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PAPER

VARIATIONS IN NUTRITIVE COMPOSITION OF THREE SHELLFISH SPECIES

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ABSTRACT

Nutritive composition, fatty acid profile and health-related lipid indices of natural-born European flat oyster (*Ostrea edulis*), variegated scallop (*Chlamys varia*) and smooth scallop (*Flexopecten glaber*) in 108 samples originating from the Adriatic sea, recovered on a monthly basis were investigated. Out of three shellfish species, the lowest share of saturated fatty acids, the most favourable ratio of polyunsaturated over saturated fatty acids, the most favourable atherogenic and thrombogenic index, and the most favourable ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids were seen in oysters, sampled during springtime. No statistically significant inter-seasonal differences between basic chemical parameters and fat quality indices were established.

Keywords: European flat oyster, lipid quality indices, seasonal variations, smooth scallop, variegated scallop

1. INTRODUCTION

Aquaculture is the fastest-growing food production sector worldwide, shellfish thereby being an important component of global aquatic food supply. The production of marine organisms mainly takes place in sheltered areas or coastal embayments (POGODA, *et al*., 2011), oyster cultivation thereby being a particularly good example of an extensive production of high value-added products (GIBBS, 2004). Due to the high nutritional and gastronomic value of these products, consumer demand for cultivated, but also wild shellfish continuously increases. In general, shellfish is a highly nutritious foodstuff, since it contains appreciable quantities of digestible proteins, essential amino acids, bioactive peptides, long-chain polyunsaturated fatty acids, astaxanthin and other carotenoids, vitamin B12 and other vitamins, minerals including copper, zinc, inorganic phosphate, sodium, potassium, selenium, iodine and also other nutrients, which offer a variety of health benefits to consumers (VENUGOPAL and GOPAKUMAR, 2017). In comparison to other shellfish, European flat oyster (*Ostrea edulis*) represents a product with a higher nutritional value and is hence much higher priced than other shellfish (FAO, 2011).

Literature data have shown that seasonal metabolic activities of shellfish molluscs result from complex interactions between food availability, environmental and growth factors, type of shellfish, but also other parameters (GABBOTT, 1983). For example, lipid changes seen throughout an annual cycle may be related to the increase in energy and nutritional requirements during gonad development (LUZZANA *et al*., 1996), temperature changes (VARLJEN *et al*., 2004) or diet (HENDERSON *et al*., 1996). GULLIAN and AGUIRE-MACEDO (2009) pointed out that, although oysters are tolerant to a broad range of natural variables, this shellfish is susceptible to various forms of physical and chemical disturbances, which cause extreme changes in their metabolism, physiology and defence related-functions, seasonal variations thereby also changing their physiology. Variations that occur in different varieties of European shellfish over a 12-month period have not been fully explored yet.

Additionally, studies of shellfish nutritive composition have been performed only on some species or during certain seasons, mainly on the Atlantic oysters (*Crassostrea gigas*) as cultivated shellfish of great economic importance (PAZOS *et al*., 1996; SOUDANT *et al*., 1999; DAGORN *et al*., 2016). On top of that, data on natural-born oysters inhabiting the Adriatic Sea and other types of shellfish is scarce. Investigations into the shellfish composition could provide producers with a useful background information that could also well serve the needs of consumers keen to evaluate health benefits of their shellfish consumption. In view of the above, the aim of this study was to investigate into, and compare, the nutritional properties of European flat oyster (*Ostrea edulis*), variegated scallop (*Chlamys varia*) and smooth scallop (*Flexopecten glaber*) originating from natural beds in the Adriatic Sea. To the best of our knowledge, this is the first study of basic nutritional composition, fatty acid profile and lipid quality of three natural-born shellfish species populating the Adriatic Sea and seasonal variations in the above parameters witnessed throughout a year.

2. MATERIALS AND METHODS

2.1. Sampling and sample preparation

Samples of European flat oyster (*Ostrea edulis*), variegated scallop (*Chlamys varia*) and smooth scallop (*Flexopecten glaber*) were retrieved during 2016 and 2017 from the western coast of the Istrian Peninsula (Fig. 1). This area is extending from the Savudrija Cape on the north to the border of the territorial sea of the Republic of Croatia, and from the Barbariga Cape on the south to the border of the territorial sea of the Republic of Croatia. This geographic area is highly influenced by strong currents and the vicinity of the Mirna River mouth. It hosts natural beds of different species of bivalves like European flat oyster (*Ostrea edulis*), smooth scallop (*Flexopecten glaber*), variegated scallop (*Chlamys varia*), clam (*Venus verrucosa)* and Noah's Ark (*Arca noae).* Fishermen are collecting mollusks by trawling. Variegated scallop samples were collected at the B1 point (45°31'30''N; 13°27'18''E), those of smooth scallop at the B2 point (45°13'15,3''N; 13°30'00''E) and those of European flat oyster at the B3 point (45°01'14,7''N; 13°41'46,8''E) of the area detailed above.

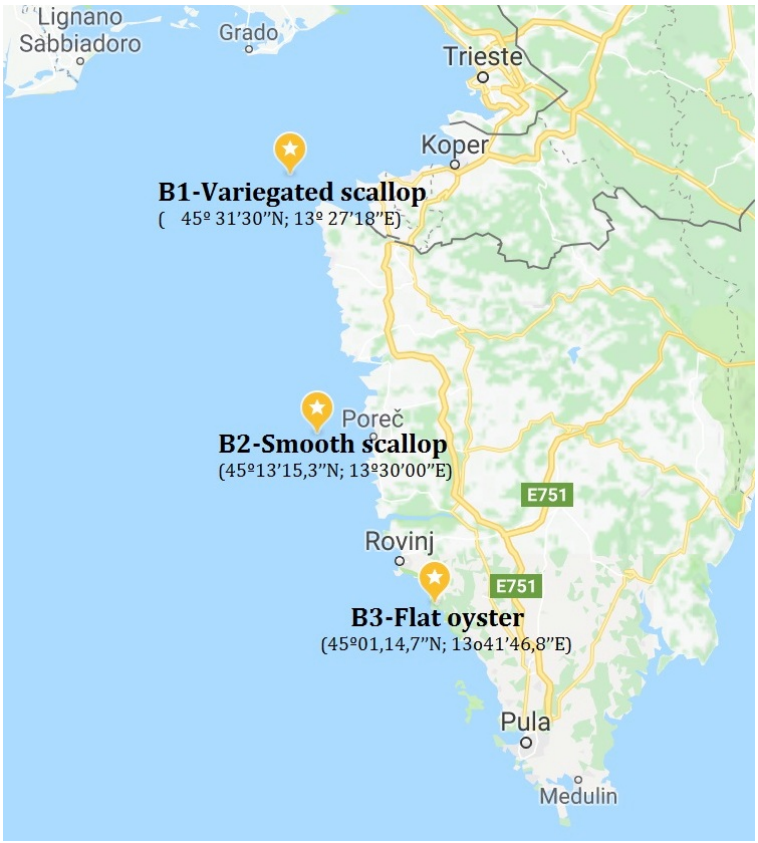

Figure 1. Sampling points at the west coast of the Istrian Peninsula.

Shellfish samples were grouped based on the recovery season, that is to say, into the group of samples retrieved during springtime (March, April & May 2016), those retrieved during summertime (June, July & August 2016), those retrieved during autumntime (September, October & November 2016) and those retrieved during wintertime (December 2016 and January & February 2017). Each month, samples containing 3 kg of each shellfish under study were sampled from the locations of their growth. In total, 108 shellfish samples (36 oyster, 36 variegated scallop and 36 smooth scallop samples) were analysed within 48 hours after sampling. From a 3 kg-shellfish sample, 300 to 400 g of muscle tissue were obtained and further homogenized using a Grindomix GM200 knife mill (Retch, Germany), so as to obtain a homogeneous sample allowing for the determination of basic chemical composition and fatty acid profile.

2.2. Determination of basic chemical composition

The moisture content was determined using gravimetric analysis. The samples were dried at 103 ± 2 °C (ISO 1442:1997) in an UF75 Plus Memmert oven (Schwabach, Germany). The total protein content was determined by virtue of the Kjeldahl method (HRN ISO 937:1999) using an 8 - Basic Digestion Unit (Foss, Höganäs, Sweden) for sample digestion and an automated device for distillation and titration (Vapodest 50s, Gerhardt, Germany). The total fat content was determined using the Soxhlet method (HRN ISO 1443:1999) that implies the digestion of samples by virtue of acid hydrolysis, followed by the extraction of fats using petroleum ether and a Soxtherm 2000 automated device (Gerhardt, Munich, Germany). The ash content was determined according to the ISO 936:1998 and made use of a LV9/11/P320 Nobertherm furnace (Lilienthal, Germany). All chemicals used for the analyses were of an analytical grade. Carbohydrate content was determined by calculation, based on the determination of water, ash, total protein and fat content. The mean of data obtained from two parallel runs in form of weight percentage (%) and with the accuracy of 0.01% was considered as a result descriptive of a single sample.

2.3. Fatty acid profile

Sample preparation method for the analysis of fatty acid methyl esters was described earlier by PLEADIN *et al.* (2015). Methyl esters of fatty acids were analysed using gas chromatography (GC) according to the EN ISO 12966-2:2011 and EN ISO 12966-4:2015. To the above effect, a 7890BA gas chromatographer equipped with flame ionization detector (FID), a 60-m DB-23 capillary column having an internal capillary diameter of 0.25 mm and the stationary phase thickness of 0.25 μm (Agilent Technologies, Santa Clara, USA) was used. The components were detected by FID at the temperature of 280 °C, hydrogen flow of 40 mL/min, air flow of 450 mL/min and nitrogen flow of 25 mL/min. The initial column temperature was 130 °C; after a minute, it was increased by 6.5 °C/min until the temperature of 170 °C was reached. The temperature was further increased by 2.75 °C/min until the temperature of 215 °C was attained. The latter temperature was maintained for 12 min and then further increased rate by 40 °C/min until the final column temperature of 230 °C was reached, the latter being maintained for 3 min. One mL of a sample was injected into a split-splitless injector at the temperature of 270 °C and with the partition coefficient of 1:50. The carrier gas was helium (99.9999%), flowing at the constant rate of 43 cm/sec. Fatty acid methyl esters were identified by comparing their retention times with those of fatty acid methyl esters contained by the standard mixture, as

described earlier by PLEADIN *et al.* (2015). The results are expressed as a percentage (%) of a particular fatty acid in total fatty acids, the accuracy thereby being 0.01%.

2.4. Nutritional quality of lipids

Data on fatty acid composition were used for the calculation of the following lipid quality indices: the atherogenic index (AI), the thrombogenic index (TI) and the hypocholesterolaemic/hypercholesterolaemic ratio (HH). The atherogenic index (AI) indicates the relationship between the sum of the main saturates and the sum of the main non-saturates. This parameter was calculated as: AI= [(C12:0 + (4 x C14:0) + C16:0)] / [∑ MUFA + PUFA n-6+ PUFA n-3] (ULBRITCTH and SOUTHGATE, 1991). The thrombogenic index (TI) is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FAs (MUFA, PUFA n-6 & PUFA n-3). The index was calculated as: TI = (C14:0 + C16:0 + C18:0) / [0.5 x ∑MUFA + 0.5 x PUFA n-6 + 3 x PUFA n-3) + (PUFA n-3/PUFA n-6)]. The ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids (HH) takes into account well-known effects of certain fatty acids on cholesterol metabolism (SANTOS-SILVA *et al.*, 2002). It was calculated as: HH = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0) (ULBRITCTH and SOUTHGATE, 1991).

2.5. Statistical analysis

Statistical analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY IBM, 2013). In order to determine the differences between the sample groups (season-based, shellfish type-based), one-way ANOVA and the robust Brown-Forsythe test were used. The decisions on statistical significance were made at the significance level of $p<0.001$ and $p < 0.05$.

3. RESULTS AND DISCUSSION

This study provides data on nutritional composition of three natural-born shellfish species originating from the Adriatic Sea, with a special emphasis on fatty acids and health-related lipid indices determined over four seasons of a one-year period. Literature data have revealed that habitats, season, feed, species, but also gametogenesis and spawning cycle, can influence the proximate shellfish composition (GABBOTT, 1983).

Basic chemical composition of the investigated shellfish determined in this study is shown in Table 1. As regards the moisture content, oysters had a significantly lower average value (around 82 g/100 g) as compared to variegated (around 84 g/100 g, $p = 0.0028$) and smooth scallops (around 87 g/100 g, $p < 0.001$), representing the shellfish richer in nutrients in comparison to the other two. However, oysters were the only one out of the three shellfish species in which significant seasonal moisture content variations were found (higher moisture content in autumn as compared to spring and summer). Moisture contents similar to those we found, with values of 82.1 g/100 g during summer and 81.4 g/100 g during winter, were reported by MARTINO and CRUZ (2004) for oysters of the *Crassostrea rhizophorae* species.

Oysters also had significantly higher carbohydrate content (average value around 4.3 g/100 g) as compared to variegated (around 3.2 g/100 g) and smooth scallops (1.4 g/100

g). However, the significant seasonal carbohydrate content variations weren't found for any shellfish ($p > 0.05$).

Table 1. Basic chemical composition of the analysed shellfish types during a one-year period.

Shellfish	Season	Mean ± SD (g/100 g)				
		Moisture	Protein	Ash	Fat	Carbohydrate
European flat oyster (n=36)	Spring	80.30±0.53^{d}	10.99±0.27^{d}	2.04±0.16	2.37±0.32^{a,d}	4.29±0.63
	Summer	81.43±0.58^{d}	10.00±0.27	2.14±0.43	2.03±0.25^{d}	4.40±0.07
	Autumn	83.87±0.61^{b,c}	8.13±1.13^{b}	2.21±0.04	1.20±0.10^{b,c}	4.57±0.77
	Winter	81.87±1.22	10.27±1.47	2.33±0.20	1.67±0.23^{b}	3.88±0.75
	Average	81.87±1.50^{B,C}	9.85±1.36	2.18±0.24	1.82±0.50^{B,C}	4.29±0.30^{B,C}
Variegated scallop (n=36)	Spring	80.43±7.51	8.88±0.48	3.40±2.42	0.90±0.20	6.39±7.94
	Summer	85.27±0.40	9.43±0.24	2.56±0.57	0.97±0.23	1.77±0.57
	Autumn	85.23±1.31	9.90±1.52	1.94±0.20	0.93±0.12	1.99±0.44
	Winter	85.27±1.25	9.02±2.03	2.06±0.35	0.77±0.15	2.87±1.81
	Average	84.05±3.95^{A,C}	9.31±1.18	2.49±1.23	0.89±0.17^{A,C}	3.24±1.83^{A}
Smooth scallop (n=36)	Spring	86.57±0.35	9.32±1.26	1.70±0.17	1.07±0.31^{d}	1.34±0.29
	Summer	86.70±0.79	8.71±0.39	2.55±0.61	0.77±0.15	1.28±0.33
	Autumn	87.63±0.95	8.65±0.88	1.91±0.16	0.30±0.10^{b}	1.51±0.46
	Winter	87.03±1.42	8.68±0.96	2.27±0.71	0.53±0.23	1.49±0.53
	Average	86.98±0.92^{A,B}	8.84±0.32	2.11±0.53	0.67±0.35^{A,B}	1.40±0.11^{A}

Results are expressed as the mean value (mean ± SD) of six results (3 months per season; each month, one sample was taken and analysed in duplicate).
Statistically significant difference ($p < 0.05$) within the same column for every shellfish type separately:
^{a}*vs.* winter; ^{b}*vs.* spring, ^{c}*vs.* summer, ^{d}*vs.* autumn; ^{A}*vs.* European flat oyster, ^{B}*vs.* variegated scallop, ^{C}*vs.* smooth scallop.

The average protein content was almost equal in all three studied shellfish species, ranging from 8.84 g/100 g in smooth scallops to 9.85 g/100 g in oysters. The proportion of proteins significantly differed among the shellfish species only in summer. Oysters and smooth scallops contained the highest protein levels in spring (10.99 g/100 g and 9.32 g/100 g, respectively) while variegated scallops presented with the highest protein levels in autumn (9.90 g/100 g), although the only statistically significant difference ($p = 0.014$) was that in the protein content of oysters, which was higher in those collected in spring as compared to those collected in autumn. Three shellfish species had quite similar average ash contents, ranging from 2.11 g/100 g in smooth scallop to 2.49 g/100 g in variegated scallop and showing no statistically significant differences, neither across seasons nor across species.
Based on linear correlation coefficient and slope values from correlation equations related to moisture and fat ($y = -0.1818x+16.453$; $R^2 = 0.6275$), moisture and protein ($y = -0.1153x+19.232$; $R^2 = 0.0885$) as well as moisture and ash ($y = -0.0679x+7.986$; $R^2 = 0.1681$), it is clear that fat content shows the strongest inversely proportional relationship with the moisture content found in the three shellfish. Therefore, a decrease in proportion of water is primarily reflected in an increase of fat content, especially in case of oysters. Oysters had

a significantly higher average fat content (1.82 g/100 g) as compared to variegated (0.89 g/100 g) and smooth scallops (0.67 g/100 g) ($p < 0.001$), as well as the highest share of fat in winter ($p = 0.002$), spring ($p = 0.001$), summer ($p = 0.001$) and autumn ($p < 0.001$) in comparison to other shellfish types (*data not shown*). As regards seasonal influence, both oysters and smooth scallops showed a significantly higher fat content in spring than in autumn ($p = 0.005$ and $p < 0.05$, respectively), while in variegated scallops no significant variations were found. Since fats have been shown to be involved in spawning-related biochemistry of marine species (REN *et al.*, 2003), the observed variability in fat levels in different sampling times was to be expected.

In comparison to the results of LIRA *et al.* (2013) that revealed these species to have a higher fat content in winter than in summer, our study failed to confirm such a pattern. However, in the study quoted above, the composition of oysters was analysed using the Brazilian cultivated *Crassostrea rhizophorae* oysters sampled in only two seasons - winter and summer. Nevertheless, our spring sampling could be compared to their winter sampling, confirming the same variability pattern. Also, it should be emphasized that the majority of studies were conducted on the Pacific oysters (*Crassostrea gigas)* in particular months or seasons, either rendering the inter-comparison impossible or limiting its extent (PAZOS *et al.*, 1996; SOUDANT *et al.*, 1999; DAGORN *et al.*, 2016).

In oysters, 27 fatty acids were identified, in all four investigated seasons mostly in the following order of representation: palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9*c*) and docosahexaenoic acid (DHA; C22:6n-3) (Table 2). The fatty acid composition and the prevalence of certain fatty acids could be compared to the results of some earlier studies performed on different oyster types (LINEHAN *et al.*, 1999; EZGETA-BALIĆ *et al.*, 2012; HURTADO *et al.*, 2012; LIRA *et al.*, 2013; POGODA *et al.*, 2013; DAGORN *et al.*, 2016). The highest SFA content was determined in summer and autumn, whereas the highest PUFA content was determined in spring. It could be assumed that in our samples fatty acid composition of the muscle tissue indicates the differences in selective incorporation of dietary PUFAs. The study of bivalve food sources populating the Adriatic Sea (EZGETA-BALIĆ *et al.*, 2012) confirmed that bivalves feed on mixed food, the quality of which strongly depends on seasonal changes in food composition. During the period of high phytoplankton presence (spring/summer), bivalve species mainly ingest phytoplankton, but also zooplankton and detritus. During the period of low phytoplankton presence (autumn/winter), bivalves rely on zooplankton and detritus.

In line with the findings of EZGETA-BALIĆ *et al.* (2012), we confirmed that oysters accumulate a significant amount of PUFAs during springtime. As oppose to the results of LIRA *et al.* (2013) (although obtained on *Crassostrea rhizophorae,* not *Ostrea edulis*), who determined the DHA (C22:6n-3) content to be twice higher in winter than in summer, in our study the highest DHA oyster content was observed in spring, with moderately high levels in winter and autumn and the lowest level in summer. The ratios in favour of DHA over EPA throughout the year confirm the presence of animal component in oyster diets (EZGETA-BALIĆ *et al.*, 2012).

Generally, variegated and smooth scallops were shown to harbour a significantly lower number of fatty acids in comparison to oysters, which could be explained by the fact that food selection is an active process and that different species have different affinities when it comes to food, i.e. various preferences for microalgae (GONZÀLEZ-ARAYA *et al.*, 2012).

Table 2. Fatty acid composition (% of total fatty acids) of European flat oyster (*Ostrea edulis*).

	Season			
Fatty acids	**Spring**	**Summer**	**Autumn**	**Winter**
C8:0	0.21±0.18	< LOD	< LOD	0.08±0.14
C10:0	0.26±0.23	< LOD	< LOD	< LOD
C12:0	0.06±0.11	< LOD	< LOD	0.60±1.04
C14:0	7.32±0.73	6.51±1.74	5.30±1.28	5.01±1.48
C15:0	1.45±0.00	1.54±0.37	1.43±0.31	1.24±0.45
C16:0	31.57±2.41	39.56±7.40	36.54±3.68	34.90±3.75
C17:0	3.15±0.22	4.42±0.87	4.20±0.67	3.33±1.03
C18:0	6.72±5.88	14.75±1.92	20.27±2.86	14.09±7.13
C20:0	< LOD	< LOD	< LOD	0.11±0.19
C23:0	0.28±0.48	< LOD	< LOD	0.56±0.49
C14:1	< LOD	0.43±0.74	< LOD	< LOD
C16:1n-7*t*	0.50±0.07	< LOD	0.47±0.81	0.48±0.48
C16:1n-7*c*	4.26±0.67	3.14±0.71	2.96±0.50	3.14±0.83
C17:1	0.21±0.18	< LOD	< LOD	< LOD
C18:1n-9*c*	8.03±0.20	10.18±0.75	9.08±4.91	14.82±9.69
C18:1n-7	2.96±0.24	3.13±0.39	3.12±0.26	2.13±1.85
C20:1n-9	0.60±0.02	1.63±2.12	< LOD	0.12±0.20
C24:1n-9	< LOD	< LOD	< LOD	0.25±0.43
C18:2n-6*c*	2.72±0.26	2.26±0.44	1.74±1.60	4.31±3.53
C18:3n-6	0.40±0.69	< LOD	< LOD	< LOD
C20:4n-6	0.89±0.19	0.41±0.70	0.37±0.64	0.54±0.66
C18:3n-3	4.35±1.40	1.71±1.72	1.78±1.58	1.11±1.42
C18:4n-3	5.22±0.91	1.69±1.62	1.47±1.41	1.62±2.39
C20:4n-3	0.49±0.43	< LOD	< LOD	0.22±0.38
C20:5n-3	7.31±1.58	3.40±2.96	5.03±1.23	5.15±3.85
C22:5n-3	0.60±0.52	< LOD	< LOD	< LOD
C22:6n-3	10.44±2.05	5.21±4.62	6.25±2.29	6.18±5.28
SFA	51.02±7.19	66.79±11.40	67.74±8.74	59.93±7.99
MUFA	16.55±1.12	18.52±2.88	15.63±3.92	20.95±7.68
n-6	4.01±0.88	2.67±1.14	2.11±2.12	4.85±3.41
n-3	28.42±5.67	12.02±10.78	14.52±6.01	14.28±13.01
PUFA	32.43±6.46	14.69±11.63	16.64±7.38	19.13±11.66

Results are expressed as the mean value (%, mean ± SD) of six results obtained for total fatty acids (3 months per season; each month, one sample was taken and analysed in duplicate);
LOD (limit of detection) = 0.05%.
SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids.

RABY *et al.* (1997) found that different species ingest microalgae of different sizes, which is an indicator of their active food selection, the size of microalgae thereby being the major factor influencing the ingestion of food particles. Although the three shellfish types were collected from different locations in the Adriatic Sea (along the coast of the Istrian

Peninsula), the influence of water temperature, salinity and other environmental factors on fatty acid composition should be negligible, due to the small distances between the sampling locations (the same area of the Adriatic Sea). So, the differences in nutritive composition of three shellfish investigated in this study are probably mainly coming as a result of their different diet preferences.
Table 3 presents the fatty acid composition of variegated scallop determined in various seasons. Same as with oysters, the most dominant fatty acids in variegated scallop were C16:0, C18:0 and C18:1n-9*c*, whereas DHA was not detected. In this shellfish, no statistically significant inter-seasonal differences ($p > 0.05$) in individual fatty acid, SFA, MUFA and PUFA contents as were found. However, PUFA contents were highly variable within the same annual period, as can be seen from high intra-seasonal standard deviations. Higher PUFA content was observed in winter in comparison to summertime.

Table 3. Fatty acid composition (% of total fatty acids) of variegated scallop (*Chlamys varia*).

	Season			
Fatty acids	**Spring**	**Summer**	**Autumn**	**Winter**
C8:0	0.52±0.90	< LOD	< LOD	0.40±0.69
C10:0	0.35±0.61	< LOD	< LOD	< LOD
C12:0	< LOD	< LOD	< LOD	0.50±0.87
C14:0	8.13±4.77	10.02±1.33	7.18±3.36	3.72±0.96
C16:0	41.20±6.34	44.34±1.10	44.60±1.81	44.56±4.67
C17:0	2.52±4.36	< LOD	< LOD	< LOD
C18:0	31.68±12.47	24.20±4.35	38.73±10.02	35.70±9.55
C16:1n-7	3.67±3.29	3.31±2.95	2.95±2.60	0.70±1.21
C18:1n-9c	7.86±3.61	15.27±2.63	4.48±3.93	12.27±6.89
C18:1n-7	< LOD	1.96±1.75	0.78±1.35	< LOD
C18:2n-6c	1.69±2.93	0.90±1.56	< LOD	2.15±3.73
C18:3n-3	2.38±4.13	< LOD	1.28±2.21	< LOD
SFA	84.40±8.64	78.56±4.91	90.51±8.46	84.87±11.83
MUFA	11.53±6.32	20.54±5.07	8.21±7.11	12.98±8.10
n-6	1.69±2.63	0.90±1.56	< LOD	2.15±3.73
n-3	2.38±4.13	< LOD	1.28±2.21	< LOD
PUFA	4.07±7.05	0.90±1.56	1.28±2.21	2.15±3.73

Results are expressed as mean value (%, mean ± SD) of six results obtained for total fatty acids (3 months per season; each month, one sample was taken and analysed in duplicate);
LOD (limit of detection) = 0.05%.
SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids.

Fatty acid composition of smooth scallop seen in various seasons is presented in Table 4. Same as with variegated scallop, the most dominant fatty acids in smooth scallop were C16:0, C18:0 and C18:1n-9*c*, while DHA presence was not detected. A statistically significant inter-seasonal difference was determined only for C18:0 found in summer (lower value) as compared to that in autumn (higher value) ($p = 0.001$), which can also be achieved thanks to low intra-seasonal variability witnessed in these two seasons. As for

the content of other individual fatty acids, SFA, MUFA and PUFA, no statistically significant inter-seasonal differences were found ($p > 0.05$). PUFAs were not quantified, while a higher SFA content was observed in winter in comparison to summertime.

Table 4. Fatty acid composition (% of total fatty acids) of smooth scallop (*Flexopecten glaber*).

Fatty acids	Season			
	Spring	Summer	Autumn	Winter
C14:0	8.63±3.10	5.91±1.81	1.10±1.90	5.80±3.08
C16:0	44.67±0.89	46.01±3.45	45.29±0.43	44.30±2.45
C17:0	1.88±2.66	< LOD	< LOD	< LOD
C18:0	29.12±11.47	33.47±1.33*	47.22±1.14*	39.49±5.57
C14:1	< LOD	1.17±2.02	< LOD	< LOD
C16:1n-7	4.78±1.60	1.19±2.06	< LOD	2.00±3.46
C18:1n-9*c*	8.99±0.49	12.25±2.44	6.39±1.31	8.41±3.13
C18:1n-7	1.93±2.73	< LOD	< LOD	< LOD
SFA	84.29±4.82	85.39±5.78	93.61±1.31	89.59±2.11
MUFA	15.71±4.82	14.61±5.78	6.39±1.31	10.41±2.11
PUFA	< LOD	< LOD	< LOD	< LOD

Results are expressed as mean value (%, mean ± SD) of six results obtained for total fatty acids (3 months per season; each month, one sample was taken and analysed in duplicate);
LOD (limit of detection) = 0.05%.
SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids.
* Statistically significant difference ($p < 0.05$).

The representation of all fatty acid groups (SFA, MUFA and PUFA) significantly differed ($p < 0.05$) between the analyzed shell species. Oysters contained the smallest proportion of SFAs ($p < 0.001$) and the highest ($p < 0.001$) share of PUFAs as compared to other shellfish types. The share of MUFA was significantly higher in oysters than in smooth scallop ($p = 0.031$).

It is known that shellfish fatty acid composition usually reflects a fatty acid composition of their diet (phytoplankton or zooplankton), although shellfish have shown a certain ability to elongate (e.g. C16:1 to C18:1, C18:1 to C20:1, C20:5 to C22:5, C20:4 to C22:4) or desaturate (e.g. C20:3 to C20:4) fatty acids (ALBENTOSA *et al.*, 1996; DELAPORTE *et al.*, 2005). Given that basic shellfish nutrient composition and fatty acid profile are influenced by many parameters, both a correct interpretation of the obtained results and a plausible comparison with the results of other studies require the knowledge on ecological characteristics of areas in which shellfish are cultivated or natural-born.

The results pertaining to the nutritional fat quality indices (n-6/n-3, PUFA/SFA, AI, TI and HH) determined for each shellfish type in each season and in total, the latter being expressed as the mean value descriptive of the entire one-year study period, are presented in Table 5. Ratios n-6/n-3 and PUFA/SFA are the parameters most commonly used for the assessment of nutritional fat quality. Literature has shown that in case of lower n-6/n-3 ratios, the body is more able to make use of n-3 fats (WOOD *et al.*, 2008). Ratio n-6/n-3 has been suggested to be a good tool for comparing relative nutritional values of different species, but this index is of a limited value should the share of individual fatty acids be

unknown. Due to the fact that fatty acids containing C20 and C22 are more valuable from the nutritional standpoint as compared to fatty acids containing C18, and taking into account their predominance over other n-3 fatty acids, EPA and DHA are largely responsible for the changes in n-6/n-3 ratio, the latter otherwise being considered as a reliable indicator that enables comparison of relative nutritive lipid values (PLEADIN *et al.*, 2017). As recently reviewed by WEYLAND *et al*. (2015), beneficial effects of these fatty acids have been reported for a number of disorders, including cardiovascular, neuropsychiatric and inflammatory diseases, as well as some cancers (mainly colorectal, mammary and prostatic cancer).

Table 5. Nutritional fat quality indices established for the analysed shellfish types during a one-year period.

Shellfish type	Season	Fat quality indices (target values)				
		n-6/n-3	PUFA/SFA	AI	TI	HH
European flat oyster (n=36)	Spring	0.14±0.01	0.65±0.21	1.27±0.26	0.46±0.16	0.89±0.17
	Summer	0.11±0.10	0.24±0.20	2.32±1.53	2.92±3.72	0.54±0.28
	Autumn	0.13±0.14	0.26±0.13	1.95±0.91	1.30±0.79	0.60±0.28
	Winter	0.77±1.02	0.34±0.24	1.43±0.32	1.17±0.62	0.81±0.17
	Average	0.29±0.53	0.37±0.24	1.74±0.89^{B,C}	1.46±1.90 ^{B,C}	0.71±0.25^{B,C}
Variegated scallop (n=36)	Spring	0.24±0.41	0.05±0.09	6.12±3.82	14.29±15.52	0.25±0.16
	Summer	n.d.	0.01±0.02	4.08±0.96	7.68±2.33	0.30±0.03
	Autumn	n.d.	0.02±0.03	3.80±3.36	6.36±7.32	0.11±0.10
	Winter	n.d.	0.03±0.05	5.68±3.40	16.33±10.03	0.32±0.26
	Average	0.24±0.41	0.03±0.05	4.92±2.84^{A}	11.17±9.61^{A}	0.23±0.15^{A}
Smooth scallop (n=36)	Spring	n.d.	n.d.	5.15±0.74	11.17±4.38^{d}	0.17±0.00
	Summer	n.d.	n.d.	5.48±2.86	13.19±5.81^{d}	0.24±0.07
	Autumn	n.d.	n.d.	8.08±2.45	30.12±5.95^{b,c}	0.14±0.03
	Winter	n.d.	n.d.	6.65±1.74	17.82±4.54	0.17±0.08
	Average	n.d.	n.d.	6.45±2.22^{A}	18.70±8.93^{A}	0.18±0.06^{A}

Results are expressed as mean value (%, mean ± SD) of six results obtained for total fatty acids (3 months per season; each month, one sample was taken and analysed in duplicate);
SFA saturated fatty acids; PUFA polyunsaturated fatty acids;
HH hypo-/hyper-cholesterolaemic fatty acids ratio = (C18:1n-9+C18:2n-6+C20:4n-6+C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3)/(C14:0+C16:0)
AI atherogenic index = [(C12:0+(4xC14:0)+C16:0)]/(∑ MUFA+PUFA n-6+PUFA n-3)
TI thrombogenic index = (C14:0+C16:0+C18:0)/[(0.5 x ∑ MUFA+ 0.5x PUFA n-6+ 3x PUFA n-3)+(PUFA n-3/PUFA n-6)]
Statistically significant difference ($p< 0.05$) within the same column for every shellfish type separately:
^{a}*vs.* winter; ^{b}*vs.* spring, ^{c}*vs.* summer, ^{d}*vs.* autumn; ^{A}*vs.* European flat oyster, ^{B}*vs.* variegated scallop, ^{C}*vs.* smooth scallop
n.d. (not detected) - fatty acids needed for calculation were not detected (< LOD).

According to health recommendations, n-6/n-3 ratio should be lower than 4, thereby reducing the incidence of chronic food-related illnesses (CORDAIN *et al.*, 2005; SIMOPOULOS, 2002). In an annual form, this index was calculable only for oysters, while for variegated scallop it could be provided only for the samples recovered during springtime. In both cases, the determined n-6/n-3 ratios fell within the recommended

boundaries, although high intra-seasonal variations were evident. In an annual form, n-6/n-3 ratio was not calculable for smooth scallop because of the absence of PUFAs (values below the LOD).

PUFA/SFA ratio is recommended to be higher than 0.4, so as to reduce the risk of cardiovascular, autoimmune and other chronic diseases (SIMOPOULOS, 2002). Generally, for two shellfish types in which PUFAs were present in values above the LOD (oyster and variegated scallop), the determined PUFA/SFA ratios were significantly lower than the recommended minimum, except in oysters during springtime (0.65±0.21). Same as with n-6/n-3 ratio, high intra-seasonal variations were noticeable. Some authors are of the opinion that an index such as PUFA/SFA may prove inadequate for the evaluation of nutritional value of fats, because some SFAs do not increase plasma cholesterol. Therefore, MENSINK and KATAN (1992), and DALEY *et al.* (2010), suggested that C12:0 and C14:0 have a more pronounced total cholesterol raising effect than C16:0, whereas C18:0 is neutral when it comes to the concentration of total serum cholesterol, with no apparent impact on either LDL or HDL. Myristic acid (C14:0) has a 4-6 times higher potential to increase cholesterol concentrations as compared to C16:0 (ULBRITCTH and SOUTHGATE, 1991; BRESSAN *et al.*, 2011). On top of that, PUFA/SFA index ignores the effects of MUFAs, which may have more profound health benefits in terms of coronary disease prevention (ORELLANA *et al.*, 2009).

Therefore, two additional indices, which take into account different effects that a single fatty acid might have on the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation, i.e. the atherogenic (AI) and the thrombogenic index (TI), were calculated, too. The atherogenic index takes into account the fact that some saturates are considered to be pro-atherogenic (since they facilitate the adhesion of lipids onto the cells the immune and the circulatory system are composed of), while non-saturates are considered to be anti-atherogenic (since that inhibit the formation of plaques and diminish the levels of esterified fatty acids, cholesterol, and phospholipids, therefore preventing micro- and macro-coronary disease) (ULBRITCTH and SOUTHGATE, 1991). The thrombogenic index (TI) shows the tendency towards blood clotting. It is assumed that AIs and TIs below 1 are beneficial to human health (PLEADIN *et al.*, 2017). According to the data reported in Table 5, only oysters approach the recommended values, while other two shellfish types exceed the maximum limits by far.

In order to gain insight into the effect of fatty acids on blood cholesterol, an additional indicator of nutritional quality, i.e. the ratio between hypocholesterolaemic and hypercholesterolaemic fatty acids (HH), was calculated. It is preferable for that index to be higher (SANTOS-SILVA *et al.*, 2002). The obtained HH values ranged from 0.18±0.06 in smooth scallop and from 0.23±0.15 in variegated scallop, whereas the highest HH index was determined in oysters during springtime (0.89±0.17).

In case of oysters and variegated scallop, no statistically significant seasonal differences ($p > 0.05$) in any of the fat quality indices were determined. In smooth scallop, a significant seasonal difference was determined only for TI ($p < 0.05$; $p = 0.016$), with a significantly higher TI in autumn in comparison to spring and summer. With the exception of n-6/n-3 ratio, which was actually determined only in oysters, a significant difference in TI, HH and PUFA/SFA indices was determined across the studied shellfish types, while a statistically significant difference in the AI value among the analysed shellfish over different seasons failed to be seen. In spring, oysters had significantly higher PUFA/SFA and HH ratios than other shellfish types. In winter and autumn, oysters also showed a significantly higher HH ratio as compared to variegated and smooth scallop. The TI established for smooth scallop in autumn was significantly higher as compared to other

shellfish; in winter, the latter index determined for smooth scallop was also higher than that found for oysters. As in the study by LIRA *et al.* (2013), fatty acids-related nutritional quality indices were more favourable in winter in comparison to summer period.

4. CONCLUSION

The proportion of fat found in all shellfish types under study was low, with the highest average representation in oysters. The proportion of proteins and total minerals in meat of all three shellfish was found to be similar. The representation of saturated fatty acids was generally found to be high, with an unfavourable PUFA/SFA ratio that might increase the risk of chronic diseases. Out of the three shellfish species under study, the lowest SFA content, the most favourable PUFA/SFA ratio and the most favourable AI, TI and HH indices were established in oysters. Although oysters harvested in springtime contained the highest proportion of fats and proteins, and therefore presented with the most favourable PUFA/SFA, AI, TI and HH indices in that particular season, intra-seasonal variations were huge, so that statistically significant inter-seasonal differences in these parameters in oysters harvested in different times of the year failed to be found. During springtime, smooth scallop also showed the highest representation of fats and proteins, and hence also the most favourable AI and TI indices, but inter-seasonal variations were proven to be either statistically insignificant or significant only in comparison to one out of the three remaining seasons. As for variegated scallop, none of the seasons could be considered as the most favourable when it comes either to fat and protein content or to fat nutritional quality indices. In summary, no statistically significant inter-seasonal differences in basic chemical parameters and fat quality indices descriptive of an edible part of the three shellfish were determined.

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