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Chemical Composition and Omega 3 Human Health Benefits of Two Sea Cucumber Species of North Atlantic

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ABSTRACT
The main goal of this study was to investigate the seasonal chemical changes of two sea cucumber species caught from Portugal. The potential contribution to human health of lipids was also evaluated. For this, the chemical composition (protein, ash, and fat) and lipids composition of *Holothuria arguinensis* and *Holothuria forskali* was followed in summer and winter. Both species presented adequate levels of protein and ash and low content of total lipids, although their lipid profile showed a high proportion of polyunsaturated fatty acid (PUFA). Eicosapentaenoic acid (EPA) was abundant in all samples, and a favorable and similar n-3/n-6 ratio was found for both species. The atherogenic and thrombogenicity indices and hypocholesterolaemic/hypercholesterolaemic ratio suggests a high-quality for *H. arguinensis* and *H. forskali*. In general, significant differences were observed between species but not between summer and winter. Some significant variations were observed between summer and winter in the lipid profile of both species and in the chemical composition of *H. forskali*. Results indicate that these species have adequate nutritional quality for human consumption and may be suitable for the development of reliable fatty acid guides in sea cucumbers.

KEYWORDS
Fatty acid; lipid quality indices; chemical composition; season effect; holothuroidea; Portugal

Introduction

Fish and marine invertebrates have been consumed by Europeans since prehistory (Hoffmann 2005). Although there are species of sea cucumber (Echinodermata: Holothuroidea) distributed over almost all seas and oceans (Demetropoulos and Hadjichristophorou 1976; Katsanevakis et al. 2014; Reich 2012), they are not part of the gastronomic culture of European countries (Çakli et al. 2004). However, there is local and isolated consumption. For example in Greece, *Holothuria tubulosa* has been used as bait for anglers for over a century, and in Catalonia in Spain, *Parastichopus regalis* is fished for human consumption. Turkey is the main country in the Mediterranean that catches sea cucumbers, *Holothuria mammata*, *Holothuria polii*, and *H. tubulosa*, for export (Antoniadou and Vafidis 2011; Ramón et al. 2010). On the other hand, the use of sea cucumber as a human food source has been strongly linked to Asia for hundreds of generations, as the continent that most imports, consumes, and exports this product (Ferdouse 2004).

Sea cucumbers are considered a food with a high nutritional value and suitable for human consumption, since they contain higher amounts of protein and lower amounts of fat than most foods. However, similar to other marine products (EFSA 2012; Wang et al. 2006), sea cucumbers are a source of lipids that

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are not found in other organisms, such as omega 3 polyunsaturated fatty acids (n-3 PUFA). A number of clinical studies have clearly shown the importance of n-3 PUFA for achieving optimum state of health. Health benefits have been attributed primarily to n-3 eicosapentaenoic acids (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Calder 2008; Cawood et al. 2010; Miles and Calder 2012). Humans cannot synthesize adequate levels of n-3 PUFA because of the lack of the desaturase enzymes required to produce the simplest members of this family, including α-linolenic acid (18:3 n-3) and linoleic acid (18:2 n-6), as well as precursors for the synthesis of more highly unsaturated n-3 (EPA, DHA) and n-6 fatty acids (arachidonic acid (ARA, 20:4 n-6)) (Tapiero et al. 2002). Thus, these FAs are considered "essential fatty acids" (EFAs) that need to be obtained through food. The primary sources of EPA and DHA are seafood and products derived from it, even though amounts can vary significantly (Biandolino et al. 2019a, 2010).

As in the other marine animals, the abundance of n-3 PUFA in sea cucumbers varies according to species, sex, degree of sexual maturity, size, region, water temperature, type of diet, and season, with fatty acids being the result of the balance between dietary consumption and biosynthesis (Castell et al. 2004; Ferreira et al. 2010; Sieiro et al. 2006).

There are five identified, commercial sea cucumber species in Portuguese Atlantic coast: *Holothuria arguinensis* (Domínguez-Godino et al. 2015; Siegenthaler et al. 2015), *Holothuria forskali* (Pratas et al. 2017), *Holothuria mammata* (Marquet et al. 2017), *Holothuria tubulosa* (Bertoncini et al. 2008), and *Parastichopus regalis*, (González-Wangüemert et al. 2014). However, there are few studies on their chemical composition, with only two works carried out in the north of Portugal (Pereira et al. 2013; Santos et al. 2016) and one in the south of the country (Roggatz et al. 2016). Despite the small number of studies, there is evidence for the potential and nutritional quality of these species. *H. arguinensis* and *H. forskali* are common holothurian species in the Mediterranean and north of the Atlantic and represent a significant part of the macrozoobenthic biomass. To the best of our knowledge, no relevant information has been published about the biochemical composition of *H. arguinensis* and *H. forskali* of southwestern Portuguese Atlantic coast.

In the last decades, there has been a world-wide intensification of catches, growth in price (130.00 euros/Kg/dried), and consumption of sea cucumbers (Maggi and González-Wangüemert 2015). This change in trade was mainly due to the significant economic development of China, which increased the purchasing power of its populations and provided a greater acquisition of luxury foods (Sicuro and Levine 2011). González-Wangüemert et al. (2018a) and Dereli and Aydin (2020) reported sea cucumber populations to be at risk of becoming extinct due to the intensity of illegal fishing in Portugal and Turkey, respectively. The production of sea cucumber in aquaculture is a mitigation alternative to supply natural stocks and supply Asian demand (Chen 2004). However, it has only recently become significant on the world stage. The Asian species *A. Japonicus* corresponds to 94% of aquaculture production, with the remaining 6% being represented by tropical species (Eriksson and Clarke 2015). The species from Mediterranean and temperate climates are not accounted in world aquaculture production, although there are studies that indicate the possibility and interest (Léonet et al. 2009; González-Wangüemert 2018a; Rakaj et al. 2018; Domínguez-Godino and González-Wangüemert 2019; Rakaj et al. 2019; Laguerre et al. 2020). In addition to the increased interest from the luxury food market, there is also an increase in scientific interest in the different classes of compounds that give sea cucumbers potential for the development of new food and nutraceutical products (Bordbar et al. 2011; Ferdouse 2004; Sicuro et al. 2012). Due to these factors, in recent years there has been a large number of studies carried out on the nutritional profile of various species of sea cucumber (Khotimchenko 2015; Künili and Çolakoğlu 2019; Li et al. 2019).

In this context, the main objective of this study was to obtain information about the main chemical compounds in the body tissue of the holothurian species *H. arguinensis* and *H. forskali* from the southwest region of mainland Portugal as a source of protein, ash, and fat and compare the contents in two seasons. *H. arguinensis* and *H. forskali* were also studied as a source of essential fatty acids. For this purpose, the lipid class, fatty acids composition, and lipid nutritional quality indexes (LNQI) were evaluated.

Material and methods

Sampling procedures

Fresh samples of sea cucumber were captured by scuba diving in coastal zone between Sesimbra and Sado Estuary (Setúbal, Portugal; 38°25′23.50"N; 9°0′45.06"W) (Figure 1) in two seasons, winter (January/2019) and summer (July/2019). A total of 126 animals were captured, *H. arguinensis* (summer = 30; winter = 32) and *H. forskal*i (summer = 34; winter = 30). After capture, to avoid excessive variability in measurements as a result of muscle contraction and evisceration, a standardized procedure was adopted, which consisted of placing individual animals in bags. The entire contents of each bag were weighed to avoid losses in the coelomic liquid (live weight, LW, ±0.01 g). The animals were placed on ice to measure length after reaching total muscle relaxation (total length, accuracy of ±0.1 mm). Body wet weight after dissection and removal of internal organs and coelomic fluid was measured (gutted body weight, GBW, ±0.01 g), and samples were cleaned under running water. After that, GBW was ground, correctly identified, and stored at −80°C until analysis. Samples were lyophilized in a Biobase device (BK-FD10P) for 72 hours.

Protein, ash, moisture, and carbohydrate

The moisture and ash contents were determined by drying at 105°C and combustion of dry samples for 16 hours at 500°C until constant weight, respectively (AOAC 2000). The protein level was quantified using a combustion analysis method with the FP-528 DSP LECO nitrogen analyzer (LECO, St. Joseph, MI, USA) calibrated with EDTA (Saint-Denis and Goupy 2004). Carbohydrates were calculated by the difference in the sum of the values of fat, ash, moisture, and protein content.

Lipid analyses

Crude fat was determined following the extraction Bligh and Dyer method (Bligh and Dyer 1959).

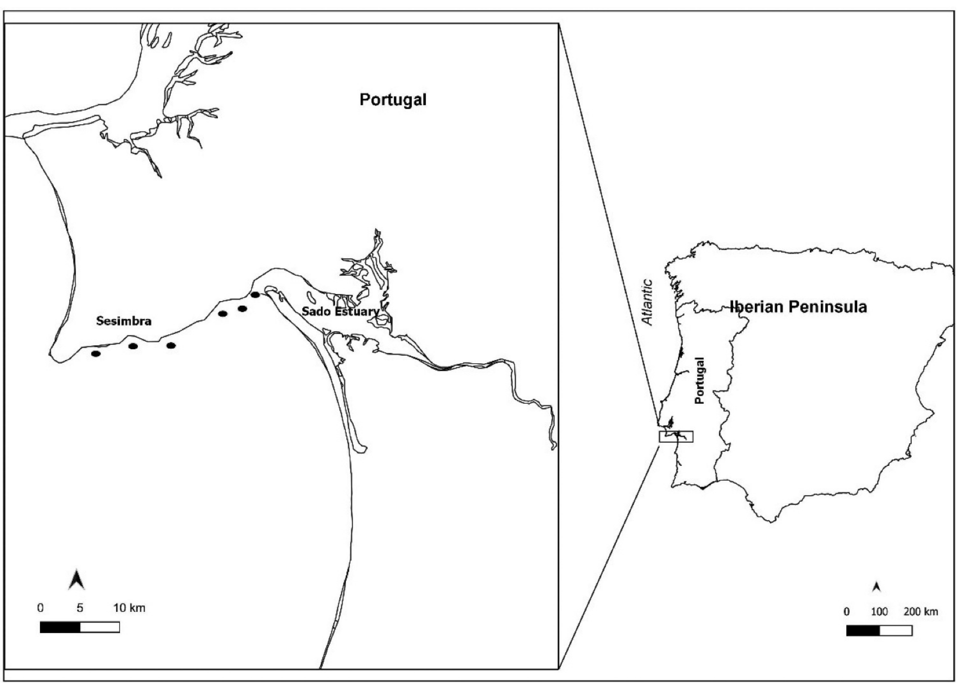

Figure 1. Map showing dots as sampling areas of sea cucumber on the southwest Atlantic Coast, Portugal.

Lipid class determination

Lipid class determination followed the methodology described by Costa et al. (2015). The relative weight of each lipid class was determined by analytical thin-layer chromatography (TLC). An eluent mixture of hexane/diethyl ether/acetic acid (65:35:1 by volume) and a plate coated with 0.25 mm silica gel G was used. Lipid class identification was done by comparison with standards (Sigma Chemical Co., St. Louis, MO, USA). The relative percentage of each lipid class was determined using a GS-800 densitometer and version 4.5.6 of Quantity One 1-D Analysis software from Bio-Rad (Hercules, CA, USA).

Separation of lipid class fractions

For fresh samples of *H. arguinensis* and *H. forskali*, triacylglycerol (TAG) and phospholipid (PL) classes were fractionated from the fat using a solid phase silica column (Isolute SPE column, UK). Firstly, TAG was separated using a mixture of hexane-diethyl ether (1:1), followed by PL separation, using methanol.

The different classes were fractionated using a preparative TLC. This involved applying 5 µl of a 10 mg/ml solution in several points of the TLC. The plate was placed in an elution vessel containing hexane:diethyl ether:acetic acid (65:35:1). After eluting, the plate was sprayed with 10% phosphomolybdic acid in ethanol and placed in the oven for 1 h to activate. Visualization was achieved in a UV chamber (CAMAG, UV cabinet II). Lipid fractions were identified using sigma standards – glyceryl-trioleate (TAG), glyceryl 1,3-dipalmitate (diacylglycerol, DAG), DL-a-monoolein (monoacylglycerol, MAG), and L-α-phosphatidylcholine (PL). To calculate the relative proportions of each lipid fraction present, a scanner (Densitometer GS800) and version 2.4 of the Quantity One program (NY, USA) were used.

Fatty acid composition

Fatty acid methyl esters (FAME) were analyzed by gas chromatography, using a CP 3800 Varian Star gas chromatograph (Walnut Creek, CA, USA), equipped with an auto-sampler and a split injector (100:1) and a flame ionization detector, both set at 250°C. The separation was carried out with helium (Air Liquide, Lisbon, Portugal) as carrier gas in a DB-WAX capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Agilent Technologies, Santa Clara, CA, USA) with a temperature program for the column starting at 180°C and increasing to 200°C at 4°C/min, holding for 10 min at 200°C, heating to 210°C at the same rate, and holding at this temperature for 14.5 min. FAME were identified by comparing their retention time with those of Sigma–Aldrich standards (PUFA-3, menhaden oil and PUFA-1, marine source from Supelco Analytical).

Lipids nutritional quality indexes (LNQI)

To assess the tendency of sea cucumbers to influence the incidence of coronary heart disease, the following indexes were used: atherogenicity index (AI), thrombogenicity index (TI), cholesterolemic index (h/H), PUFA/SFA, and n-3/n-6.

PUFA/SFA refers to the ratio between polyunsaturated and saturated fatty acids; n-3/n-6 was calculated as Σ n-6 PUFA/Σ n-3 PUFA. AI and TI indices were calculated using the equations of Ulbricht and Southgate (1991):

$$(\mathrm{AI}) = (12{:}0 + 4\,\mathrm{x}\,14{:}0 + 16{:}0) / (\mathrm{n}-6\,\mathrm{PUFA} + \mathrm{n}-3\,\mathrm{PUFA} + \mathrm{MUFA})$$

$$(\mathrm{TI}) = (14{:}0 + 16{:}0 + 18{:}0) / \big(0.5\,\mathrm{x}\,\mathrm{MUFA} + 0.5\,\mathrm{x}\,\mathrm{n}-6\,\mathrm{PUFA} + 3\,\mathrm{x}\,\mathrm{n} \\ -3\,\mathrm{PUFA} + \mathrm{n}-3\,\mathrm{PUFA} / \mathrm{n}-6\,\mathrm{PUFA}\big).$$

Cholesterolemic index (h/H) was calculated as per Santos-Silva et al. (2002):

$$(h/H) = (C18:1n-9 + C18:2n-6 + C18:3n-3 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:6n-3)/(C12:0 + C14:0 + C16:0).$$

Unsaturated fatty acids (UNS) were calculated by the sum of PUFA and MUFA. For all index calculations, the concentration of fatty acids was expressed in g/100 g and mg/100 g of total fatty acids. The values expressed in mg/100 g were calculated based on the conversion factors proposed by Weihrauch et al. (1977) (factor = 0.712).

Energy values determination

The energy value, expressed as kcal/100 g edible part, was estimated using FAO (1989) factors: 9.02 and 4.27 kcal/g for fat and protein, respectively

Statistics

All data were expressed as mean values ± SD. Analyses were performed in three replicates, and each one was measured for three repetitions. All data were analyzed using the STATISTICA v6 software (Statsoft, Inc., Tulsa, OK, USA). The normality and homoscedasticity were evaluated by Shapiro-Wilk test. When either assumption was met, all data (means of moisture, ash, protein, lipid content, lipid class, fatty acids, and LNQI) were examined by analysis of variance (two-way ANOVA) to verify whether there were differences among the analyzed seasons and species. The post hoc Tukey's test for multiple comparison was applied in case of a significant F ratio ($p < .05$). Multivariate statistical treatment of the whole set of data was performed after logarithmic transformation to reduce the variability of data.

Results

Biometric data

The results obtained for the average size and weight of *H. arguinensis* and *H. forskali* are shown in Table 1. Weight (LW and GBW) and size were significantly different ($p < .05$) between *H. arguinensis* and *H. forskali*. Considering the two seasons as a whole, *H. arguinensis* showed the largest mean total weight (LW: 499.5 g; GWB: 218.6 g) and the longest mean length (22.5 cm), compared to *H. forskali* (LW: 140.9 g; GWB: 80.1 g; length: 16.7 cm).

H. forskali showed no significant difference ($p > .05$) in weight (live and gutted) and a significant difference ($p < .05$) in size between seasons. Length was shorter in the summer and weight highest in the winter. *H. arguinensis* showed no difference in weight (live and gutted) and size between winter and summer ($p = .47$).

Values are presented as average ± SD. Means in the same line bearing different letters are significantly different ($p < .05$). LW: Live weight; GBW: gutted body weight.

Table 1. Average wet weight and length of *H. arguinensis* and *H. forskali* species from Portugal.

	H. arguinensis		H. forskali	
	Summer	Winter	Summer	Winter
LW (g)	484.3 ± 226.1^a	514.6 ± 190.2^a	149.7 ± 45.7^b	132.0 ± 28.0^b
GBW (g)	218.2 ± 107.9^a	218.9 ± 83.2^a	84.4 ± 20.8^b	75.8 ± 12.1^b
Length (cm)	21.7 ± 4.3^a	23.3 ± 5.2^a	15.5 ± 2.6^c	17.9 ± 3.1^b

Chemical composition

Table 2 shows the average values for the main constituents (g/100 g wet weight [ww] and dry weight [dw]) – moisture, fat, protein, and ash – and the energy values of the two investigated holothurian species in summer and winter. Significant differences in the main constituents were observed between species ($p < .05$). *H. arguinensis* showed higher mean values for protein (9.2 g/100 g), ash (5.3 g/100 g), and fat content (0.05 g/100 g) than *H. forskali* (8.1 g/100 g, 3.3 g/100 g, 0.01 g/100 g, respectively).

H. arguinensis did not show differences in moisture (p = .49), fat (p = .36), protein (p = .24), and ash (p = .45) between winter and summer, unlike *H. forskali* ($p < .05$) which did have differences between seasons.

Protein content varied between 7.8 g/100 g and 11.3 g/100 g for *H. arguinensis* and 6.3 g/100 g and 9.8 g/100 g for *H. forskali*. Both species also showed high levels of ash, between 3.3 g/100 g and 8.8 g/100 g for *H. arguinensis* and 2.9 g/100 g and 3.7 g/100 g for *H. forskali. H. arguinensis* and *H. forskali* are very low-fat species, with a fat content less than 2% (Ackman 1990). High average levels of moisture were found for *H. arguinensis*, around 87 g/100 g, and 90 g/100 g for *H. forskali*.

The energy values were low when compared with other marine organisms. In this case, the highest energy value was found for *H. arguinensis* in summer (44.7 kcal/100 g ww) and the lowest for *H. forskali* in winter (34.2 Kcal/100 g ww).

Mean ±SD. ww: wet weight; dw: dry weight. Different superscript letters in the same line represent statistical differences ($p < .05$) between species and seasons (ww).

Lipid class

The results obtained from the evaluated lipid classes, TAG, free fatty acids (FFA), sterols (cholesterol, CH) and PL of *H. arguinensis* and *H. forskali* are shown in Table 3. TAG is the largest lipid class of both sea cucumbers, showing differences between species and seasons ($p < .05$), with the highest value found for *H. arguinensis* in winter (49.8%). FFA also exhibited high values, with significant differences between species and seasons ($p < .05$) and showing highest values in the summer (*H. arguinensis* 35.8%; *H. forskali* 36.8%).

As regards cholesterol, a significant difference was found between species ($p < .05$). *H. arguinensis* showed no significant differences (p = .08) between winter and summer, unlike *H. forskali* ($p < .05$). PL was the least representative lipid class, with values below 15% and no differences (p = .27) between *H. arguinensis* and *H. forskali*. Differences ($p < .05$) in PL were found only between winter and summer for *H. arguinensis*.

TAG – Triacylglycerols; FFA – Free Fatty Acid; CH – Cholesterol; PL – Phospholipids. Means in the same line with different letters, for the same species correspond to statistical differences ($p < .05$) for each lipid class.

Fatty acid composition

The average of fat content was 3.5% (dw) for *H. arguinensis* and 1.1% (dw) for *H. forskali* (Table 2), with differences between species ($p < .05$). A wide variety of fatty acids were detected in the total lipids

Table 2. Chemical composition (mean ±SD. ww; dw) and energy values (kcal/100 g) of *H. arguinensis* and *H. forskali*.

	H. arguinensis (g/100 g)				*H. forskali* (g/100 g)			
	Summer (ww)	Winter (ww)	Summer (dw)	Winter (dw)	Summer (ww)	Winter (ww)	Summer (dw)	Winter (dw)
Moisture	87.2 ± 2.6^a	86.5 ± 1.5^a	-	-	90.8 ± 3.5^b	89.6 ± 0.2^c	-	-
Protein	9.5 ± 0,7^a	8.9 ± 1.0^a	74.3 ± 5.6	66.2 ± 7.5	8.3 ± 0.5^b	7.8 ± 1.1^c	90.7 ± 5.1	74.7 ± 10.9
Ash	5.4 ± 2.0^a	5.4 ± 2.1^a	42.0 ± 15.2	40.2 ± 15.4	2.9 ± 0.3^c	3.5 ± 0.2^b	31.4 ± 3.0	33.3 ± 1.5
Fat	0.5 ± 0.0^a	0.4 ± 0.0^a	3.9 ± 0.5	3.0 ± 0.3	0.1 ± 0.0^b	0.1 ± 0.0^b	1.1 ± 0.4	1.0 ± 0.5
Energy (Kcal/100 g)	44.7	41.6	352.4	309.7	36.3	34.2	397.2	327.9

Table 3. Relative percentage of each lipid class for *H. arguinensis* and *H. forskali* samples.

	H. arguinensis		*H. forskali*	
Lipid classes	Winter	Summer	Winter	Summer
TAG	49.8^{a}	33.5^{d}	44.2^{b}	42.2 ^{c}
FFA	23.8^{d}	35.8^{b}	31.8 ^{c}	36.8^{a}
CH	14.7^{a}	15.8^{a}	10.3^{b}	6.0 ^{c}
PL	11.7^{b}	14.9^{a}	13.7^{ab}	14.9^{a}

Table 4. Total fatty acid methyl esters (FAME) (dry weight) profile and also expressed as mg/100 g of total FAME (wet weight and dry weight), of the body wall of the sea cucumber *Holothuria arguinensis* and *Holothuria forskali.*

	H. arguinensis						*H. forskali*					
	Summer			Winter			Summer			Winter		
Fatty acids	Total FAME	mg/ 100 g (ww)	mg/ 100 g (dw)	Total FAME	mg/ 100 g (ww)	mg/ 100 g (dw)	Total FAME	mg/ 100 g (ww)	mg/ 100 g (dw)	Total FAME	mg/ 100 g (ww)	mg/ 100 g (dw)
14:0	1.1^{ac}	3.92	30.54	0.8^{a}	2.28	17.09	1.6^{b}	1.14	12.53	1.4^{bc}	1.00	9.97
16:0	3.0^{b}	10.68	83.30	2.9^{b}	8.26	61.94	3.1^{b}	2.21	24.28	4.3^{a}	3.06	30.62
18:0	5.5^{a}	19.58	152.72	4.8^{b}	13.67	102.53	5.1^{ab}	3.63	39.94	4.9^{b}	3.49	34.89
20:0	2.4^{a}	8.54	66.64	2.7^{a}	7.69	57.67	2.3^{a}	1.64	18.01	2.4^{a}	1.71	17.09
21:0	1.2^{b}	4.27	33.32	1.5^{ab}	4.27	32.04	1.9^{a}	1.35	14.88	1.8^{a}	1.28	12.82
22:0	1.1^{a}	3.92	30.54	1.4^{a}	3.99	29.90	1.6^{a}	1.14	12.53	1.6^{a}	1.14	11.39
Others*	6.2^{a}	22.07	172.16	6.2^{a}	17.66	132.43	6.2^{a}	4.41	48.56	6.0^{a}	4.27	42.72
ΣSFA	20.5^{b}	72.98	569.24	20.3^{b}	57.81	433.61	21.8^{a}	15.52	170.74	22.4^{a}	15.95	159.49
16:1	5.0^{a}	17.80	138.84	3.5^{b}	9.97	74.76	3.1^{b}	2.21	24.28	2.3 ^{c}	1.64	16.38
18:1 n-5	0.1^{a}	0.37	2.85	0.1^{a}	0.29	2.14	0.1^{a}	0.07	0.78	0.1^{a}	0.11	0.71
18:1 n-7	3.6a	13.17	102.68	3.4a	9.68	72.61	3.2a	2.28	25.06	2.5b	1.74	17.79
18:1 n-9	1.0^{d}	3.20	24.99	1.5^{b}	4.27	32.04	1.1 ^{c}	0.78	8.62	2.4^{a}	1.71	17.09
20:1	8.0 ^{c}	28.48	222.14	8.6^{b}	24.49	183.70	9.0^{ab}	6.41	70.49	9.5^{a}	6.76	67.64
22:1	2.4b	8.54	66.64	3.1a	8.83	66.22	2.8ab	1.99	21.93	2.5b	1.78	17.80
24:1	3.4 ^{c}	12.10	94.41	4.6^{a}	13.10	98.26	3.9^{b}	2.78	30.54	3.9^{b}	2.78	27.77
Others**	0.3^{a}	1.07	8.33	0.3^{a}	0.85	6.41	0.3^{a}	0.21	2.35	0.3^{a}	0.21	2.14
ΣMUFA	23.8^{b}	84.73	660.88	25.1^{a}	71.48	536.14	23.5^{b}	16.73	184.05	23.5^{b}	16.73	167.32
16:3 n-3	7.4^{a}	26.34	205.48	5.7^{b}	16.23	121.75	4.4^{d}	3.13	34.46	5.0 ^{c}	3.56	35.60
18:2 n-6	0.6^{b}	2.14	16.66	1.1^{a}	3.13	23.50	0.4^{b}	0.28	3.13	1.0^{ab}	0.71	7.12
18:3 n-3	0.4	1.42	11.11	0.9	2.56	19.22	0.2	0.14	1.57	0.5	0.36	3.56
20:2 n-6	1.5^{a}	5.34	41.65	1.7^{a}	4.84	36.31	1.3^{a}	0.93	10.18	1.4^{a}	1.00	9.97
20:4 n-6	17.8^{b}	63.37	494.27	17.9^{b}	50.98	382.34	16.4 ^{c}	11.68	128.44	18.9^{a}	13.46	134.57
20:5 n-3	14.0^{a}	49.84	388.75	10.2^{d}	29.05	217.87	13.5^{b}	9.61	105.73	10.7 ^{c}	7.62	76.18
22:5 n-3	0.3^{a}	1.07	8.33	0.3^{a}	0.85	6.41	0.3^{a}	0.21	2.35	0.2^{a}	0.14	1.42
22:4 n-6	5.4 ^{c}	19.22	149.95	7.2^{b}	20.51	153.79	9.1^{a}	6.48	71.27	8.6^{a}	6.12	61.23
22:5 n-6	1.1^{b}	3.92	30.54	1.2^{b}	3.42	25.63	2.2^{a}	1.57	17.23	2.0^{a}	1.42	14.24
22:6 n-3	1.3^{ab}	4.63	36.10	1.1^{b}	3.13	23.50	1.5^{a}	1.07	11.75	1.1^{b}	0.78	7.83
Others***	3.4^{b}	12.10	94.41	3.0^{b}	8.54	64.08	2.3^{b}	1.64	18.01	2.1^{b}	1.50	14.95
ΣPUFA	53.2^{a}	189.39	1477.26	50.3 ^{c}	143.25	1074.41	51.6^{b}	36.74	404.13	51.5^{b}	36.67	366.68
Fat	97.5^{a}	347.10	2707.38	95.6^{b}	272.27	2042.02	96.8^{a}	68.92	758.14	97.4^{a}	69.35	693.49

of sea cucumber during winter and summer, with a similar profile between *H. arguinensis* and *H. forskali* (Table 4). The fatty acid profile of *H. arguinensis* and *H. forskali* was dominated by PUFA, followed by monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA).

There was no difference ($p = .79$) in the average PUFA content between *H. arguinensis* (51.7%) and *H. forskali* (51.6%). However, there was a significant difference ($p < .05$) between summer and winter for *H. arguinensis*. Among PUFA of both species, arachidonic acid (ARA, 20:4 n-6) was the most abundant, followed by EPA (20:5 n-3) and hexadecatrienoic acid (16:3 n-3). A high EPA mean value was found for *H. arguinensis* (12.1%) and *H. forskali* (12.1%), similar between species ($p = .97$) but different between seasons ($p < .05$). In this study, the DHA was low, 1.2% for *H. arguinensis* and 1.3% for *H. forskali*, with no difference between species ($p = .54$). *H. arguinensis* showed no difference ($p = .25$) in DHA content between winter and summer, unlike *H. forskali*, which was different between seasons ($p < .05$).

The average amount of MUFA and SFA was similar, accounting for 24.5% and 20.4% in *H. arguinensis* and 23.5% and 22.1% in *H. forskali* respectively. Stearic acid (18:0) was the major SFA found in *H. arguinensis* and *H. forskali*, followed by palmitic acid (16:0) and arachidic acid (20:0). Total SFA showed differences ($p < .05$) between *H. arguinensis* and *H. forskali* but not between seasons (*H. arguinensis* $p = .42$; *H. forskali* $p = .41$). The two species exhibited variable MUFA content ($p < .05$); however, *H. forskali* showed no difference ($p = .98$) between seasons. Paullinic acid (20:1) was the most abundant MUFA in both species.

Only the most important fatty acids are listed. Results are mean ± SD (n = 3). Means in the same line with different letters correspond to statistical differences ($p < .05$) for each lipid class. SFA*12:0, 13:0, 14:0 isobr, 15:0 isobr, 15:0 anteiso, 17:0 isobr, 17:0, 19:0 isobr, 19:0, 23:0, 24:0. MUFA**17:1. PUFA***16:2 n-4, 16:3 n-4, 16:4 n-3, 18:3 n-6, 18:3 n-4, 18:4 n-3, 20:3 n-3, 20:4 n-3, 22:2 n-6, 21:5 n-3, 22:5 n-3.

Lipid nutritional quality indexes

The quantification of FAME expressed (mg/100 g) for *H. arguinensis* and *H. forskali* is exhibited in Table 4. A dose of 100 g of dry weight sea cucumber *H. arguinensis* provides 1477.3 mg of PUFA in the summer, and *H. forskali* provides 404.1 mg. In winter, the values decrease slightly for both species to 1074.4 mg and 366.7 mg, respectively. *H. arguinensis* supplies 660.9 mg/100 g (dw) of MUFA in summer and 536.1 mg/100 g (dw) in winter. *H. forskali* provides 184.1 mg/100 g (dw) in summer and 167.3 mg/100 g (dw) in winter. The availability of SFA is 569.2 mg/100 g (dw) in summer and 433.6 mg/100 g (dw) in winter. Meanwhile, *H. forskali* provides 170.7 mg/100 g (dw) in summer and 159.5 mg/100 g (dw) in winter.

The lipids nutritional quality indexes of *H. arguinensis* and *H. forskali* are shown in Table 5. In this study, the two species showed values of n-3 proportional to n-6; consequently, the n3/n6 ratio was close to 1. There were no statistical differences ($p = .86$) between n-3 PUFA from *H. arguinensis* (0.8) and *H. forskali* (0.7) during winter and summer, indicating a similar level.

Table 5. Nutritional quality indices in *H. arguinensis* and *H. forskali* species in summer and winter (mean values). Recommended Daily Portion (RDP)(g) of *H. arguinensis* and *H. forskali* for the recommended amount of EPA + DHA.

	Summer	Winter
Holothuria arguinensis		
Σ n − 3	25.1[a]	19.5[b]
Σ n − 6	27.2[b]	29.9[a]
n-3/n-6	0.9[a]	0.7[b]
PUFA/SFA	2.6[a]	2.6[a]
UNS/SFA	3.8[a]	3.9[a]
EPA+DHA	15.4[a]	11.6[b]
AI	0.10[a]	0.08[a]
TI	0.09[a]	0.10[a]
h/H	8.6[a]	8.9[a]
EPA+DHA Beneficial RDP (g) ww	460.2	862.8
EPA+DHA Beneficial RDP (g) dw	58.9	116.6
Holothuria forskali		
Σ n − 3	21.2[a]	18.6[b]
Σ n − 6	29.9[b]	32.3[a]
n-3/n-6	0.7[a]	0.6[a]
PUFA/SFA	2.4[a]	2.3[a]
UNS/SFA	3.5[a]	3.4[a]
EPA+DHA	15.2[a]	12.0[b]
AI	0.13[b]	0.13[b]
TI	0.11[a]	0.13[a]
h/H	7.11[a]	6.11[b]
EPA+DHA Beneficial RDP (g) ww	2305.4	2856.9
EPA+DHA Beneficial RDP (g) dw	212.1	297.6

The European Food Safety Authority (EFSA 2015) recommends a daily intake of 250 mg of EPA +DHA for adults. To obtain the recommended intake of EPA+DHA and based on the results obtained in this study, it is estimated that consumption of 58.9 g (dw) or 460.2 (ww) of *H. arguinensis* in summer and 112.1 g (dw) or 2305.4 (ww) of *H. forskali* is enough to attain the daily intake. The amount of n-6 and n-3 PUFA is lower in the winter compared with summer, increasing the estimated eating intake to 116.6 g (dw) or 862.8 (ww) of *H. arguinensis* and 297.6 g (dw) or 2856.9 (ww) of *H. forskali. H. arguinensis* and *H. forskali* showed significantly higher values for the PUFA/SFA ratio, 2.6 and 2.4, respectively. The UNS/SFA ratio had a high value for both *H. arguinensis* (3.9) and *H. forskali* (3.5). The thrombogenicity index and the calculated atherogenicity index showed similar levels for *H. forskali* and *H. arguinensis*: IT = 0.12 and 0.10; AI = 0.13 and 0.8, respectively. The h/H showed no significant difference for *H. arguinensis* between winter and summer ($p > .05$), in contrast to *H. forskali* ($p < .05$). The mean values are considered quite high for both species, 8.8 and 6.6, respectively.

Means with different letters (a, b, c) within each row differ significantly ($p < .05$). AI = Atherogenicity Index; TI = Thrombogenicity Index; h/H = Cholesterolemic Index. UNS = PUFA+MUFA

Discussion

Biometric data

A study carried out by González-Wangüemert et al. (2016) on *H. arguinensis* caught in summer in four different locations in southern Portugal showed lower length and weight (GBW) values than those found in this work (21.7 cm; 218.2 g) for three of these locations: Tavira (17.5 cm; 55.3 g), Praia de Faro (18.6 cm; 154.4 g), and Olhos d' Água (19.5 cm; 172.3 g), and higher only in Sagres (28.5 cm; 260.5 g). Similar results were found by Olaya-Restrepo et al. (2017) for the same region and season. The data from this work were similar to commercial species of sea cucumber in the Pacific and tropical waters (Laboy-Nieves and Conde 2006; Toral-Granda et al. 2008). The physiological performance of sea cucumbers and their growth is largely influenced by environmental conditions and food supply (Hamel and Mercier 1996; Sonnenholzner 2003). However, the length and weight can also be influenced by over-harvesting, where larger animals are mainly caught. In many places in southern Portugal, there is an excessive harvest of sea cucumber (González-Wangüemert et al. 2018a). This may be the cause of the biometric data of *H. arguinensis* in the south being less than the central region of the country and other commercial species.

Tuwo and Conand (1997) found a larger size (27 cm) for *H. forskali* collected in the North Atlantic and Mediterranean than the present study (16.7 cm), but the weight (wet weight) was similar. In general, the weight and length of *H. forskali* are below most of commercial sea cucumber species, except for *Stichopus chloronotus* (18 cm; 100 g), *Holothuria atra* (20 cm; 200 g) (Toral-Granda et al. 2008), and *Stichopus naso* (18 cm; 194 g) (Veronika et al. 2018).

Chemical composition

It is well known that the chemical composition of sea cucumbers varies according to the species, age, tissue, habitat, and season (González-Wangüemert et al. 2018b). In the present study, only the effect between species and season was evaluated. The high values of moisture found for *H. arguinensis* and *H. forskali* were already expected, as marine animals usually contain a high water content. Similar values were found for other sea cucumber species, including *Apostichopus japonicus* (formerly known as *Stichopus japonicus*) (91 g/100 g) (Lee et al. 2012), *Actinopyga mauritiana* (85 g/100 g), *Holothuria scarba* (86 g/100 g), *Bohadschia marmorata* (84 g/100 g), and *Holothuria leucospilota* (82 g/100 g) (Oedjoe 2017; Omran 2013).

According to Regulation (EC) No. 1924/2006, foods that have a protein content greater than 20% of the energy value are rich in protein. In this study, both species showed protein values above 80% of the energy value and are therefore a rich source of protein. *H. arguinensis* exceeded the average of 70 g/100 g (dw) and *H. forskali* 90 g/100 (dw). High values of protein content seem to be characteristic of holothurians. Similar results to this work were found for other species. For example, Wen et al. (2010) in a study of 8 commercial species of sea cucumber purchased from a local retail market in Guangzhou, China reported the protein content ranging from 40.7 (g/100 g) (dw) to 63.3 (g/100 g) (dw).

A large proportion (ca. 70%) of this protein is comprised of collagen that can be converted into gelatine by boiling and can act as a bioactive substance (Zhao et al. 2007). Due to the high amount of protein, sea cucumber products can be sold as a food supplement in the form of tablets or capsules (Chen 2003). The protein contents found for both species are very important when considering the quality of meat and the texture of aquatic organisms (Huda et al. 2010). One study carried out by Taboada et al. (2003) suggests that *H. forskali* is a source of high-quality protein, with beneficial effects on serum triglyceride levels. The average values of crude protein and total fat observed in this study for *H. arguinensis* were slightly higher than those found by Roggatz et al. (2016) for the same species in the south of Portugal.

The high ash content of sea cucumber is probably due to the presence of abundant microscopic elements of the skeleton, called ossicles, present in the body wall of the sea cucumber. Ossicles are formed inside cell vesicles and are composed mainly of calcium carbonate (Smiley 1994). *H. arguinensis* and *H. forskali* showed high ash content, and some minerals present in sea cucumbers can be found in greater quantities in those than for example in packaged milk (Fredianto et al. 2019).

Low total fat content found in this study for *H. arguinensis* and *H. forskali* are similar to those reported in the literature for commercial sea cucumber species from the Mediterranean (Mehmet et al. 2011), Atlantic (Zhong et al. 2007), and Asia (Anisuzzaman et al. 2019; Bechtel et al. 2013; Widianingsiha et al. 2015). Due to the low total fat content, the energy value is considerably lower when compared with 24 fish species available in the Portuguese market, which can vary from 60 Kcal/100 g for pink cusk-eel (*Genypterus blacodes*) to 262 Kcal/100 g for chum salmon (*Oncorynchus keta*) (Marques et al. 2019). *H. arguinensis* and *H. forskali* did not exceed 44.7 kcal/100 g and 34.2 Kcal/100 g, respectively.

Lipid class

Storage lipids were the largest portion of the total lipids of *H. arguinensis* and *H. forskali*, found mainly in TAG, followed by FFA, PC, and CH. Triacylglycerols are generally considered to be a storage product rather than membrane lipids (Sul et al. 2000). The higher TAG values in winter, show that this lipid class can constitute an energy reserve during a period of nutritional scarcity, contributing to the energy needs of the metabolism (Beninger 1984). The level of FFA was also high. In fact, while storage lipids averaged 41.7% and 43.2% for *H. arguinensis* and *H. forskali*, respectively, free lipids averaged 29.8% and 34.3% of the total content of lipids for the same species mentioned, respectively. The high levels found in this work for FFA could be due to the hydrolysis of TAG and PL, created by enzymatic reactions during storage and processing (Lu et al. 2008). Elevated FFA values were also found in echinodermata; for example, approximately 25% in sea urchins (Liyana-Pathirana et al. 2002). There are few studies on the class of lipids in sea cucumbers, and as far as we can tell, only in the work of Zhou et al. (2018) are FFA values indicated. The FFA value for *Apostichopus japonicus* is lower (below 6%) than the FFA value for the two species of sea cucumber in the present work. However, food resources can affect the lipid content of some Holothurians due to selective feeding and changes in food supply in the marine environment (Neto et al. 2006). There was no significant difference ($p > .05$) of TAG (41.7%, 39.50%), FFA (29.8%, 34.30%), and PL (13.3, 14.31), between *H. arguinensis* and *H. forskali*, respectively. However, TAG and FFA had significant differences between summer and winter ($p < .05$). Cholesterol and PL showed significant differences between seasons only for *H. forskali*.

Marine organisms contain different lipid classes that vary in the proportion of TAG, FFA, sterols (cholesterol), and PL. These lipids are composed of different fractions, which include a portion of fatty acids moiety (Cardoso et al. 2016a). Among these FA groups, n-3 PUFA represents a large portion, especially EPA and DHA (Médale et al. 2003). Total lipids and the amount of EPA and DHA varies in different body parts of sea cucumber, the largest amount being in the spawns, followed by the intestines and body wall (Zhou et al. 2018).

Fatty acid composition

Like other species of sea cucumber, *H. arguinensis* and *H. forskali* have great potential for aquaculture and are well recognized as a good food for human health (Anderson et al. 2011; Fredalina et al. 1999). As expected, lipid composition of *H. arguinensis* and *H. forskali* presented essential fatty acids such as EPA and DHA with health advantages. Previous studies suggest that some species of sea cucumber are capable of synthesizing EPA and DHA from either ALA or LA through elongation and desaturation (Li et al. 2016, 2017b). In the body wall of sea cucumbers, EPA and DHA can represent 10% to 20% of the total PUFA in the commercial species (Kasai 2003; Lou et al. 2012), which is similar to the values reported for some fish (Cardoso et al. 2016b). However, there are reported variations in fatty acid composition between different species of sea cucumber in different regions, which may be related to factors such as diet, environmental conditions, and processing methods (Nishanthan et al. 2018; Zhang et al. 2017).

The pattern found for *H. arguinensis* and *H. forskali* in which PUFA predominated, followed by MUFA and SFA, was also observed in low-fat fish (<4%), such as walleye pollock, smelt, canary rockfish, and pink salmon (Huynh and Kitts 2009) and fish from colder waters, since fish from warmer waters have a higher SFA content (Belling et al. 1997; Osman et al. 2001).

However, the fatty acid profile (PUFA, MUFA, SFA) of Holothuroidea is quite variable. Bilgin and Tanrikulu (2018) found higher proportions of PUFA (36%) and lower SFA (19%) for *H. tubulosa* from Aegean Sea (Turkey) caught in April. Aydın et al. (2011), in a profile work of FAs of *H. tubulosa, H. polii*, and *H. mammata* in the same region over one year, also found comparable proportions among the three holothurian species for SFA, MUFA, and PUFA, with PUFA as the dominant class. In Australia, *Australostichopus mollis* caught in June also showed higher values for PUFA (54%), followed by SFA (24%) and MUFA (23%) (Liu et al. 2017a). In the work of Biandolino et al. (2019b) with two species of sea cucumber from the Ionian Sea (southern Italy) caught in April, SFA, MUFA, and PUFA of *H. polii* made a similar contribution to the total of FA, with 35, 33, and 32%, respectively; whereas PUFA was the dominant group for *H. tubulosa* (38%). However, some sea cucumber species contain very low PUFA and relatively high SFA and MUFA values. For example, *H. fuscogilva* caught in April from Indonesia had higher amounts of SFA (59.50) and MUFA (32.19) but lower contents of PUFA (8.32) (Fawzya et al. 2015). Ridzwan et al. (2014) analyzed the lipid content of four species of Malaysian sea cucumber, with *H. scabra, H. leucospilota*, and *H. atra* showing higher SFA values, followed by PUFA and MUFA. Only *S. horrens* presented PUFA with higher values; however, capture season of sea cucumber is not indicated.

ARA was the most commonly found fatty acid in *H. arguinensis* and *H. forskali*, similar to most other sea cucumber species. In the study by Svetashev et al. (1991) of the lipid composition of sea cucumber species captured in tropical and temperate waters, all 12 species showed high amounts of ARA. However, the quantity was considerably higher for tropical species than for temperate species. Although ARA is the most representativen-6 PUFA, studies show that the two most important PUFA are the n-3 EPA and DHA, which are responsible for anti-inflammatory properties, thus compensating for proinflammatory effects of n-6 PUFAs (Calder 2010, 2015). The presence of EPA and DHA in several species of sea cucumber are associated with a reduced incidence of coronary heart disease and some types of cancer (Harper and Jacobson 2005; Roynette et al. 2004). EPA and DHA are considered fundamental FA, as they cannot be biosynthesized by humans to a level of efficiency sufficient to meet adequate needs (Arts et al. 2001). The importance of EPA and DHA present in seafood is evident, having a strong relationship between food intake of DHA and EPA and the increase in calcium absorption with a consequent increase in bone density (Kruger and Schollum 2005). DHA is selectively concentrated in the synaptic and retinal membranes and is believed to be related to visual function, brain development, behavior, and learning, with some studies showing that the concentration of DHA in pregnant women is related to the visual properties and cognitive development of children (Ahmmed et al. 2019 ; Dyall and Michael-Titus 2008).

Stearic acid (18:0) was the major SFA of *H. arguinensis* and *H. forskali*, followed by palmitic acid (16: 0) and arachidic acid (20: 0). Aro et al. (1997) verified that in humans, a diet containing 18:0 appears to be less harmful than diets containing trans-fatty acids. Apparently, and unlike other SFA, 18:0 does not increase levels of total cholesterol in the blood and low-density lipoprotein (LDL). On the contrary, 18:0 reduced LDL and TAG did not affect the LDH/HDL ratio and increased HDL cholesterol. Among SFA, 16:0 is the most common fatty acid found in the human body and can be synthesized endogenously or provided in the diet. Under normal conditions, there is a defined concentration and strict homeostatic control of 16:0 in the tissues, since changes in intake do not significantly influence tissue concentration (Song et al. 2017). Its strict control seems to be related to its essential role in several biological functions. The disruption of the homeostatic balance is usually related to uncontrolled endogenous biosynthesis due to different pathophysiological conditions, such as atherosclerosis, neurodegenerative diseases, and cancer regardless of their food contribution (Carta et al. 2017). Contrary to what usually occurs in fish, where the percentage of 20:0 is usually below 1% (Sargent and Whittle 1981), in sea cucumbers the percentage of these SFA was higher.

The relationship between monounsaturated acid intake and cancer risk shows that MUFA can play a potential role in reducing the risk of various types of cancer (Zamora-Ardoy et al. 2004). The most representative MUFA was eicosenoic acid (20:1), followed by hexadecenoic acid (16:1) and vaccenic acid (18:1 n7). In accordance with this study, Pereira et al. (2013) also reported 20:1 as dominant MUFA of *H. forskali* caught in September from Peniche, Portugal. On the other hand, Santos et al. (2016) reported 18:1 as dominant MUFAs for the same species and location, with animals captured over 10 months. This difference is probably related to changes in the season that influence feed availability, because their exogenous origin is recognized (Ackman 1982). Roggatz et al. (2016) report 18:1 as dominant MUFAs for *H. arguinensis* from Northeastern Atlantic and southern Portugal, similar to that found for the same species in this work.

Lipid nutritional quality indexes

One dose (100 g) of *H. arguinensis* and *H. forskali* provides a greater amount (mg/100 g) of PUFA, MUFA, and SFA in summer compared to winter. The greater availability of PUFA, MUFA, and SFA in the summer may be related both to environmental factors and to the trophic ecology of *H. arguinensis* and *H. forskali*, since they feed mostly from the sediment (Slater et al. 2011; Yokoyama 2013). The low rainfall in the summer favors the accumulation of organic matter in the sediment and also because animals live along the coast, where there is significant influence of continental waters in the marine environment (Zipperle and Reise 2005). This is in contrast to winter, when there is an even greater amount of continental water that removes and agitates the sediment (Cave and Henry 2011; Encarnação et al. 2013; Moore 1999).

Regarding LNQI, the ratio of n-3/n-6 fatty acids is an index for comparing interspecies nutritional quality (Piggott and Tucker 1990). The high content of n-3 and n-6 PUFA is favorable to human health (Simopoulos 2002). In this study, as mentioned above, the two species presented values of n-3 proportional to n-6; and the n-3/n-6 ratio was near to 1. *H. arguinensis* and *H. forskali* provide similar levels of n-3 FA (0.8 and 0.7, respectively), and these values are in the range suggested by Simopoulos (2009) as an ideal standard of food intake (0.25–1.0). A more balanced proportion of n-3/n-6 is recommended for the prevention of cardiovascular disease (CVD). The Food and Agriculture Organization and the World Health Organization recommends a daily ratio of n-3/n-6 in the diet of 1:4 (FAO/WHO 2003). In this study, *H. arguinensis* and *H. forskali* showed an n-3/n-6 ratio better than the values recommended above. Similar value was found by Künili and Çolakoğlu (2019) for *H. tubulosa* (ratio n-3/n-6 = 0.9). These values indicate that these species of holothurian can be a good contribution to a balanced diet. There are studies that report a smaller proportion of the n-3/n-6 ratio. Haider et al. (2015) analyzed the n-3/n-6 ratio of two species collected in Pakistan at the same time.

They found higher values for *Holothuria arenicola* (0.6) than for *Actinopyga mauritiana* (0.2). These results may suggest that the difference between species better explains the n-3/n-6 ratio than the geographical area and substrate for sea cucumbers. Santos et al. (2016) reported a slightly lower proportion (0.5) of n-3/n-6 for *H. forskali* caught north of Portugal than that found in the present study. Roggatz et al. (2016) reported 0.4 for *H. arguinensis*, and Wen et al. (2010) determined proportions for several species of Chinese sea cucumber (*Stichopus herrmanni*: 0.3; *Thelenota ananas*: 0.6; *Thelenota anax*: 0.6; *Holothuria fuscogilva*: 0.4; *Holothuria fuscopunctata*: 0.3; *Actinopyga mauritiana*: 0.4; *Actinopyga caerulea*: 0.5; *Bohadschia argus*: 0.3). EPA and DHA are the most important nutritional components of n-3 PUFA, with seafood being the only significant source of intake. Although *H. arguinensis* and *H. forskali* have low DHA values, the sum of EPA+DHA showed good value for health benefits.

There is no study, as far as we could ascertain, that indicates the amount (g) of *H. arguinensis* and *H. forskali* recommended to satisfy the daily intake of EPA+DHA. However, values indicated for other species were higher than those found for the two species in the present study. Biandolino et al. (2019b) found that the required daily intake of *Holothuria tubulosa* is 578 g and *Holothuria polli* is 445 g, also based on the recommendation of the European Food Safety Authority (EFSA, 2015).

Foods that contain a PUFA/SFA ratio less than 0.45 (recommended minimum) are considered undesirable for human health, as they can promote an increase in cholesterol (HMSO 1994). *H. arguinensis* and *H. forskali* showed significantly higher values for the PUFA/SFA ratio, 2.6 and 2.4, respectively.

The UNS/SFA ratio was calculated, since the PUFA/SFA index does not take into account important metabolic changes in MUFAs. In fact, the UNS/SFA ratio also had a high value for both *H. arguinensis* (3.9) and *H. forskali* (3.5), being above 70% of the total FAs.

AI, TI, and h/H are commonly used to assess the nutritional value of the lipid fraction and its potential effect on the development of coronary heart disease (Ulbricht and Southgate 1991). Thrombogenicity index shows the tendency to form clots in blood vessels. This is defined as the relationship between pro-thrombogenetic (SFA) and anti-thrombogenetic fatty acids (MUFA, PUFA n-6, and PUFA n-3) (Garaffo et al. 2011; Senso et al. 2007). The atherogenicity index indicates the relationship between the sum of the main SFA and that of the main groups of unsaturated FA, the first being considered pro-atherogenic (favoring the adhesion of lipids to the cells of the immune and circulatory system) and the last anti-atherogenic (inhibiting the aggregation of plaque and decreasing the levels of esterified fatty acids, cholesterol, and phospholipids, preventing the appearance of micro and macrocoronary diseases) (Garaffo et al. 2011; Senso et al. 2007). High values of TI and AI indicate high atherogenic and thrombogenic fatty acids and are considered important underlying risk factors for coronary heart disease. Therefore, the lower the AI and TI values, the healthier the food. On the other hand, lower h/H values are harmful to human health. The values found for AI and TI for *H. arguinensis* (TI = 0.10; AI = 0.8) and *H. forskali* (TI = 0.12; AI = 0.13) were similar between species. These values are below those found for some species of brackish and fresh water fishes, including *Amblygaster clupeiodies* (AI = 1.88), *Siganus lineatus* (AI = 2.68), *Stoleophorus commensoni* (AI = 2.33), *Etroplus maculatus* (TI = 1.42), *Glossogobius giuris* (TI = 2.08), *Stoleophorus indicus* (TI = 1.66), and *Stoleophorus commensoni* (TI = 1.86) (Devadawson et al. 2016), and indicate potential for stimulating platelet aggregation. The average of h/H was considered quite high for both species, 8.8 and 6.6, respectively. Thus, the low values of AI and TI and the high value of h/H show the protective potential for coronary artery disease of *H. arguinensis* and *H. forskali*.

Conclusions

To our knowledge, this study was the first paper on the chemical composition and fatty acid profile of two important commercial species of sea cucumber, *H. arguinensis* and *H. forskali* in southwest Portugal. Data show that sea cucumber species contained essential nutritional components for human health, with an adequate content of protein and a high level of ash but low content of total lipids and carbohydrates. Moreover, the fatty acid profile showed a higher level of PUFA in both species, compared to MUFA and SFA. Storage lipids were the largest portion of total lipids, found mainly in TAG, FFA, PC, and CH, respectively.

The results also demonstrate that the FA composition of *H. arguinensis* and *H. forskali* are a good source of essential lipids. There were significant differences between species but not between seasons. It appears that there is no published data on AI, IT, and h/H of *H. arguinensis* and *H. forskali*, so the data presented in this study will be the basis for future research in this field. The nutritional value of these two species of sea cucumber can be useful as nutrients for increasing consumer's health or for the development of functional foods. The information of the present work can be important for nutritionists, health professionals, and the food and nutraceutical sector industry.

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