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Food Quality and Safety of Mediterranean Sea Cucumbers *Holothuria tubulosa* and *Holothuria polii* in Southern Adriatic Sea

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ABSTRACT

The aim of this paper was to determine the food quality and safety of two Mediterranean sea cucumbers species, *Holothuria tubulosa* (HT) and *Holothuria polii* (HP) collected in the Italian Southern Adriatic Sea. Three hundred samples were collected in the spring of the 2008 and some of them were analyzed for their proximate composition, fatty acid composition, amino acid composition and heavy metals content. Proximate composition was similar in both species: HT: dry matter 16.19%; crude protein 44.58%; ash 46.43 %; HP: dry matter 22.03%; crude protein 36.99%; ash 48.22%. Lipid content resulted very low in both species and an interesting content of polyunsaturated fatty acids (PUFA) was found. Among PUFA, eicosapentaenoic acid (EPA) and eicosatrienoic acid were present in high percentages in both species. Considering the total amino acids composition, glutamic acid, glycine and aspartic acid were the most abundant amino acids. In terms of food safety, the heavy metals concentrations in the analyzed species were under the limits for the Italian legislation. These results confirmed that the HT and HP could be considered as a suitable seafood for human consumption.

Key words: *Holothuria tubulosa*, *Holothuria polii*, seafood, heavy metals

INTRODUCTION

Sea Cucumbers (SC) are commercialized and worldwide known as "beche-de-mer" ("trepang" o "haisom") (Conand, 1990) and their importance in terms of trade has been extensively reported in several FAO reports during last decade (Lovatelli *et al.*, 2004; Toral-Granda *et al.*, 2008; Purcell *et al.*, 2010). SC are considered an important seafood in the Indo-Pacific region, including Philippines, Malaysia, Japan, Korea and China that is the world largest sea cucumber producer (Chen, 2004). In the northern hemisphere SC fishery is mainly developed in four countries: Canada, United States of America, Russian Federation and Iceland (Hamel and Mercier, 2008a) where *Cucumaria frondosa* represents the most extensively fished and farmed SC species (Hamel and Mercier, 2008b). In the Mediterranean area SC are almost completely unexploited natural resource except in Turkey where the species *Holothuria tubulosa*, *H. sanctori*, *H. polii* and *H. mammata* are usually consumed (Cakly *et al.*, 2004) and in Southern Spain where the consumption of royal SC (*Stichopus regalis*) is considered a delicacy (Ramon *et al.*, 2010). In this moment Mediterranean SC not considered as potential seafood by Italian consumers, therefore the aim of this paper was to investigate the food quality and safety of the Mediterranean sea cucumbers, *H. tubulosa* and *H. polii*, as preliminary step for the development of seafood market for Italian fishermen and aquaculture producers.

MATERIALS AND METHODS

Sample collection: Three hundred SC (150 for each species) were collected from an area of the Southern Adriatic Sea in the April 2008 in Italy. Upon the arrival in the laboratory, a part of the SC samples were cleaned, gutted and dried in a draft oven at 70°C for 24 h, whereas the remaining samples were frozen for the glycosaminoglycan analysis.

Proximate composition and glycosaminoglycans determination: A sub-sample of 5 animals per species was used to determine their proximate composition according to standard methods (AOAC, 1995). The gross energy content (GE) was determined using an adiabatic calorimetric bomb (IKA C7000, Staufen, Germany). Total nitrogen content was determined using a nitrogen analyzer (Rapid N III, Elementar Analysensysteme GmbH, Germany) according to the Dumas method and the crude protein was calculated as total N×6.25. For the glycosaminoglycans (GAGs) determination, tissues were homogenized, digested by papain and deproteinized with trichloroacetic acid; GAGs were precipitated by adding 4 volumes of cold ethanol, lyophilized and dissolved in distilled water (Cappelletti *et al.*, 1980). GAGs were quantified by the carbazole method using glucuronolactone as standard (Bitter and Muir, 1962) and their concentration was expressed as glucuronic acid per g of wet tissue.

Fatty acid (FA) composition: Lipid sample extraction was carried out using a chloroform: methanol (2:1, v/v) mixture following the method of Folch *et al.* (1957). The total lipid content was determined gravimetrically. After evaporation to dryness, the lipid extract fraction was transesterified to methyl esters in boron trifluoride catalyzed by a methanol: benzene solution (1: 2 v/v) at 90°C for 20 min. When the methylation was completed, 2 mL of distilled water was added to the mixture. FA methyl esters were analyzed by gas-liquid chromatography using an HP 6890 series gas chromatograph (Hewlett Packard, USA) equipped with an Omegawax 250 capillary column (Supelco, USA). The column temperature program was as follows: from 150 to 250°C at 4°C/min, then maintained at 250°C. Helium was used as the carrier gas at a flow of 1 mL min^{-1}. Methyl esters of FAs were identified by the FAME mix (Supelco) as the standard and the results are reported as average percentages of total identified methyl ester FAs. Lipid analyses were carried out in triplicate and results are expressed as average percentages of the Dry Weight (DW) of the animals.

Amino acid composition: The total amino acid composition was performed by an amino acid analyser via acid hydrolysis using a Beckman System Gold HPLC system, according to the procedure described by Cavallarin *et al.* (2005).

Heavy metals determination: Concentrations of eight heavy metals (As, Cd, Cr, Cu, Fe, Se, Zn, Pb) were determined on five pooled samples (two kg each) of each species. The determination of the heavy metals has been measured with atomic absorption spectrophotometry with background correction by Zeeman effect. Values were expressed as mg per kg of wet tissue.

RESULTS

The SC body composition is similar in the considered species (Table 1) and the main features are the high quantity of protein, very low fat content and relevant quantity of ash, this latter due to calcareous endoskeleton, typical of echinoderms. SC skeleton is reduced to microscopic ossicles embedded beneath the skin. In order to separate the calcareous part, the SC meal obtained was sifted and successively ash content was measured on sifted meal, the ash content was similar. This fact indicates that these ossicles are not mechanically separable. After proximate analyses, glycosaminoglycans (GAGs) content was also analyzed considering their potential effect in preventing inflammation in human, higher GAGs content was found in *H. polii* 4.32±0.3 mg kg^{-1}, while in *H. tubulosa* were 2.88±0.1 mg kg^{-1}. Lipid content in SC meals is very low (Table 1) and polyunsaturated fatty acids (PUFA) represent the highest percentage. Among PUFA, eicosapentaenoic acid (EPA) and eicosatrienoic acid are more frequent in both species, while among monounsatured fatty acid; palmitoleic acids represent the largest quantity, stearic and palmitic are the most representative saturated fatty acids (Table 2). Total amino acid composition was

Table 1: Proximate composition and glycosaminoglycans (GAG$_s$) content of sea cucumbers

Item	*H. tubulosa*	*H. polii*
Dry matter (DM) (%)	16.19±1.51	22.03±3.07
Crude protein (% DM)	44.58±1.01	36.99±0.62
Crude lipid (% DM)	0.71±0.12	0.55±0.12
Ash (% DM)	46.43±0.51	48.22±1.09
Gross Energy (MJ kg^{-1} DM)	9.70±0.21	8.10±0.20
GAG$_s$ (mg kg^{-1})	2.88±0.10	4.32±0.30

Values are as Mean±SD of DM (n = 3)

Table 2: Fatty acid composition of sea cucumbers

Item	*H. tubulosa*	*H. polii*
C14:0	3.14±1.00	3.74±0.10
C15:0	0.49±0.05	0.67±0.14
C16:0	8.96±1.92	10.24±0.22
C16:1	9.44±2.85	9.82±1.13
C17:0	0.96±0.21	1.04±0.01
C17:1	2.20±1.08	1.72±1.67
C18:0	9.28±1.63	9.90±0.89
C18:1	2.86 ±0.14	3.47±0.53
C18:2 n6	1.27±0.17	1.35±0.18
C18:3 n6	2.47±0.94	2.74±0.79
C18:3 n3	0.54±0.02	0.64±0.05
C20:0	3.02 ±0.89	3.29±0.14
C20:1 n9	2.52±0.66	2.71±0.13
C20:2	1.38±0.13	1.37±0.34
C20:3 n6	2.99±0.24	3.22±0.02
C20:3 n3	20.55±4.22	18.09±1.76
C20:5 n3	15.43±3.85	12.94±0.48
C22:0	2.24±0.43	2.45±0.30
C22:1 n9	1.76±0.68	1.50±1.32
C23:0	1.50±1.35	2.75±1.35
C22:6 n3	5.43±0.74	5.31±0.91
C24:1 n9	2.20±0.42	2.77±0.56

Values are as Mean±SD of dry weight (n = 3)

Table 3: Amino acid composition (% on the total) of sea cucumbers

Item	*H. tubulosa*	*H. polii*
Aspartic acid	5.60±0.00	4.49±0.02
Threonine	2.74±0.28	1.87±0.13
Serine	1.81±0.13	1.46±0.08
Glutamic acid	8.03±0.34	6.18±0.38
Glycine	10.36±2.72	7.42±0.19
Alanine	4.69±0.41	3.71±0.23
Cysteine	0.22±0.06	0.46±0.06
Valine	1.23±0.12	1.37±0.21
Methionine	0.62±0.04	0.62±0.06
Isoleucine	0.88±0.03	0.72±0.03
Leucine	1.55±0.03	1.37±0.16
Tyrosine	0.63±0.14	1.03±0.44
Phenylalanine	0.48±0.34	0.72±0.12
Histidine	0.82±0.12	0.88±0.11
Lysine	1.11±0.15	0.77±0.07
Arginine	4.14±0.13	3.58±0.21
Proline	4.53±1.09	4.90±1.33
EAA	11.90±1.11	13.60±1.38
NEAA	29.88±3.19	36.68±5.54
EAA/NEAA	0.40	0.37

Values are as Mean±SD on DM (n= 3), EAA: Essential amino acids, NEAA: Nonessential amino acids

Table 4: Heavy metals (mg kg^{-1} DM) content of sea cucumbers

Item	*H. tubulosa*	*H. polii*
Arsenic (As)	22.35±0.08	33.30±0.85
Cadmium (Cd)	0.07±0.00	0.07±0.00
Chromium (Cr)	0.86±0.04	0.88±0.59
Copper (Cu)	2.50±0.14	2.50±0.14
Iron (Fe)	24.50±0.57	19.40±0.28
Lead (Pb)	1.16±0.04	0.65±0.03
Selenium (Se)	4.18±0.16	4.24±0.04
Zinc (Zn)	17.40 ± 0.42	14.90±0.57

Values are as Mean±SD of DM (n = 3), DM: Dry matter

investigated and shown in Table 3. Glutamic acid, glycine and aspartic acid are dominant amino acids, similarly to as reported by Zhong *et al.* (2007) in *C. frondosa* and by Chen (2004) in *H. insignis*. Considering that these animals are filter feeders and especially that they are possible candidates for human consumption, the heavy metals content was investigated in order to prevent any possible negative effect (Table 4). The level of heavy metals resulted very low, under the limit for Italian legislation Italian limits (Reg. CE n. 1881/2006) and there were a differences on As content, that was higher in *H. polii* and Fe content that was higher on *H. tubulosa*.

DISCUSSION

The most common SC species in Mediterranean Sea are *H. tubulosa*, *H. forskalii* and *H. polii* (Ocana and Tocino, 2005; Shakouri *et al.*, 2009) which are considered edible in Turkey

(Cakly *et al*., 2004) or considered as potential sources of pharmacological compounds (Ismail *et al*., 2008; Mokhlesi *et al*., 2012). European consumption of SC regards mainly high quality dried and prepared seafood which are popular among the Chinese community that is a potential target for SC production. Moreover the SC consumption is also increasing in Europe and in particular in Catalonia were royal sea cucumber (*S. regalis*) is the most expensive seafood product and it can reach up to 130 € kg^{-1} (Ramon *et al*., 2010). The SC consumption in China is traditionally related to its high protein and low fat content, similarly Mediterranean SC considered in this research shows high protein content and very low lipid content, comparable with tropical ones (Zhong *et al*., 2007) while water content is interestingly lower respect to the Asian species *Apostichopus japonichus* (Dong *et al*., 2006). Our data on proximate body composition confirmed those previously found in other Mediterranean countries (Cakly *et al*., 2004). These characteristics confirmed their suitability for human consumption also for *H. polii* which is here investigated for the first time. *H. polii* is externally similar to *H. tubulosa*, the species are recognizable only by specific external and anatomic characteristics, consequently the future harvesting and farming will consider both species. In the Southern Italy some cases of consumption of SC are reported until the second world war and the local fishermen were not able to distinguish these two species. For these reasons an investigation on *H. polii* was also considered essential for this study. The main difference found in these species is the ash content, that it is considerably higher than the Pacific species of edible SC (*C. frondosa*) as reported by Zhong *et al*. (2007), where ash is about 30% on dry matter. However this higher ash content has not adverse effects on human consumption as *H. tubulosa* is already commonly eaten. Even considering their low fat content, less than 1%, FA composition was investigated. SC fatty acid composition resulted very interesting and its contents of n3 PUFA, in particular EPA is 5-10 times higher than blue mussels (Prato *et al*., 2010) even if EPA values are much lower respect other studies carried out in Pacific SC (*C. frondosa*) where EPA content was almost 50% of total FA (Zhong *et al*., 2007). In Malesian species (*S. chloronotus*) the major FA are palmitic acid, stearic acid and EPA (Fredalina *et al*., 1999). Palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1) and timnodonic acid (C20:5) are also found in blue mussels (Fuentes *et al*., 2009) and in some abyssal echinoderms. In general, the bottom sediment feeder organisms, as SC, should contain high levels of branched chain FA for their potential role in wound healing (Prato *et al*., 2010) and EPA could be associated with the ability to initiate tissue repair since EPA is involved in prostaglandin inhibition. In *H. tubulosa* and *H. polii* there were no trace of arachidonic acid that is major FA in almost all the tropical species except *E. godeffroyi* where EPA was the main one. It is probable that arachidonic acid in *H. tubulosa* and *H. polii* is only a precursor in the synthesis of EPA which occurs at a high level. The lower presence of EPA in *H. tubulosa* and *H. polii* respect *C. frondosa* could be related also to the period of study, essential FA content increases typically during reproductive period in coincidence to ovarian maturation period. This fact should be further investigated, considering that the effect of seasonal change in SC body composition has been already demonstrated (Dong *et al*., 2006). Body chemical composition of sessile animals strictly depends on the food resources available and therefore on the season of harvest (Orban *et al*., 2002). Proximate composition of other echinoderms is influenced by breeding or collection sites (Fuentes *et al*., 2009) and by seasons (Orban *et al*., 2002). Considering their effect on quality of final product, amino acid composition was investigated in these species. Amino acids are not only protein constituents but in the free form, give rise to sweetness, bitterness, sourness and umami taste. In particular glutamic acid is responsible for the distinctive flavour and taste of many foods. Thus, the composition of free amino acids can significantly influence the quality of marine foods. Zhong *et al.* (2007) already reported the importance of free amino acids in *C. frondosa*, the presence of high quantity of glutamic acid in *H. tubulosa* and *H. polii* could have a positive effect on taste of final product. The major edible part of SC is the body wall mainly consisting of collagen and glycosaminoglycan (GAGs) (Cui *et al.*, 2007). Several GAGs isolated from the body wall of sea cucumber have appeared as a potentially useful for pharmacological applications (Wu *et al.*, 2010) and the GAGs observed in *H. tubulosa* and *H. polii* are interesting and in high quantity in comparison with other marine invertebrates coming from same area. As SC can live from 4 to 8 years, they can accumulate contaminants eventually present in the sediments where they live, consequently heavy metals content has been investigated in other studies (Iogoren-Emiroglu and Gunay, 2007; Warnau *et al.*, 1998, 2006). Moreover, it is very well known that eating fish and seafood is one of the major potential sources of heavy metal (Etesin and Benson, 2007) and in particular mercury (Naqvi *et al.*, 2003; Khaniki *et al.*, 2005). In the present study the heavy metals content in SC was low and it under limits of Italian legislation for bivalves mollusks (Reg. No 1881/2006 UC, 19/12/2006), as previously reported in *H. polii* in the same area (Storelli *et al.*, 2000, 2001). Contaminants were measured in SC body wall but it is known that many metals are selectively distributed among different body compartments in *P. lividus* and their concentrations are influenced by season (Warnau *et al.*, 2006). SC are also considered good sources of biological compounds (Ridzwan *et al.*, 2001, 2003; Mokhlesi *et al.*, 2012) and in particular antioxidant. Antioxidant analyses showed that *H. tubulosa* and *H. polii* were differently from other studies who found a relevant amount of antioxidants in *C. frondosa* (Mamelona and Pelletier, 2010). First result obtained in this study is that SC present in the area of study are suitable for human consumption and in particular *H. tubulosa* is potential candidate for future harvesting and farming. Even if the presented results consider a single sampling period they confirmed that these species represent an unexploited marine resource useful for human consumption, exportation and future farming along Italian coasts.

CONCLUSION

The results of this study confirm that the Mediterranean sea cucumbers, *H. tubulosa* and *H. polii*, are good source of nutrients with a low content of contaminants and therefore these seafood could developed a niche seafood market for Italian fishermen and aquaculture producers has already happened in other Mediterranean countries.

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