

# Proximate composition and *in vivo* digestibility of the integument of *Parastichopus regalis* (Cuvier, 1817) collected from the Mostaganem area in the western Mediterranean Sea

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## Abstract

The exploitation of sea cucumbers in Algeria began a few years ago but only targets species of the genus *Holothuria*. Another species belonging to the order Synallactida (*Parastichopus regalis*) with important nutritional and economic value is also present along the Algerian coast. This species is caught in depths of over 50 m by professional fishers as bycatch but it is not consumed or sold. Studies devoted to this species are few. The aim of this study was to evaluate the quality of this species' tegument through the evaluation of moisture, lipid and ash contents as well as its digestibility by the gastric enzyme pepsin. Forty-two individuals of *P. regalis* were collected in four stations of the Mostaganem region. The integument of *P. regalis* individuals were freeze-dried to determine the water content, lipid content, ash content and its *in vitro* digestibility by pepsin. The results obtained showed a high-water content ( $90.98 \pm 0.71$  %), a low lipid content ( $1.40 \pm 0.57$ %), an ash content of  $40.45 \pm 0.54$ % and a good digestibility by pepsin ( $46.96 \pm 3.08$ %). These results were compared and discussed with those found in the literature.

**Keywords:** Sea cucumber, integument, approximate composition pepsin, occidental Algerian basin

## Introduction

The depletion of sea cucumbers of the genus *Holothuria* has led to the exploration of new areas in several regions of the world, including Algeria (Mezali and Slimane-Tamacha 2020). But this exploitation has not yet considered species belonging to other genera such as *Parastichopus regalis*, which is the only representative of the order Synallactida recently found along the Algerian coast (Benzait et al. 2020; Khodja et al. 2021). This is probably due to its inaccessibility as this species lives in depths of 50 m or more. Indeed, *P. regalis* is caught incidentally by small-scale fisheries and trawlers that consider it as worthless bycatch (Benzait et al. 2020). On the other hand, in Spain, *P. regalis* is highly valued for its five muscle bands and represents the most expensive seafood product in Catalan markets, reaching a price of EUR 130/kg (Ramón et al. 2010). Several other representatives of this family are exploited worldwide, including *Parastichopus tremulus* and species of the genera *Thelenota* and *Stichopus* (Dissanayake and Stefansson 2010). Sea cucumbers also have important nutritional values and have long been used in traditional medicine in Asian countries (Khotimchenko 2015) because they contain bioactive compounds that reduce the risk of certain chronic diseases, and they improve health by meeting some basic nutritional needs (Pangestu and Arifin 2018). These bioactive compounds include polyunsaturated fatty acids and triterpene glycosides (saponins) (Caulier et al. 2016; Mecheta et al. 2020). In the Mediterranean, several studies have detailed the composition of holothurian species of the genus *Holothuria* (Wen et

al. 2010; Aydin et al. 2011; Roggatz et al. 2015; Gonzalez-Wangüemert et al. 2018; Mecheta and Mezali 2019; Mecheta et al. 2020). However, few studies have been done on species of the genus *Parastichopus*. As far as we know, the chemical composition of *P. regalis* has been studied only by Santos et al. (2015) in Portugal and by Roggatz et al. (2018) in Spain. Hence, the interest of this study whose purpose is to evaluate the quality of *P. regalis* integument through the study of its moisture, lipid and ash contents as well as its digestibility by the gastric enzyme pepsin.

## Methodology

This study was carried out on 42 individuals of *Parastichopus regalis* from four stations in the region of Mostaganem on the western Algerian coast ( $36^{\circ}02'36.66''\text{N}$  and  $00^{\circ}01'49.38''\text{W}$ ;  $36^{\circ}05'4.62''\text{N}$  and  $00^{\circ}00'29.40''\text{E}$ ;  $35^{\circ}59'6.54''\text{N}$  and  $00^{\circ}01'5.34''\text{E}$ ;  $36^{\circ}27'58.68''\text{N}$  and  $00^{\circ}38'25.86''\text{E}$ ). Sampling was carried out in June 2020 by small-scale fisheries in the area. In the laboratory, measurements were carried out the contracted body length (CBL) of the individuals ( $\pm 0.10$  mm), wet weight of the integument (TWW) ( $\pm 0.001$  g) and thickness of the integument (TT) ( $\pm 0.01$  mm). After these measurements were recorded, the integument was frozen for biochemical analysis.

Five frozen integuments were selected for biochemical analysis. These integuments were freeze-dried (Christ Alpha 2-4 LD plus) to remove all water without altering their chemical composition by high temperatures, then weighed

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dry (DTW) to determine the moisture content according to the following formula and the wet/dry weight ratio:

$$\text{Moisture content (\%)} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

The *in vitro* digestibility of the integument of *P. regalis* individuals was performed using a digestive enzyme (pepsin 1:10,000). This step was performed in three replicates on three different individuals following the protocol of Escudero et al. (2010) and modified by Wen et al. (2015). For this study, 0.5 g of dry integument was homogenised twice in 2 ml of distilled water for 1 min at 6000 rpm/min. The whole was centrifuged twice for 1 min at 6000 rpm/min, then the pH was adjusted to 2 with HCL (1 M) before adding pepsin 1:10,000 with an amount of 5% based on the dry weight of the integument. The mixture was kept at 37°C for 2 h, then the pH was adjusted to 7 with NaOH (1 M) to inactivate the pepsin. The undigested integument was dried at 60°C for 72 h and then weighed. The digestibility of the integument by pepsin was calculated according to the following formula:

$$\text{DT (\%)} = \left( \frac{\text{Wt} - \text{Wi}}{\text{Wt}} \right) \times 100$$

Where DT = digestibility of the integument by pepsin, Wt = dry weight of the integument before pepsin digestion, and Wi = dry weight of the integument not digested by pepsin.

The extraction of lipids from the integument of *P. regalis* was performed according to the method of Folch et al. (1957) and later modified by Low and Ng (1987) on two individuals. A total of 1 g of freeze-dried integument samples was dissolved in 120 ml of 2:1 (v/v) chloroform/methanol mixture. The mixture was homogenised for 2 h and then filtered through Whatman No 1 filter paper, and 30 ml (v/4) of 0.73% NaCl was added. The solution was left to decant for 2 h then the chloroform was filtered over sodium sulphate and recovered in a previously weighed flask. The methanol was washed with 40 ml of chloroform and 10 ml of 0.58% NaCl and left to decant for 30 min. The chloroform containing the lipids was also filtered over sodium sulphate, recovered and then removed at 53°C. The lipid content was deduced by weight difference.

To determine the ash content of *P. regalis* integument, approximately 1 g of sample was incinerated completely at 600°C for 6 h. The ash content expressed as a percentage is the difference between the weight of the samples before and after incineration. This step was performed on two individuals.

## Results and discussion

The contracted length of the 40 *P. regalis* individuals varied between 80 and 230 mm (mean 140.55 ± 45.27 mm). The integument thickness had a mean value of 3.67 ± 1.40 mm and the wet integument weight ranged from 7.22 to

133.86 g (mean 56.25 ± 28.48 g). Dried individuals had an average weight of 6.07 ± 1.35 g.

The ratio between the wet (TWW) and dry (TDW) weights of the integument was 9.27. This value is very close to the standard value (10:1) established for holothurians by Newell and Courtney (1965). This ratio was calculated in other species of the genus *Holothuria* in the central and western Algerian region by Mezali (1998) and Mecheta and Mezali (2019). The ratio obtained for *P. regalis* was higher than that of *Holothuria poli* (6.44 and 7.02 obtained by Mezali 1998) and Mecheta and Mezali (2019), respectively, *H. tubulosa* (6.44 and 8.69 obtained by Mezali 1998) and Mecheta and Mezali (2019), respectively, and *H. forskali* (8.98 obtained by Mezali 1998), close to that of *H. sanctori* (9.39 obtained by Mezali 1998) and lower than that of *H. arguinensis*, which possesses the highest ratio of 15.78 (Mecheta and Mezali 2019). According to Mezali (1998, 2001), the variation in this ratio between species may be related to their physiology and the chemical composition of their integument. In fact, the percentage of water content and the percentage of ossicles in the integument that differ between species can influence dry weights and create considerable variation. Habitat may also play a role in the variation of this ratio. In fact, unlike species of the genus *Holothuria* that inhabit coastal areas and have a rigid integument, *P. regalis* is found on muddy substrates (Elakkeri et al. 2021) and its integument is soft. When this ratio is accurately calculated for commercial species, it can be used to estimate the weight of fresh sea cucumbers from previously processed ones (Ngaluafé and Lee 2013).

## Approximate composition

The moisture content (Table 1) in the integument of *P. regalis* was 90.98 ± 0.71%, this value is close to that observed in the same species in Spain (Roggatz et al. 2018). According to Chang-Lee et al. (1989), the moisture content in holothurians is between 82.0 and 92.6%, and is high compared to that of fish and molluscs, although higher or lower values have been observed in some species (Table 1). Such is the case for *Holothuria tubulosa*, *H. poli* and *Stichopus variegatus*, with moisture contents of 81.09 ± 1.97%, 93.53 ± 1.95% and 93.36 ± 0.02%, respectively (Mecheta and Mezali 2019; Ridhowati et al. 2018). The value observed in the species studied remains within the range of rates reported in the literature. Within the same species, fluctuations in the moisture content can be observed depending on the sampling period, such as the case of the species *Isostichopus* sp., where the moisture content varies but not significantly by 3% between the months of January and July (Vergara and Rodríguez 2016). This monthly variation has also been observed in the species *Holothuria scabra* (Özer et al. 2004). In the case of our comparison, all individuals were collected in summer except in the case of *P. regalis* from the Spanish coast and *S. variegatus*, which were collected from fall-winter and winter-summer, respectively (Roggatz et al. 2018; Ridhowati et al. 2018). Thus, the observed interspecific water content variations are simply due to species and genus differences.

**Table 1.** Approximate composition of the integument of *Parastichopus regalis* compared to other sea cucumber species.

Species	Moisture content (%)	Lipid content (%)	Ash content (%)	References
<i>Parastichopus regalis</i>	90.98 ± 0.71	1.40 ± 0.57	40.45 ± 0.54	Present study
<i>P. regalis</i>	-	3.63 ± 0.11	-	Santos et al. (2015)
<i>P. regalis</i>	91.40	1.27	36.80	Roggatz et al. (2018)
<i>Holothuria arguinensis</i>	88.13 ± 4.16*	2.57 ± 0.28	47.31 ± 0.08	Mecheta et al. (2020)
<i>H. poli</i>	93.53 ± 1.95*	5.53 ± 0.59	41.78 ± 1.82	Mecheta et al. (2020)
<i>H. sanctori</i>	-	3.07 ± 0.50	31.58 ± 0.10	Mecheta et al. (2020)
<i>H. scabra</i>	84.91	-	-	Özer et al. (2004)
<i>H. tubulosa</i>	81.09 ± 1.97*	3.81 ± 0.25	40.77 ± 0.60	Mecheta et al. (2020)
<i>Stichopus herrmanni</i>	-	0.80 ± 0.02	37.90 ± 0.33	Wen et al. (2010)
<i>S. variegatus</i>	93.36 ± 0.02	-	-	Ridhowati et al. (2018)
<i>Thelenota ananas</i>	-	1.90 ± 0.01	25.10 ± 0.30	Wen et al. (2010)

\*Mecheta and Mezali (2019)

The lipid level (Table 1) obtained from *P. regalis* was 1.40 ± 0.57%. This value is more or less close to that of *Stichopus herrmanni* and *Thelenota ananas* (0.80 ± 0.02% and 1.9 ± 0.01%, respectively) (Wen et al. 2010). The chemical composition of marine organisms in general can be influenced by several factors such as physiological characteristics, habitat, life cycle and environmental conditions, as well as biological factors such as diet and reproductive cycle (Taboada et al. 2003; Diniz et al. 2012). According to Khotimchenko (2015), the organic and non-organic compositions of fresh holothurians vary with species, season, habitat and probably stage of ontogeny.

Two studies approached the lipid level in the integument of *P. regalis* in the Mediterranean. The level obtained in individuals from Portugal is higher (3.63 ± 0.11%) (Santos et al. 2015) than those obtained in Spain by Roggatz et al. 2018 (1.27%), and those obtained in this study. This variation in lipid levels observed within the same species is explained by several factors but particularly by temperature and availability of food resources due to the selective behaviour of holothurians (Taboada et al. 2003; Neto et al. 2006). Indeed, the three values obtained correspond to samples from different regions and different periods, with the highest lipid level observed in September 2012 (Santos et al. 2015) and the lowest between fall and winter 2011 (Roggatz et al. 2018). The intermediate value was obtained in our study in summer 2020.

The ash content (Table 1) obtained in the integument of *P. regalis* is 40.45 ± 0.54%, which is slightly higher than that obtained by Roggatz et al. (2018) for the same species in Spain (36.80%) as well as those of the other two species of the same family, *Stichopus herrmanni* and *Thelenota ananas* with contents of 37.9 ± 0.33 % and 25.10 ± 0.30%, respectively (Wen et al. 2010). Compared to other species of the genus *Holothuria* from the Algerian west coast, only *H. arguinensis* has a higher ash content than the species studied (47.31 ± 0.08%), *H. tubulosa* and *H. poli* both have almost similar levels (40.77 ± 0.60% and 41.78 ± 1.82%, respectively) and *H. sanctori* has lower values (Mecheta et al. 2020). The results

of the ash content should be taken with caution because the number of ossicles could influence this value.

In general, sea cucumbers are high in protein, low in lipids and contain several minerals and vitamins (Çakli et al. 2004; Mecheta et al. 2020). This chemical composition is influenced by the nutrition of these organisms, their geographical location, the handling procedure of these organisms after they are harvested, and the method by which they are processed into beche-de-mer (Chang-Lee et al. 1989; Özer et al. 2004; Aydin et al. 2011).

### *In vitro* digestibility of the *P. regalis* integument by pepsin

An assessment of the quality of a protein source is usually based on the amino acid composition and digestibility of the protein in the digestive tract (Santé-Lhoutellier et al. 2017). The *in vitro* pepsin digestibility of *P. regalis* integument of 46.96 ± 3.08% is the same as that observed in fish (Wen et al. 2010) and close to that of chicken and beef (44.67% and 42.75%, respectively) (Table 2). Compared to other holothurian species from the Algerian west coast, the integument of *P. regalis* is more digested by pepsin than that of *H. poli* or *H. tubulosa* (34.68 ± 8.66% and 25.96 ± 2.04%, respectively) (Mecheta and Mezali 2019). Despite the fact that *P. regalis* has a low protein content (14.7% according to Roggatz et al. 2018) compared to *H. arguinensis* (66.41% according to Mecheta et al. 2020), their integuments are almost equally digested by pepsin. This digestion may not be related to the protein level but instead to its composition. Indeed, pepsin cleavage is more influenced by the amino acid residue in the P1 position, and when the amino acids His, Lys, Arg or Pro are in this position they prohibit this cleavage (Hamuro et al. 2018). These amino acids represent approximately 19.6%–26.4% of the protein in *H. arguinensis*, *H. tubulosa*, *H. poli* and *P. regalis*, and have low lysine/arginine levels (Roggatz et al. 2015; Gonzalez-Wangüemert et al. 2018; Roggatz et al. 2018).

**Table 2.** Digestibility of *P. regalis* integument by pepsin compared to other sea cucumber species and to commonly consumed meats.

Species	Digestibility (%)	References
<i>Parastichopus regalis</i>	46.96 ± 3.08	Present study
<i>Holothuria arguinensis</i>	53.56 ± 3.41	Mecheta and Mezali (2019)
<i>H. poli</i>	34.68 ± 8.66	Mecheta and Mezali (2019)
<i>H. tubulosa</i>	25.96 ± 2.04	Mecheta and Mezali (2019)
Pork	47.22	Wen et al. (2015)
Fish	46.98	Wen et al. (2015)
Chicken	44.67	Wen et al. (2015)
Beef	42.75	Wen et al. (2015)

## Conclusion

The sea cucumber *Parastichopus regalis* has a high-water content, low lipid content and good digestibility by pepsin. Thus, *P. regalis* from the Algerian coast could constitute a new nutritional and economic source to be exploited. Before that, however, studies on its reproduction must be carried out in order to establish management measures that guarantee the sustainability of the stocks such as the determination of fishing closure periods and minimum commercial sizes, which are not available for any species exploited in Algeria.

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