Zn<sup>66</sup>) and 105% (Na<sup>23</sup>). Chemicals were of ultrapure grade (Merck Suprapur, Darmstadt, Germany), and all the glasswares were treated prior to the analysis with 10% v/v HNO<sub>3</sub> for 24 h and afterwards rinsed with Milli-Q® water. Standard solutions were obtained daily using ultrapure deionized water (<0.1 µs at 25°C).

### Lipid nutritional quality indices (LNQI)

The data from fatty acid composition analysis were used to determine the nutritional quality of the lipid fraction by means of three indices using the following calculations:

Atherogenicity index [Ulbricht & Southgate, 1991]:

$$AI = \frac{(C14:0 + 4 \times C14:1 + C16:0)}{\Sigma MUFAs + \Sigma PUFAs}$$

Thrombogenicity index [Ulbricht & Southgate, 1991]:

$$TI = \frac{(C14:0 + C16:0 + C18:0)}{[(0.5 \times \Sigma MUFAs + 0.5 \times \Sigma n6 PUFAs + 3 \times \Sigma n3 PUFAs + (n3/n6)]}$$

Fatty acid hypocholesterolemic/hypercholesterolemic ratios [Santos-Silva et al., 2002]:

$$HH = \frac{(C18:1cis9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3)}{(C14:0 + C16:0)}$$

### Statistical analysis

Analyses were performed in three replicates and each one was measured for three repetitions. Results were expressed as mean values ± standard deviation.

The normality of the data was evaluated by Kolmogorov-Smirnov test. The homogeneity of variances was assessed by Levene test. When either assumptions were met, all data (means of proximate composition, lipid class, minerals, fatty acids and LIQN) were examined by analysis of variance (one-way ANOVA) to verify whether there were differences among the analysed species. The multiple comparison (Tukey's test) was applied when the variance analysis indicated significant differences. The level of significance was set as 0.05.

A principal component analysis (PCA) based on Pearson's correlation matrix relationships between variables, was performed. Multivariate statistical treatment of the whole set of data was performed after logarithmic transformation to reduce the variability of data.

All statistical analyses were performed with the software package Past version 1.0 and STATISTICA® (StatSoft Inc., Tulsa, OK, USA).

## RESULTS AND DISCUSSION

### Biometric parameters and meat yield

Table 1 shows results of the biometric measurements, as well as meat yield of eight commercial bivalves. In this study, bivalves exhibited meat yield values ranging from 31.4% in *F. glaber* to 44.5% and 41.2% in *M. varia* and *M. galloprovincialis*, respectively.

Previous studies demonstrated that the percent meat yield or edibility of bivalves as a good commercial quality indicator varies seasonally and geographically, depending on food availability and the timing of the gametogenic cycle [Anibal et al., 2011; Celik et al., 2012; Okumus & Stirling, 1998; Orban et al., 2007].

The results obtained were comparable to those observed for *F. glaber*, in Lapseki Bay in Canakkale (Turkey), by Berik & Çankiriligil [2013] (MY: 34.68%), in the same period. Mussels and oysters showed high MY compared with findings from other studies in the Mediterranean area; e.g. mussel from Adriatic Sea, Dardanelles (Turkey) and Black Sea showed values in the range of 18–25% [Bongiorno et al., 2015; Yildiz et al., 2006]. In turn, Fuentes et al. [2009] reported the MY values of 31% and 34% for *M. galloprovincialis* from Galicia and Valencia (Spain), respectively, during the summer period. Moreover, *Ostrea edulis* from Dardanelles displayed values of about 8% in February [Yildiz et al., 2011]. As regard the other species no literature data exist.

### Proximate analysis

The proximate composition of bivalves meat is shown in Table 1. As expected, the main component of all studied species is the moisture, whose content indicates flesh freshness. The highest values were found in *M. galloprovincialis* and *F. glaber*, and *L. tuberculata* (84.10, 83.67, and 82.60 g/100 g, respectively). These values did not differ statistically between each other (p≥0.05), and the lowest was found in *M. barbatus* (79.84 g/100 g).

The highest ash content, which indicates the amount of inorganic compounds in the tissues of bivalves, was found in *L. tuberculata*, *A. noae* and *S. marginatus* (5.33 – 4.26 g/100 g; differences between values were insignificant, p≥0.05). Significantly lower (p<0.05) ash content was noted in *M. galloprovincialis* (2.62 g/100 g), although four other species exhibited not statistically different values (p≥0.05). Despite these values, *A. noae* showed, for the most minerals investigated, values lower than those of *M. galloprovincialis*, probably due to the presence of other minerals not investigated herein.

High protein levels and low lipid contents characterized the overall proximate profile of bivalves studied herein. *A. noae* showed the highest protein content with 12.25g/100 g. *A. noae* and *S. marginatus* represent a good source of protein considering the WHO Daily Value (DV) recommendation of 0.80g/kg/day [WHO, 2007].

The total lipid amount, also, exhibited differences among the species, with the highest values determined in *M. barbatus*, *L. tuberculata*, and *O. edulis* (2.98, 2.76, and 2.70 g/100 g, respectively) and the lowest ones in *F. glaber*, *A. noae*, and *S. marginatus* (1.04, 1.18, and 1.25 g/100 g, respectively).

TABLE 1. Biometric measurements, meat yield, proximate composition, lipid classes and mineral content of eight commercial bivalves species on a wet weight basis.

Specification	<i>A. noae</i>	<i>F. glaber</i>	<i>L. tuberculata</i>	<i>M. varia</i>	<i>M. barbatus</i>	<i>M. galloprovincialis</i>	<i>O. edulis</i>	<i>S. marginatus</i>
Shell length (mm)	53.0±3.2	44.0±1.5	43.5±1.6	42.2±3.1	48.3±4.60	59.6±6.0	82.5±2.0	100.5±10.5
Shell width (mm)	29.0±1.5	41.4±3.6	33.4±3.5	37.4±5.0	25.5±2.6	27.8±3.3	61.2±2.0	18.5±0.5
Total wet weight (g)	10.8±0.5	12.5±1.1	13.4±0.7	7.84±0.7	9.7±0.9	11.6±0.8	57.2±1.5	21.3±1.3
Meat yield (%)	40.3±2.3	31.4±3.9	35.2±2.3	44.5±4.5	40.0±1.8	41.2±4.3	37.5±2.3	39.0±3.5
Moisture (g/100g)	80.74±0.8 <sup>c,d</sup>	83.67±0.6 <sup>a,b</sup>	82.60±1.0 <sup>a,b,c</sup>	81.33±1.9 <sup>b,c,d</sup>	79.84±2.7 <sup>d</sup>	84.10±0.6 <sup>a</sup>	81.47±1.2 <sup>b,c,d</sup>	80.56±1.7 <sup>c,d</sup>
Ash (g/100g)	4.81±1.2 <sup>a,b</sup>	3.16±0.2 <sup>c,d</sup>	5.33±0.8 <sup>a</sup>	3.39±0.7 <sup>b,c,d</sup>	3.77±0.9 <sup>b,c,d</sup>	2.62±0.2 <sup>d</sup>	3.72±1.4 <sup>b,c,d</sup>	4.26±0.5 <sup>a,b,c</sup>
Protein (g/100 g)	12.25±1.8 <sup>a</sup>	7.08±0.4 <sup>c,d</sup>	8.57±0.0 <sup>b,c</sup>	8.68±0.1 <sup>b,c</sup>	6.88±0.0 <sup>c,d</sup>	6.58±0.5 <sup>d</sup>	8.10±1.2 <sup>c,d</sup>	9.96±1.4 <sup>b</sup>
Lipid (g/100 g)	1.18±0.0 <sup>d</sup>	1.04±0.3 <sup>d</sup>	2.76±0.4 <sup>a,b</sup>	2.26±0.6 <sup>b,c</sup>	2.98±0.2 <sup>a</sup>	2.15±0.3 <sup>c</sup>	2.70±0.3 <sup>a,b,c</sup>	1.25±0.1 <sup>d</sup>
PL (%)	83.47±2.5 <sup>a,b</sup>	89.59±2.7 <sup>a</sup>	66.29±3.5 <sup>c</sup>	69.14±3.4 <sup>c</sup>	73.32±4.5 <sup>c,b</sup>	69.83±3.8 <sup>c</sup>	69.54±3.4 <sup>c</sup>	64.35±4.5 <sup>c</sup>
TAG (%)	10.79±3.6 <sup>c</sup>	7.20±3.7 <sup>c</sup>	22.97±1.2 <sup>b</sup>	21.29±1.0 <sup>b</sup>	21.60±1.1 <sup>b</sup>	24.35±1.2 <sup>b</sup>	21.56±1.3 <sup>b</sup>	31.57±1.0 <sup>a</sup>
CL (%)	5.74±1.4 <sup>b</sup>	3.21±1.1 <sup>c</sup>	10.74±3.5 <sup>a</sup>	9.57±1.9 <sup>a</sup>	5.08±1.7 <sup>b,c</sup>	5.82±1.2 <sup>b</sup>	8.90±1.8 <sup>a</sup>	4.08±1.4 <sup>c</sup>
Na (mg/100 g)	91.92±3.1 <sup>c</sup>	301.61±10.1 <sup>a</sup>	226.47±7.5 <sup>c</sup>	167.45±5.6 <sup>d</sup>	237.33±7.9 <sup>b,c</sup>	240.3±8.0 <sup>b</sup>	244.52±8.2 <sup>b</sup>	167.37±5.6 <sup>d</sup>
Mg (mg/100 g)	19.30±0.6 <sup>f</sup>	47.00±1.6 <sup>a</sup>	37.33±1.2 <sup>c</sup>	24.80±0.8 <sup>e</sup>	40.08±1.3 <sup>b</sup>	35.72±1.2 <sup>c</sup>	29.79±1.0 <sup>d</sup>	29.75±1.0 <sup>d</sup>
K (mg/100 g)	49.63±1.6 <sup>b</sup>	34.46±1.2 <sup>d</sup>	25.97±0.9 <sup>e</sup>	45.18±1.5 <sup>c</sup>	74.89±2.5 <sup>a</sup>	34.32±1.1 <sup>d</sup>	46.45±1.6 <sup>e</sup>	77.17±2.6 <sup>a</sup>
Ca (mg/100 g)	9.7±0.35 <sup>c</sup>	21.78±0.7 <sup>c</sup>	21.59±0.7 <sup>c</sup>	14.00±0.5 <sup>d</sup>	14.45±0.5 <sup>d</sup>	8.12±0.3 <sup>f</sup>	35.50±1.2 <sup>a</sup>	27.15±0.9 <sup>b</sup>
Fe (mg/100 g)	12.50±0.4 <sup>a</sup>	7.64±0.2 <sup>d</sup>	4.58±0.1 <sup>e</sup>	5.18±0.2 <sup>f</sup>	6.54±0.2 <sup>e</sup>	6.30±0.2 <sup>e</sup>	11.24±0.4 <sup>b</sup>	9.57±0.3 <sup>c</sup>
Zn (mg/100 g)	3.5±0.1 <sup>d</sup>	4.31±0.1 <sup>d</sup>	0.79±0.0 <sup>f</sup>	23.4±0.8 <sup>c</sup>	25.42±0.8 <sup>b</sup>	2.49±0.1 <sup>e</sup>	30.67±1.0 <sup>a</sup>	1.95±0.1 <sup>e</sup>
Cu (mg/100 g)	0.32±0.0 <sup>d</sup>	0.52±0.0 <sup>c</sup>	0.80±0.0 <sup>b</sup>	5.00±0.1 <sup>a</sup>	0.55±0.0 <sup>c</sup>	0.22±0.0 <sup>d</sup>	4.83±0.2 <sup>a</sup>	0.19±0.0 <sup>d</sup>

Note: Data are the mean values of three replicates ± standard deviation. Moisture and ash are expressed as % of body mass; proteins and lipids as g/100 g. Mineral as mg/100 g. Phospholipids (PL), Triacylglycerols (TAG) and Cholesterol (CHL) as % of total lipid. Means within the same row without a common lowercase letter differ significantly ( $p < 0.05$ ).

To the best of our knowledge, no information is available on the nutritional quality for most of the studied species. Thus, we compared our findings with some other bivalves species of commercial size from different geographical areas.

Protein content in *M. galloprovincialis* was comparable to that found in mussels from Bay of Biscay and Ebro Delta (Spain) [Azpeitia *et al.*, 2016; Fuentes *et al.*, 2009]. Higher values were found, in the same study period, by Bongiorno *et al.* [2015] for bivalves from the North Adriatic Sea (8.9–10.9%), by Dernekbası *et al.* [2015] for species from the Black Sea (10–11%), by Fuentes *et al.* [2009] for bivalves from two different Spanish areas, Galicia and Valencia (10%), and by Yildirim & Ercan [2016] for species from Gulluk Gulf, Turkey (10%).

Lipids exert important biological functions as energy storage compounds, structural components of the cell membranes and as signalling molecules [Zhukova, 2014]. Lipid contents in species studied herein were higher than most data reported in literature [Azpeitia *et al.*, 2016; Bongiorno *et al.*, 2015; Fuentes *et al.*, 2009; Yildirim & Ercan 2016], but agreed well with those reported by Dernekbası *et al.* [2015] and Fuentes *et al.* [2009] from Valencia (2.10%).

Asha *et al.* [2014] reported a slightly higher protein and lipid content for *Crassostrea madrasensis* compared with those found in *O. edulis* in this study (9.41 vs 8.10 g/100 g of pro-

tein and 3.25 vs 2.79 g/100 g of lipid, respectively). In addition, Berik *et al.* [2017] for *F. glaber* and Orban *et al.* [2007] for *Chamelea gallina* reported a higher protein and lipid content (10.75 g/100 g and 1.84 g/100 g, respectively for *F. glaber* and 10.8 g/100 g and 1.59 g/100 g, respectively, for *C. gallina*).

#### Lipid classes

Phospholipids (PL), the major lipid class found herein, differed among species with *F. glaber* and *A. noae* showing the highest proportion (89.59% and 83.47% of total lipids, respectively) ( $p < 0.05$ ) (Table 1). Generally, PLs in bivalves are stable with respect to lipid class proportions, irrespective of growth rates or diets. Such stability is linked to the structural role of the PL in cell membranes where they give the desired structure to the membrane and determine its permeability [Caers *et al.*, 2000].

The level of triacylglycerols (TAG) in bivalves species ranged from 7.20% to 31.57% of total lipids. The highest TAG content was noted in *S. marginatus*. In turn, *F. glaber* and *A. noae* showed the lowest TAG proportion. TAGs play important roles as energy reserves that are mainly influenced by reproduction and/or by nutrition. Low levels of TAGs have been considered an indicator of low nutritional status [Okumus & Stirling, 1998].

The cholesterol (CL) maintains both membrane structural integrity and fluidity; moreover, it plays a key role in lipid metabolism [Los & Murata, 2004].

Among the bivalves examined, *L. tuberculata* and *O. edulis* had the highest cholesterol content (8.90–10.74% of total lipids), while *F. glaber* and *S. marginatus* and *M. barbatus* had the lowest (3.21–5.08% of total lipids) (Table 1). It is noticeable that people who are designing low cholesterol diets for health purposes must pay attention to these differences, even though the flesh of the examined bivalves exhibited a relatively low cholesterol content.

### Minerals

Contents of minerals (Na, K, Ca, Mg, Cu, Zn, and Fe) in fresh soft tissue of the marine bivalves found in this study are shown in Table 1.

Significant differences ( $p < 0.05$ ) were found in the distribution of metals among the species analyzed, though the Na, K, Mg, and Ca were predominant in the bivalves. The only exceptions are represented by *A. noae* where  $Fe > Ca$  and by *M. barbatus* and *M. varia* where  $Zn > Ca$ . Na, in particular, was the most abundant element with contents up to 3 orders of magnitude higher than the other minerals. Fe, Zn, and Cu, instead, were found generally at lower levels and followed the order  $Fe > Zn > Cu$  except in *M. varia*, *M. barbatus*, and *O. edulis* where Zn was found at higher levels than Fe. As regards the minimum and maximum contents, the highest contents of Na and Mg were found in *F. glaber* (301.61 and 47.00 mg/100 g, respectively), while the lowest levels of Na and Mg were found in *A. noae* (91.92 and 19.30 mg/100 g, respectively). The highest Ca and Zn levels were found in *O. edulis* (35.50 and 30.67 mg/100 g, respectively), while the lowest values of Ca and Zn level were found in *M. galloprovincialis* (8.12 mg/100 g) and *L. tuberculata* (0.79 mg/100 g), respectively.

The highest levels of Cu were detected in *M. varia* (5.00 mg/100 g) and *O. edulis* (4.83 mg/100 g), while *S. marginatus*, *M. galloprovincialis*, and *A. noae* had the lowest level of Cu (Table 1).

Fe was found at higher contents in *A. noae* (12.50 mg/100 g) and *O. edulis* (11.24 mg/100 g), while in the other species was uniformly distributed.

Lastly, *S. marginatus* and *M. barbatus* had the highest contents of K (77.17 and 74.89 mg/100 g, respectively) while its level was the lowest in *L. tuberculata* (25.97 mg/100 g).

In comparison with the literature data, Na, Ca, Fe, Cu, and Zn contents found in the *M. galloprovincialis* were higher than those noted for bivalves from Trieste Gulf (162 mg/100 g for Na; 487 mg/100 g for Ca; 119 mg/100 g for Fe; 5.1 mg/100 g for Cu; and 59 mg/100 g for Zn) and from Galician waters (218 mg/100 g for Na; 40 mg/100 g for Ca; 1.0 mg/100 g for Fe; 0.15 mg/100 g for Cu; and 2.3 mg/100 g for Zn) [Bongiorno et al., 2015; Fuentes et al., 2009, respectively]. As regards K, its levels in the *M. galloprovincialis* were similar to those reported by Fuentes et al. [2009] (36 mg/100 g) and lower than those obtained by Bongiorno et al. [2015] (41 mg/100 g). Concerning Mg contents, Fuentes et al. [2009] reported for *M. galloprovincialis* a higher value (56 mg/100 g), while Bongiorno et al. [2015] a lower value (14 mg/100 g).

Na, Ca, K, Mg, and Zn contents measured in oysters were much lower than levels found in oysters of the Croatian coasts (715 mg/100 g for Na, 155 mg/100 g for Ca, 248 mg/100 g for K, 90 mg/100 g for Mg, and 68 mg/100 g for Zn) [Bilandžić et al., 2016]. On the other hand, Fe levels were higher (4.6 mg/100 g) and Cu contents were similar (5.4 mg/100 g) to those obtained by Bilandžić et al. [2016]. No data on the minerals and trace metals studied in fresh soft tissue of *L. tuberculata*, *S. marginatus*, *M. varia*, and *F. glaber* were found in the literature. Although, Berik et al. [2017] in a recent paper, reported data on the mineral content of *F. glaber*, they are referred to digestive gland and adductor muscle, thus a direct comparison with data of this study is impossible. Only a few papers report data on the trace metals in *M. barbatus* and in particular regarding the Cu and Zn levels herein were much higher than levels found in bivalves from Croatian coasts (7.1 mg/100 g for Zn and 0.31 mg/100 g for Cu) by Cuculic et al. [2010]. Differences found in mineral levels in bivalves could be due to the different physiological state, abiotic factors (size, age, and sex of organisms), biotic factors (salinity, temperature, pH, and dissolved oxygen), genetic characteristics, chemical forms of metals, and contamination of the area [Fuentes et al., 2009]. Anyway, considering their WHO Daily Value recommendation [WHO, 2004], these bivalves can be good sources of minerals especially for Fe that in all products analyzed constituted more than 20% of the DV.

### Fatty acids

Twenty-eight fatty acids, including C12:0 to C22:6 *n*-3, that exceeded a minimum of 0.1% of total fatty acids in a minimum of one bivalve sample, were identified (Table 2).

Fatty acid composition differed significantly among some species ( $p < 0.05$ ), although, saturated fatty acids (SAFAs) were the most predominant fatty acids in all samples, followed by polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs). The content of SAFAs ranged from 40.73% in *M. varia* to 50.51% in *F. glaber* ( $p < 0.05$ ). The main SAFAs were palmitic acid (C16:0, from 22.52% in *S. marginatus* to 30.50% of total FAs in *O. edulis*), myristic acid (C14:0, from 4.21% to 11.8% of total FAs in *O. edulis* and *L. tuberculata*, respectively), and stearic acid (C18:0, from 4.85% to 10.51% of total FAs in *L. tuberculata* and *F. glaber*, respectively). These findings are in agreement with other studies on *M. galloprovincialis*, *A. noae*, and *M. barbatus* [Azpeitia et al., 2016; Ezgeta-Balić et al., 2012].

Palmitic acid is an important component of FAs, since its desaturation and elongation lead to biosynthesis of essential FAs (C18:2 *n*-6 and C18:3 *n*-3 in a first instance and later to C20:4 *n*-6, C20:5 *n*-3, and C22:6 *n*-3) [Angioni & Addis, 2014].

MUFAs are often referred to as “good” fats because they help in reducing both total and low density lipoprotein-LDL blood cholesterol levels and protect against heart disease [Siri-Tarino et al., 2015]. In the present study, MUFAs represented about a quarter of the total fatty acids, with *S. marginatus* and *A. noae* containing significantly ( $p < 0.05$ ) the highest level (24.35–26.63% of total FAs) and *M. galloprovincialis* the lowest (18.00% of total FAs) ( $p < 0.05$ ). The latter result was higher than that reported by Azpeitia et al. [2016] for

TABLE 2. Fatty acid composition (% of total fatty acids) of eight edible bivalves Mediterranean species.

Fatty acids	<i>A. noae</i>	<i>F. glaber</i>	<i>L. tuberculata</i>	<i>M. varia</i>	<i>M. barbatus</i>	<i>M. galloprovincialis</i>	<i>O. edulis</i>	<i>S. marginatus</i>
C12:0	0.33 ± 0.05	0.47 ± 0.02	0.37 ± 0.14	0.36 ± 0.15	0.69 ± 0.25	0.41 ± 0.11	0.22 ± 0.01	1.05 ± 0.04
C14:0	7.61 ± 0.71	8.94 ± 3.25	11.79 ± 1.41	7.56 ± 1.02	9.22 ± 1.11	6.94 ± 0.18	4.21 ± 0.97	9.65 ± 0.66
C15:0	nd	2.09 ± 0.14	0.95 ± 0.11	nd	1.80 ± 0.32	1.16 ± 0.07	1.52 ± 0.35	nd
C16:0	27.21 ± 2.22	24.72 ± 0.58	23.43 ± 2.23	23.91 ± 1.42	24.31 ± 0.22	27.77 ± 0.68	30.50 ± 6.04	22.52 ± 1.58
C17:0	2.74 ± 0.22	2.93 ± 0.53	1.44 ± 0.10	1.94 ± 0.16	2.56 ± 0.31	1.82 ± 0.18	3.01 ± 0.02	3.89 ± 0.58
C18:0	7.37 ± 0.22	10.51 ± 0.87	4.85 ± 0.13	6.98 ± 0.73	5.14 ± 0.45	5.21 ± 0.00	8.08 ± 0.31	5.26 ± 0.58
C20:0	nd	nd	nd	nd	nd	0.32 ± 0.00	nd	nd
C21:0	nd	0.85 ± 0.25	0.78 ± 0.15	nd	nd	0.73 ± 0.17	0.56 ± 0.07	1.34 ± 0.00
ΣSAFA	<b>45.26 ± 3.30<sup>ab</sup></b>	<b>50.51 ± 2.14<sup>c</sup></b>	<b>43.62 ± 3.19<sup>ab</sup></b>	<b>40.75 ± 2.78<sup>a</sup></b>	<b>43.72 ± 1.26<sup>ab</sup></b>	<b>44.36 ± 1.07<sup>ab</sup></b>	<b>48.11 ± 5.47<sup>bc</sup></b>	<b>43.71 ± 1.61<sup>ab</sup></b>
C14:1	1.07 ± 0.16	nd	0.21 ± 0.16	nd	nd	0.29 ± 0.04	nd	nd
C15:1	nd	0.31 ± 0.09	nd	nd	nd	nd	nd	nd
C16:1	10.06 ± 0.86	8.38 ± 0.79	6.06 ± 0.72	4.94 ± 0.34	11.53 ± 0.39	7.62 ± 0.07	2.78 ± 0.53	5.70 ± 0.11
C17:1	nd	nd	0.97 ± 0.11	1.05 ± 0.08	2.15 ± 0.29	1.13 ± 0.06	nd	1.23 ± 0.30
C18:1n7	3.60 ± 0.60	3.99 ± 0.14	3.01 ± 0.45	3.15 ± 0.29	4.11 ± 0.09	2.08 ± 0.06	2.54 ± 0.36	2.07 ± 0.15
C18:1n9t	nd	nd	nd	nd	1.02 ± 0.15	nd	nd	2.94 ± 0.22
C18:1n9c	7.86 ± 0.29	10.54 ± 3.43	11.73 ± 1.72	11.38 ± 0.90	4.17 ± 0.40	5.64 ± 0.12	9.70 ± 0.58	12.13 ± 0.92
C20:1n9	1.51 ± 0.15	0.74 ± 0.21	0.92 ± 0.14	0.63 ± 0.05	0.69 ± 0.01	0.77 ± 0.08	3.55 ± 0.69	1.33 ± 0.65
C22:1n9	0.38 ± 0.03	nd	nd	nd	nd	0.47 ± 0.00	0.56 ± 0.11	1.23 ± 0.18
ΣMUFA	<b>24.35 ± 0.96<sup>de</sup></b>	<b>23.85 ± 3.97<sup>d</sup></b>	<b>22.90 ± 0.62<sup>cd</sup></b>	<b>21.15 ± 1.33<sup>bc</sup></b>	<b>23.67 ± 0.05<sup>cd</sup></b>	<b>18.00 ± 0.17<sup>a</sup></b>	<b>19.13 ± 0.27<sup>ab</sup></b>	<b>26.63 ± 0.65<sup>e</sup></b>
C18:2n6c	3.19 ± 0.08	2.49 ± 0.42	2.45 ± 0.10	2.17 ± 0.11	2.48 ± 0.08	1.50 ± 0.01	2.57 ± 0.28	2.04 ± 0.69
C18:3n6	nd	0.79 ± 0.20	0.43 ± 0.22	0.59 ± 0.18	1.08 ± 0.01	0.45 ± 0.02	0.36 ± 0.06	0.93 ± 0.50
C18:3n3	5.36 ± 1.14	2.94 ± 0.21	4.47 ± 0.28	3.97 ± 1.02	3.72 ± 0.97	5.17 ± 1.09	6.24 ± 1.48	3.66 ± 0.60
C18:4n3	4.27 ± 0.15	2.85 ± 0.11	5.88 ± 0.10	6.57 ± 0.52	3.99 ± 0.05	4.76 ± 0.04	3.09 ± 0.07	4.78 ± 0.42
C20:2	0.23 ± 0.10	nd	nd	nd	nd	nd	nd	nd
C22:0 + 20:3n6	0.47 ± 0.15	0.80 ± 0.13	0.14 ± 0.16	nd	0.82 ± 0.01	0.39 ± 0.05	0.41 ± 0.24	1.21 ± 0.24
C20:3n3 + 22:1	0.35 ± 0.09	nd	0.15 ± 0.05	nd	0.23 ± 0.03	0.28 ± 0.01	0.37 ± 0.13	nd
C20:4n6	2.30 ± 0.16	2.93 ± 0.96	1.92 ± 0.36	2.22 ± 0.17	3.79 ± 0.08	2.16 ± 0.12	1.85 ± 0.45	3.51 ± 0.25
C20:5n3	8.45 ± 0.97 <sup>cd</sup>	6.53 ± 2.00 <sup>ab</sup>	7.11 ± 0.83 <sup>abc</sup>	8.16 ± 1.02 <sup>bcd</sup>	9.27 ± 0.15 <sup>d</sup>	11.74 ± 1.05 <sup>e</sup>	7.46 ± 1.45 <sup>abc,d</sup>	6.10 ± 0.59 <sup>a</sup>
C22:5n3	0.74 ± 0.04	nd	nd	nd	nd	0.61 ± 0.12	1.21 ± 0.04	1.47 ± 0.40
C22:6n3	5.96 ± 1.72 <sup>a</sup>	6.30 ± 2.19 <sup>a</sup>	10.88 ± 2.67 <sup>bc</sup>	14.41 ± 4.32 <sup>c</sup>	7.21 ± 0.03 <sup>a</sup>	10.58 ± 2.05 <sup>b</sup>	9.20 ± 4.40 <sup>ab</sup>	5.95 ± 0.70 <sup>a</sup>
ΣPUFA	<b>30.38 ± 4.25<sup>ab</sup></b>	<b>25.63 ± 6.07<sup>a</sup></b>	<b>33.46 ± 3.81<sup>bc</sup></b>	<b>37.86 ± 6.15<sup>c</sup></b>	<b>32.59 ± 1.29<sup>bc</sup></b>	<b>37.64 ± 1.25<sup>c</sup></b>	<b>32.76 ± 5.75<sup>bc</sup></b>	<b>29.66 ± 2.26<sup>ab</sup></b>

Note: Data are the mean values of three replicates ± standard deviation. Means within the same row without a common lowercase letter differ significantly ( $p < 0.05$ ); nd = not detected. SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

*M. galloprovincialis* from Bay of Biscay (Spain), but similar to that found by Fernández *et al.* [2015] for *M. edulis* collected at various locations in Ireland, in the same period of the year.

The major MUFAs were palmitoleic (C16:1) and oleic (C18:1 *n*-9) acids, which is in agreement with literature data [Bongiorno *et al.*, 2015; Fuentes *et al.*, 2009]. However, the relative contents of these fatty acids differed widely among all species. The oleic acid (C18:1 *n*-9) showed the highest values, with 12.13% and 11.73% of total FAs, in *S. marginatus* and *L. tuberculata*, respectively, and the lowest ones in *M. barbatus* (4.17% of total FAs) and *M. galloprovincialis* (5.64% of total FAs) (Table 2). Ezgeta-Balic *et al.* [2012] reported values of palmitoleic and oleic acids in *A. noae*, *M. galloprovincialis*, *M. barbatus*, and *O. edulis* from Adriatic Sea lower than those observed in this study period. The predominance of these MUFAs in some examined species may have two origins: exogenous from the diets or endogenous by desaturation of C16:0 and C18:0 acids, respectively [Ekin & Başhan, 2010].

Considerable variation in the relative proportion of total PUFAs was found in the Ionian bivalve species, showing the highest level in *M. varia*, *M. galloprovincialis*, *L. tuberculata*, *O. edulis*, and *M. barbatus* (32.59–37.86% of total FAs; differences between values were insignificant,  $p \geq 0.05$ ) (Table 2). Other species showed to have the PUFAs percentage greater than 30% of total FAs, such as *L. tuberculata* (33.46%), *O. edulis* (32.76%), *M. barbatus* (32.59%), and *A. noae* (30.38%).

The composition of the seafood lipid fraction has widely been studied in terms of its PUFAs and highly unsaturated fatty acids (HUFAs), in both the *n*-3 and *n*-6 family, for its implications in reducing cardiovascular diseases and inflammations [Simopoulos, 2006; Wijendran & Hayes, 2004].

DHA (C22:6 *n*-3) and EPA (C20:5 *n*-3) were the main *n*-3 FAs found in this study, accounting for over 65% of the total *n*-3 PUFAs in most of the bivalves, followed by  $\alpha$ -linolenic (C18:3 *n*-3) and stearidonic acids (C18:4 *n*-3). However, EPA and DHA proportions significantly ( $p < 0.05$ ) differed among the bivalve species. *M. galloprovincialis* showed the highest EPA level with 11.74% of total FAs, while the highest DHA was found in *M. varia* and *L. tuberculata* (10.88–14.41% of total FAs), which is in line with literature data [Bongiorno *et al.*, 2015; Dernekbası *et al.*, 2015; Prato *et al.*, 2010; Radić *et al.*, 2014; Telahigue *et al.*, 2010]. In turn, Azpeitia *et al.* [2016] in their study with mussels (*M. galloprovincialis*) cultured in the Bay of Biscay (northern Spain) found lower levels of EPA (8.77%–8.82% of total FAs) and higher levels of DHA (18.24%–22.49%) than those determined in our study.

Among *n*-6 PUFAs, the most abundant were linoleic acid (C18:2 *n*-6) with content ranging from 1.50% (*M. galloprovincialis*) to 3.19% (*A. noae*), and arachidonic acid (C20:4 *n*-6) with contents from 1.85% (*O. edulis*) to 3.79% (*M. barbatus*) of total FAs (Table 2). These data are in accordance with those reported in literature, in the same period of the year for

*M. galloprovincialis*, *A. noae*, *M. barbatus*, *F. glaber* and *Ruditapes philippinarum* [Azpeitia et al., 2016; Dernekbası et al., 2015; Ezgeta-Balic et al., 2012; Fernández-Reiriz et al., 2017; Prato et al., 2010; Radić et al., 2014; Telahigue et al., 2010].

Essential fatty acids, such as C18:2 *n*-6 and C18:3 *n*-3, are of great physiological importance since they are the precursors for the two families: *n*-3 and *n*-6.

They are converted into long-chain highly unsaturated fatty acids such as arachidonic acid (C20:4 *n*-6), EPA and DHA via fatty acid desaturation and elongation steps. However, the conversion efficiency is low in humans, so direct uptake appears to be significantly more effective [Brenna, 2002; Burdge et al., 2003].

### Lipids nutritional quality indices (LNQI)

Due to different effects of fatty acids on health, LNQI, with consideration to the fatty acid profile and their biological functions, were estimated.

The consumption of foods containing relatively high levels of *n*-3 PUFAs and lower amounts of *n*-6 PUFAs provides a high *n*-3/*n*-6 ratio that is favourable for human health and can be used as an index for comparing the nutritional values of shellfish, such as for fish oil [Piggott & Tucker, 1990]. On the other hand, a very high intake of *n*-6 PUFAs was recognized as undesirable [Kinsella et al., 1990; Simopoulos, 2006] due to their associated negative health impacts such as an increased risk of cardiovascular disease and autoimmune diseases [Simopoulos, 2006].

A high content of the nutritionally important *n*-3 PUFAs (from 18.62% to 33.12%) and low levels of total *n*-6 PUFAs (from 4.60% to 8.17%) were obtained herein. Consequently, high *n*-3/*n*-6 ratio values characterized the FAs profile of all species from Ionian Sea examined (Table 3). They ranged from 2.65 in *F. glaber* to 7.19 in *M. galloprovincialis*.

The recommended *n*-3/*n*-6 ratio differs between authors but it is always superior to 1 [Chow, 2008]. The U.K. Department of Health recommends an ideal *n*-3/*n*-6 ratio to be 4.0, at maximum [HMSO, 1994]. A ratio higher than 4.0 is of great importance in order to diminish coronary heart diseases, plasma lipid levels and cancer risks [Kinsella et al., 1990]. However, due to

the excessive use of vegetable oil, rich in linoleic acid (18:2 *n*-6), in the human food chain and to reduced intake of seafood, this ratio is now lower, in most Western diets [Simopoulos, 2008].

In our study, *M. varia*, *L. tuberculata* and *O. edulis* had the *n*-3/*n*-6 ratio above 5, suggesting that these species could be categorized as ideal to human health consumption. Dernekbası et al. [2015] reported for *M. galloprovincialis* an *n*-3/*n*-6 ratio between 1.44 (Autumn) and 2.23 (Winter), which is much lower compared to that obtained in this study (7.19). On the other hand, Azpeitia et al. [2016] found a similar value of 6.85 in *M. galloprovincialis*, in the same study period.

Asha et al. [2014] reported an *n*-3/*n*-6 ratio of 4.66 for *C. madrasensis*, which is slightly lower than that found in *O. edulis* (5.31).

Another useful key factor for the evaluation of nutritional quality of seafood is the PUFA/SAFA ratio. A minimum value of PUFA/SAFA ratio recommended is 0.45 [HMSO, 1994], which is lower than those obtained from all bivalve species studied herein. The highest PUFA/SAFA ratio was obtained for *M. varia* (0.94) followed by *M. galloprovincialis* (0.85), *L. tuberculata* (0.77) and *M. barbatus* (0.75). These values did not differ statistically between each other ( $p \geq 0.05$ ).

SAFAs have been cited as responsible for a minor increase of HDL-cholesterol; however, such a positive effect does not prevent the harmful increase of low-density lipoprotein (LDL) cholesterol [DiNicolantonio et al., 2016].

Atherogenic index (AI), thrombogenicity index (TI) and hypocholesterolaemic/hypercholesterolaemic fatty acid ratio (HH) provide indications on the dietetic quality of lipids and their potential effect on the development of coronary disease [Ulbricht & Southgate, 1991]. Low values of AI and TI show the better nutritional quality of fatty acids, reducing the potential risk of coronary heart disease (CHD). Literature data reported values of AI from 0.20 to 2.37 and TI from 0.01 to 1.18 for different seafoods, including bivalves [Ghribi et al., 2017; Joy & Chakraborty, 2017; Turan et al., 2007]. In this study, AI and TI fell within the above-mentioned range (Table 3). The values of  $AI \leq 1$  were observed in *M. varia*, *O. edulis* (0.93) and *M. galloprovincialis* (1.0), while the highest one in *L. tuberculata* and *F. glaber* (1.24–1.26).

TABLE 3. Nutritional quality indexes of eight edibles bivalves species of commercial interest.

Specification	<i>A. noae</i>	<i>F. glaber</i>	<i>L. tuberculata</i>	<i>M. varia</i>	<i>M. barbatus</i>	<i>M. galloprovincialis</i>	<i>O. edulis</i>	<i>S. marginatus</i>
$\Sigma n-3$	25.13±4.02 <sup>b,c</sup>	18.62±4.43 <sup>d</sup>	28.50±3.13 <sup>a,b</sup>	33.12±6.28 <sup>a</sup>	24.42±1.66 <sup>b,c,d</sup>	33.08±2.08 <sup>a</sup>	27.57±7.65 <sup>a,b,c</sup>	21.96±1.51 <sup>c,d</sup>
$\Sigma n-6$	5.96±0.28 <sup>b</sup>	7.01±1.64 <sup>a</sup>	4.94±0.48 <sup>b</sup>	4.98±0.12 <sup>b</sup>	8.17±0.16 <sup>a</sup>	4.60±0.10 <sup>b</sup>	5.18±4.84 <sup>b</sup>	7.70±1.69 <sup>a</sup>
<i>n</i> -3/ <i>n</i> -6	4.21±0.65 <sup>c</sup>	2.65±0.06 <sup>d</sup>	5.77±0.21 <sup>b</sup>	6.65±1.44 <sup>a</sup>	2.99±0.14 <sup>d</sup>	7.19±0.28 <sup>a</sup>	5.31±0.98 <sup>b</sup>	2.86±0.44 <sup>d</sup>
PUFA/SAFA	0.68±0.13 <sup>b,c</sup>	0.51±0.14 <sup>c</sup>	0.77±0.15 <sup>a,b</sup>	0.94±0.13 <sup>a</sup>	0.75±0.07 <sup>a,b</sup>	0.85±0.07 <sup>a,b</sup>	0.68±0.28 <sup>b,c</sup>	0.68±0.11 <sup>b,c</sup>
UNS/SAFA	1.21±0.15 <sup>b,c</sup>	0.98±0.08 <sup>c</sup>	1.30±0.17 <sup>a,b</sup>	1.45±0.17 <sup>a</sup>	1.29±0.09 <sup>a,b</sup>	1.25±0.08 <sup>a,b</sup>	1.10±0.34 <sup>b,c</sup>	1.29±0.12 <sup>a,b</sup>
AI	1.06±0.16 <sup>a,b</sup>	1.24±0.33 <sup>a,b</sup>	1.26±0.20 <sup>a</sup>	0.93±0.12 <sup>b</sup>	1.10±0.08 <sup>a,b</sup>	1.00±0.04 <sup>a,b</sup>	0.93±0.23 <sup>b</sup>	1.10±0.08 <sup>a,b</sup>
TI	0.46±0.09 <sup>b</sup>	0.61±0.15 <sup>a</sup>	0.38±0.07 <sup>b</sup>	0.33±0.06 <sup>b</sup>	0.42±0.02 <sup>b</sup>	0.34±0.02 <sup>b</sup>	0.44±0.12 <sup>b</sup>	0.44±0.03 <sup>b</sup>
HH	0.98±0.19 <sup>b</sup>	0.95±0.16 <sup>b</sup>	1.13±0.20 <sup>a,b</sup>	1.34±0.20 <sup>a</sup>	0.87±0.06 <sup>b</sup>	1.08±0.06 <sup>a,b</sup>	1.14±0.31 <sup>a,b</sup>	1.08±0.08 <sup>a,b</sup>

Note: Values are mean (± SD). Means within the same row without a common lowercase letter differ significantly ( $p < 0.05$ ). SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, UNS = unsaturated fatty acids, AI = Atherogenic Index, TI = Thrombogenicity Index and HH hypocholesterolaemic/hypercholesterolaemic fatty acid ratio.

As regards the TI value, there were no significant differences among species (0.33–0.46) except for *F. glaber* that showed a significantly higher TI value (0.61) ( $p < 0.05$ ) (Table 3).

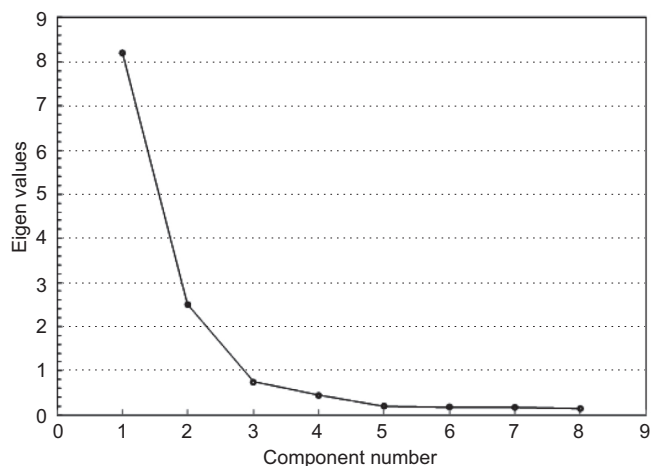


FIGURE 1. The scree plot of the eigenvalues for PCA.

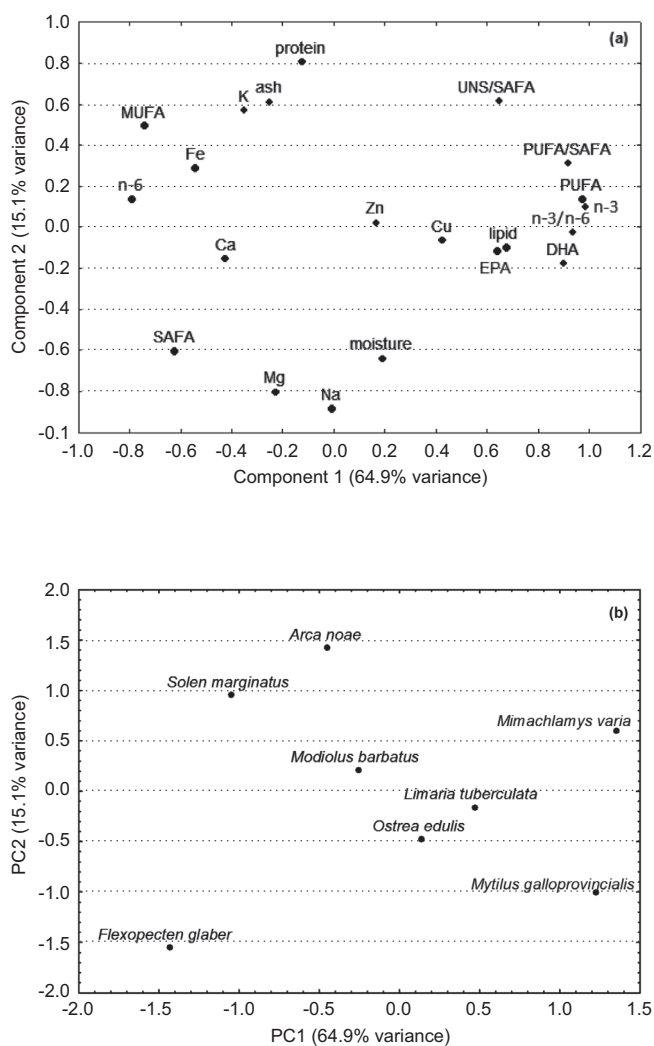


FIGURE 2. Loading of the variables on the first two principal components (a); scatter plot of different bivalve species (b).

As regards the hypocholesterolaemic/hypercholesterolaemic fatty acid ratio (HH), its higher values are desirable [Joy & Chakraborty, 2017; Ramos Filho *et al.*, 2008]. In this study, *M. varia* with 1.34 significantly differed from *A. noae*, *F. glaber* and *M. barbatus* that showed the lowest HH values (0.87–0.98) ( $p < 0.05$ ).

The similarities and differences among the nutritional parameters of bivalves species investigated were statistically assessed utilizing PCA on a data set of 8 cases (8 bivalves) and 21 variables (Na, Mg, K, Ca, Fe, Zn, Cu,  $\Sigma$ SAFA,  $\Sigma$ MUFA,  $\Sigma$ PUFA,  $n-3$ ,  $n-6$ ,  $n-3/n-6$ , UNS/SAFA, PUFA/SAFA, EPA, DHA, protein, lipid, moisture, ash).

Five principal components (PC) were extracted by covering 91% of the cumulative variance. The variance of the five principal components is 64.9%, 20.1%, 5.9%, 3.5% and 1.6%, respectively. Figure 1 reported the scree plot of the eigenvalues. In agreement to these results, the total variance explained by the two first components was 85%. Loading of variables on the first two principal components (Figure 2a) showed that moisture, Zn, Cu, EPA, lipid, UNS/SAFA, PUFA/SAFA, PUFA,  $n-3$ ,  $n-3/n-6$  and DHA had positive scores on the PC1, while K, Ca, Fe, Mg, Na, ash, protein, MUFA,  $n-6$  and SAFA were characterized by negative scores on the same factorial component. Besides protein, K, ashes, UNS/SAFA, MUFA, Fe, PUFA/SAFA,  $n-6$ , PUFA, Zn and  $n-3$  had positive scores on the PC2 while Na, Mg, SAFA, moisture, EPA, DHA, lipid, Ca, Cu and  $n-3/n-6$  had negative scores on the PC2. The scatter plot of scores on the first two principal components PC1 and PC2 (Figure 2b) shows a separation among the bivalves. In fact, *M. varia*, *M. galloprovincialis*, *L. tuberculata* and *O. edulis*, had positive scores on the PC1, while *F. glaber*, *S. marginatus*, *A. noae* and *M. barbatus* were characterized by negative scores on the same principal component. Furthermore *A. noae*, *S. marginatus*, *M. varia* and *M. barbatus* had positive scores on the PC2 while *F. glaber*, *M. galloprovincialis*, *O. edulis* and *L. tuberculata* had negative scores on the PC2. PCA showed, therefore, that *F. glaber* was associated to higher Na, Mg, SAFA and Ca than other organisms; *M. galloprovincialis*, *O. edulis* and *L. tuberculata* were associated to higher moisture, EPA, DHA, lipid,  $n-3/n-6$  and Cu, while *A. noae*, *S. marginatus* and *M. barbatus* were associated to higher protein, ash, K, MUFA, Fe and  $n-6$ . Lastly, *Mimachlamys varia* was associated to higher UNS/SAFA, PUFA/SAFA, PUFA,  $n-3$ .

## CONCLUSION

The importance of the results of this study lies in the fact that, up to now, nutritional quality data are not available in the literature for half of the eight bivalves species. Therefore, it can provide information to consumers, nutritionists, food scientist and guide the farmers to promote the culture of these species.

Edible bivalves, from the Ionian coast of Italy may be considered as food item with interesting dietetic properties due to high contents of protein, Ca, K, Na, Fe, Zn, Cu, and low cholesterol content and to the interesting fatty acid composition. Both the amount of lipid (rather low) and the proportion of saturated, monounsaturated, and polyunsaturated fatty acids in bivalves contribute to a healthful diet.



One of the purposes of this study was just to obtain information on the profile of fatty acids in these bivalve species of commercial interest, widely appreciated in the markets.

The main result of the present study is the finding that many of them can satisfy the nutritional needs of consumers for valuable *n*-3 PUFAs, that represent the fraction of the sea-food lipids that has the largest effect on human health.

All species showed elevated levels of *n*-3 PUFAs, especially EPA and DHA, plus a high *n*-3/*n*-6 ratio. *M. galloprovincialis* and *M. varia* showed the best values of these two FAs.

The *n*-3/*n*-6 ratio, PUFA/SAFA, HH, AI and TI, which are indicators of lipids nutritional quality in food, indicated that the consumption of these species could be beneficial to human health. Despite differences among bivalves investigated, all samples had the *n*-3/*n*-6 ratio within the recommended range and among them, *M. galloprovincialis*, *M. varia*, *L. tuberculata*, *O. edulis* had the best *n*-3/*n*-6 ratio. In terms of HH, AI and TI, *M. varia*, *O. edulis*, *M. galloprovincialis*, and *L. tuberculata* showed to have good potential as a nutritional food with beneficial effects for the consumer's health. Moreover, it must be emphasized that *L. tuberculata* is not very commercialized; it is considered a niche product well appreciated for its organoleptic qualities by estimators, in particular in Southern Italy. At present, no literature data of this species in the wild exist, but our results encourage further studies.

This study has shown that differences exist in fatty acid composition among specific Ionian bivalve species and this is particularly important when a nutritional evaluation of seafood is made and when recommendations of human consumption levels follow for specific health benefits. Considering the importance of exogenous (season, temperature, salinity) and endogenous factors related to the nutritional quality of bivalves, future research will extend the study period to one year.

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## CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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