J. Natn. Sci. Coun. Sri Lanka 1985 **13** *(2) : 115–130*

CHEMICAL CONSTITUENTS OF SOME SPECIES OF HOLOTHURIANS FOUND IN SRI LANKA

RAJESWARY MAGESWARAN AND VASEETHA BALAKRISHNAN
Department of Chemistry, University of Jaffna, Jaffna, Sri Lanka.

(Date of receipt : 30 January 1985)
(Date of acceptance : 10 July 1985)

Abstract : The sodium, potassium, calcium, iron, magnesium and phosphorus contents of the **six** Holothurian species *Holothuria scabra, Holothuria atra, Holothuria nobilis, Holothuria leucospilota, Bohadschia marmorata* and *Sticopus chloronotus* were determined. *H. scabra* has the highest sodium (4.29%), potassium (1.05%), calcium (7.91%) and magnesium (5.21%) contents. *B. marmorata* has the lowest amounts of sodium (0.30%) and calcium (2.05%). *H. atra* has the lowest amount of magnesium (0.73%) and *S. chloronotus* has the lowest amount of potassium (0.10%). *H. scabra* has the highest amount of the trace elements copper (9 ppm) and nickel (36 ppm), while *B. marmorata* contains the highest amount of manganese (7 ppm) and zinc (340 ppm). Of the two commercial species, *H. atra* (61 - 65%) has a higher protein content than of *H. scabra* (36 - 38%). The effect of processing on the mineral and protein contents of *H. atra* and *H. scabra* is also reported. Processed *H. atra* and *H. scabra* from the Northern coast of Sri Lanka have a higher protein content (73 – 76%) than the processed *H. scabra* (59%) from the South Pacific Islands. A possible method of differentiating *H. scabra* and *H. atra* on the basis of the mineral content is also suggested.

1. Introduction

The Holothurians are soft, cylindrical-bodied, usually dull dark-coloured and often warty species much like cucumbers (therefore called sea cucumbers) belonging to the Invertebrate class Holothuroidea (or Holothurioides) of the phylum Echinodermata.[2]

Holothurians are marine species of economic importance and their export provides valuable foreign exchange to Sri Lanka. Processed Holothurian, *Beche-de-mer* is considered a delicacy by the Chinese and as a result is exported to the countries where the Chinese population is relatively high.[3] The prices of the exported animals depend on qualities such as size, appearance, odour, colour, moisture content and resistance to spoilage. Thus knowledge of the amounts of moisture, protein, carbohydrates, fat, amino-acids and minerals present in different species of Holothurians found in Sri Lanka would be useful in evaluating their food value and the market potential. Holothurians are also used in making poultry feed.[3] The water extract of *H. scabra,* obtained during processing is used to relieve pain and to cure paralysis by some villagers in the Northern part of Sri Lanka.[3]

Of the species known, *H. scabra* is the most commercially popular. In northern Sri Lanka, this species is largely available in the coastal area of Mandaitivu, Nainativu and Mannar. Another commercially popular species *H. atra* is also available in the northern coastal area. The latter is plentiful in the coastal area of Point Pedro.

The mineral, fat, protein and moisture contents of *Beche-de-mer* processed from *H. scabra* from the South Pacific Islands have been reported.[10] Recently a group of workers have reported[4,5] the protein, fat, and saponin contents of some species of Holothurians found in Sri Lanka. In our earlier communication,[1] we reported the mineral and iodine contents of six species of fresh Holothurians found in the coastal area of Jaffna. In this paper, we report our results on the chemical constituents of the commercially popular species *H. scabra* and *H. atra* along with those of the other species. The effect of processing on the mineral content is also reported.

2. Experimental Methods and Materials

Fresh samples of *H. scabra* used in this study were collected from Mandaitivu during the months December 1982 and June 1984 and from Mannar in October 1983. *H. atra* used for this study was collected from Mandaitivu in March 1983 and June 1984 and from Point Pedro during the months of March and June 1983. Also samples of *B. marmorata, H. nobilis* and *S. chloronotus* collected from Trincomalee and *H. leucospilota* collected from Mandaitivu were analysed for comparison purposes. The different species were identified by reported methods.[3]

Processing[3] of Holothurians was carried out as follows: The specimen was first boiled in water for 30 min removed from water and allowed to air dry for 3 h. Then the dried specimen was kept under a soil bed overnight for fermentation. The outer body wall cover was removed by washing with water and the processes of boiling and air drying were repeated twice.

After the above treatment, the air-dried samples were dried in an oven 105°C to constant weight. The ash content was determined by ashing weighed quantities of oven dried samples at 450°C in a Muffle furnace until constant weight was obtained.

2.1 Preparation of Test Solutions

Oven dried samples of known weights were ashed at 450°C and dissolved (quantitatively) in a known volume of 0.6M HCl and the resulting solutions were used to determine sodium, potassium, calcium, magnesium, iron and trace elements.

2.1.1 Determination of the Amounts of Sodium and Potassium

Sodium and potassium contents of test solutions were determined Flame Photometrically[7] using a Corning 400 Flame Photometer. The Flame intensities for sodium were corrected for interference by calcium by the standard addition method.[13]

2.1.2 Determination of the Amounts of Calcium and Magnesium

Calcium and magnesium contents of test solutions were determined titrimetrically[11] using EDTA with potassium cyanide as the masking agent. Patton Reeders indicator was used for calcium determination.

Magnesium content of test solutions was estimated as follows: The total amount of magnesium and calcium present was determined[11] by titrating a known volume of test solution with standard EDTA solution with Eriochrome Black T as indicator and using potassium cyanide as masking agent.[11] Magnesium content was obtained by subtracting the amount of calcium present from this value.

2.1.3 Determination of Iron

Iron content of test solution was determined colorimetrically.[6] 1,10—Orthophenanthroline was used as the complexing agent and the colour intensity was measured using a Corning Model 252 Colorimeter with a 490 nm filter.

2.1.4 Estimation of Protein Content

The total nitrogen content of oven dried samples of holothurians was determined by the Kjeldhal method[6] and protein content was calculated by multiplying the total nitrogen content by 6.25.

2.1.5 Determination of Trace Elements

The trace elements copper, manganous, nickel, zinc, cadmium, cobalt and chromium were determined[6] using the test solution (prepared as described above) on a Varian Model 1257 Atomic Absorption Spectrophotometer.

2.1.6 Determination of Lead

Oven-dried samples were subjected to wet oxidation with concentrated nitric acid and concentrated sulphuric acid as reported previously.[6] The resulting solution was used to prepare the test solution. The amount of lead present was determined using a Corning Model 252 Colorimeter after complexing with dithizone.[6]

2.1.7 Determination of Phosphorus

Accurately weighed oven-dried samples (1—2g) were ashed with magnesium nitrate[6] and the ash was dissolved in 1M H_2SO_4 (10 ml) and the solution made up to 50 ml. Phosphorus content of this solution was determined[6] by measuring the intensity of the colour produced on complexing with vanadomolybdate reagent using a Corning Model 252 Colorimeter with 430, 470 and 490 nm filters.

2.1.8 Determination of Iodine

Iodine content of accurately weighed oven dried samples was estimated by the alcoholic potash method.[8]

2.2 Identification of aminoacids and carbohydrates

Methanol extracts of fresh samples of *H. scabra* and *H. atra* were used for the identification of carbohydrates and aminoacids. Carbohydrates and aminoacids were separated from the methanol extract by using the cation exchange resin, Zeocarb 225. The aminoacids and carbohydrates present were tentatively identified[12] by comparing with authentic samples using paper chromatography.

2.3 Separation of spicules and their analysis

Spicules were separated[3] from the flesh of the specimen by adding saturated solution of sodium hypochlorite. The mixture was left overnight and decanted (The spicules will float in the liquid – the flesh will be at the bottom). The spicules were separated from the decanted solution by centrifuging and decanting the supernatant liquid. The spicules were washed with distilled water until no more chloride ions are detected in the washings. The spicules were then weighed, ashed and dissolved in 0.6M HCl and the estimation of the minerals were carried out as before.

3. Results and Discussion

The habitats of the six holothurian species studied are given in Table 1 and their localities are given in Table 2. Of the six species studied, *H. atra* is the smallest (usually 8 – 15 cm in length) and *H. leucospilota* is the largest (30 – 80 cm). Our results on the estimation of the amounts of sodium, potassium, calcium and magnesium present in six species of Holothurians are given in Table 3. The amounts of iron, phosphorus and iodine are given in Table 4. Of the six species investigated, *H. scabra* contains the highest amounts of sodium, calcium and magnesium. But *H. atra*, and the non-

commercial species *H. leucospilota* and *S. chloronotus* contain relatively high amounts of iron and phosphorus. *H. scabra* from the Mandaitivu coast and *S. chloronotus* from Trincomalee have appreciable amounts of iodine while *H. atra, H. leucospilota* and *B. marmorata* do not have detectable amounts of iodine. It is interesting to note that seaweeds from the Mandaitivu coast also have high iodine content.[9] *H. scabra* from the South Pacific Islands[10] has higher amounts of sodium and iron than the Holothurian species from Sri Lankan coast but its calcium and phosphorus contents are much lower. Tables 3 and 4 also show the amounts of the same minerals present in different species of Holothurians after processing. Processing removes 87 – 90% of sodium and potassium from both *H. scabra* and *H. atra*. Relatively smaller amounts of calcium, magnesium, iron and phosphorus are lost during processing and iodine is lost completely. However there is a large difference in the amount of calcium, magnesium and phosphorus lost by *H. atra* and *H. scabra*. Table 5 gives the percentage of the original amounts of the minerals remaining in the animals after processing to the original value.

Table 1. The habitats of the holothurian species studied.

Species	Habitat
Holothuria scabra	Found among eel grasses in the shallow waters and in sandy bottom in deep waters.
Holothuria atra	Found among coral reefs and clear sand. It is usually found coated with sand with few naked patches.
Holothuria leucospilota	Found in deep sea (about 10 m depth).
Holothuria nobilis	Found in muddy bottom (4 - 8 m depth).
Sticopus chloronotus	Found in deep waters on pebble stone grounds (6-12 m depth).
Bohadschia marmorata	Found in deep sandy bottom where the bed can be easily seen from the surface of the water.

Table 2 : Details of the species used in the study.

Species	Locality	Month of collection	Weight of whole sample–(g)	Length of sample–(cm)	Moisture (%) in body	Weight of body (g) (without alimentary canal)
Holothuria scabra (I)	Mandaitivu	December 1982	314	21	83.10	144
Holothuria scabra (II)	Mandaitivu	June 1984	386	30	82.51	178
Holothuria scabra (III)	Mandaitivu	June 1984	188	22	82.63	95
Holothuria scabra (IV)	Mannar	October 1983	465	28	85.31	206
Holothuria atra (I)	Mandaitivu	April 1984	68	13	85.97	24
Holothuria atra (II)	Mandaitivu	April 1984	106	12	85.90	38
Holothuria atra (III)	Point Pedro	March 1983	125	15	83.21	55
**Holothuria leucospilota*	Mandaitivu	–	–	–	–	–
**Holothuria nobilis*	Trincomalee	–	–	–	–	–
**Bohadschia marmorata*	Trincomalee	–	–	–	–	–
**Sticopus chloronotus*	Trincomalee	–	–	–	–	–

*Samples from Miss P. Elanganayagam, Department of Zoology. Sun dried mixture of several animals was used (moisture content of these sun dried samples ranged from 15 – 17 %).

Table 3.

Sodium, Potassium, Calcium and Magnesium contents of Holothurians, [g/100g oven dried samples]

Species	Ash		Sodium		Potassium		Calcium		Magnesium	
	a	b	a	b	a	b	a	b	a	b
1. *Holothuria scabra* [I]	53.23	19.61	3.92	0.39	0.74	0.09	6.76	2.73	4.39	0.98
2. *Holothuria scabra* [II]	53.89	22.75	4.29	0.48	1.05	0.14	7.91	3.11	5.21	1.17
3. *Holothuria scabra* [III]	48.14	14.82	2.36	0.24	0.66	0.07	5.85	2.63	4.18	0.89
4. *Holothuria scabra* [IV]	45.81	15.17	3.70	0.40	0.30	0.02	4.28	1.84	2.12	0.54
5. *Holothuria atra* [I]	20.36	3.12	1.05	0.11	0.15	0.02	2.07	0.42	0.73	0.23
6. *Holothuria atra* [II]	21.76	3.65	1.50	0.17	0.16	0.02	2.49	0.57	0.77	0.26
7. *Holothuria atra* [III]	22.50	3.86	1.14	0.15	0.18	0.03	2.58	0.64	0.95	0.34
8. *Holothuria scabra*[10] [South Pacific Islands)	–	37.9	–	3.43	–	–	–	1.65	–	–
9. *Holothuria leucospilota*	22.35	–	0.55	–	0.24	–	2.17	–	2.13	–
10. *Holothuria nobilis*	22.00	–	0.83	–	0.17	–	2.49	–	2.04	–
11. *Bohadschia marmorata*	29.40	–	0.30	–	0.23	–	2.05	–	2.30	–
12. *Sticopus chloronotus*	36.47	–	1.90	–	0.10	–	3.00	–	1.56	–

a – ash and minerals in unprocessed samples.

b – ash and minerals in processed [as described earlier] samples

Table 4.

Iron, Phosphorus and Iodine contents of Holothurians [mg/kg oven dried samples]

Species	Iron		Phosphorus		Iodine	
	a	b	a	b	a	b
1. *Holothuria scabra* [I]	81	43	777	653	51	no detectable amount
2. *Holothuria scabra* [II]	90	51	865	700	54	" "
3. *Holothuria scabra* [III]	59	31	733	630	34	" "
4. *Holothuria scabra* [IV]	40	18	281	223	no detectable amount	
5. *Holothuria atra* [I]	146	66	1092	28	" "	" "
6. *Holothuria atra* [II]	233	104	1116	691	" "	" "
7. *Holothuria atra* [III]	452	194	985	625	" "	" "
8. *Holothuria scabra*[10] [South Pacific Islands)	–	226	–	96	–	–
9. *Holothuria leucospilota*	413	–	1255	–	no detectable amount	–
10. *Holothuria nobilis*	336	–	666	–	6	–
11. *Bohadschia marmorata*	86	–	577	–	no detectable amount	
12. *Sticopus chloronotus*	251	–	1037	–	150	–

a – minerals in unprocessed samples
b – minerals in processed samples.

Table 5. Percentage of the original amounts of minerals present after processing.

Species	Na	K	Ca	Mg	Fe	P
Holothuria scabra [I]	9.9	12.2	40.4	22.3	53.1	84.1
Holothuria scabra [II]	11.2	13.3	42.2	22.5	56.7	80.92
Holothuria scabra [III]	10.2	10.6	45.0	21.3	52.5	86.0
Holothuria scabra [IV]	10.8	6.7	43.0	25.8	45.0	79.4
Holothuria atra [I]	10.5	13.3	20.3	31.5	45.2	57.5
Holothuria atra [II]	11.3	12.5	22.9	33.8	44.6	61.9
Holothuria atra [III]	13.2	16.7	24.8	35.8	42.9	63.5

One of the reasons for the greater loss of alkali metals during processing could be the higher solubility of their salts. Another possibility is that these salts are present in relatively large amounts in the outer skin and spicules which are removed during the processing. The difference in the loss of calcium, magnesium and phosphorus between *H. scabra* and *H. atra* could be attributed to the difference in the distribution of these elements in the animal body wall, the skin and spicules of *H. atra* containing relatively larger amounts of the minerals than *H. scabra.* Table 6 gives the amount of minerals left in the animal after each stage in processing for the two species *H. scabra* and *H. atra.*

The trace elements present in different species of holothurians were also estimated using an Atomic absorption spectrophotometer. The elements chromium and cobalt could not be detected in any of the species. The Table 7 shows the amounts of copper, manganous, zinc, lead, nickel and cadmium present in four species.

Table 6

Effect of processing on the mineral contents of *H. scabra* and *H. atra*; [g/100g oven dried samples]

Stage in processing	Ash	Na	Ca	Mg	K	Fe	P
1. *Holothuria scabra* [I]							
Fresh	53.23	3.92	6.76	4.39	0.74	0.0081	0.0777
After 1st boil	44.10	2.95	6.04	3.05	0.447	0.0067	0.0798
After fermentation	33.35	1.04	4.95	2.37	0.324	0.0084	0.0779
After 2nd boil	25.69	0.47	3.28	1.27	0.210	0.0047	0.0674
After 3rd boil	19.61	0.39	2.73	0.98	0.091	0.0043	0.0653
2. *Holothuria atra* [II]							
Fresh	21.76	1.50	2.49	0.77	0.160	0.0233	0.1116
After 1st boil	14.32	1.23	1.74	0.49	0.093	0.0165	0.0957
After fermentation	7.20	0.78	1.07	0.41	0.039	0.0180	0.0842
After 2nd boil	4.07	0.36	0.89	0.33	0.032	0.0140	0.0728
After 3rd boil	3.65	0.17	0.57	0.26	0.020	0.0104	0.0691

Table 7

Amounts of trace elements present in some species of Holothurians; [mg/kg of the oven dried samples]

Species	Copper		Manganese		Zinc		Lead		Nickel		Cadmium	
	a	b	a	b	a	b	a	b	a	b	a	b
Holothuria scabra [I]	9.2	10.6	3.8	3.8	6.7	8.4	19.2	12.6	35.5	26.0	3.0	1.7
Holothuria atra [I]	2.3	2.6	2.1	3.3	8.6	9.3	7.2	5.1	14.0	12.7	1.4	0.4
Bohadschia marmorata	2.6	–	6.9	–	339.6	–	15.2	–	30.0	–	2.7	–
Holothuria leucospilota	4.3	–	6.5	–	54.2	–	7.6	–	14.3	–	1.1	–

a – amount of trace elements present in unprocessed samples.

b – amount of trace elements present in processed samples.

The elements copper, manganous and zinc are essential and nutritive, whereas lead and cadmium are toxic. Elements such as copper and zinc although essential for life in trace amounts, have an toxic action when ingested in higher amounts. The recommended levels of copper and zinc are 20 and 50 ppm respectively. The amounts of copper and zinc present in the commercial species *H. scabra* and *H. atra* are well below the recommended level. The non commercial species *B. marmorata* and *H. leucospilota* contain zinc in quantities well above the recommended level. On processing the percentage of the nutritive elements [Cu, Mn and Zn] increases slightly but remain well within the recommended level. The percentage of toxic elements [Pb and Cd] decreases [by about 30 – 46%] on processing. The mineral contents of the spicules of five species of holothurians are given in Table 8. The spicules of the commercial species *H. scabra* and *H. atra* have a relatively high calcium content. The spicules of *B. marmorata* has relatively high magnesium content while that of *S. chloronotus* is low in calcium but contain the highest amount of potassium and iron. These findings may be of chemotaxonomic significance. It is relevant to note that shape of spicules among other properties is used[3] in identification of different species of holothurians.

Table 8

Amounts of sodium, calcium, magnesium, potassium and iron present in spicules of different holothurians; [g/100g oven dreid samples]

Species	Na	Ca	Mg	K	Fe
1. *Holothuria scabra*	0.74	32.20	3.21	0.0069	0.0190
2. *Holothuria atra*	0.67	34.93	4.00	0.0012	0.0067
3. *Bohadschia marmorata*	0.82	34.56	19.00	0.0160	0.0270
4. *Sticopus chloronotus*	0.21	14.03	3.84	0.0637	0.1221
5. *Holothuria nobilis*	0.96	226.70	3.20	0.0048	0.0066

The protein contents of fresh and processed *H. scabra* and *H. atra* species are given in Table 9. It is of interest to note that although unprocessed *H. atra* has a very much higher percentage of protein than unprocessed *H. scabra,* the processed species have nearly the same percentage of protein. This may be due to a greater loss of non-protein material from *H. scabra* than from *H. atra* during processing. Also the local *H. scabra* and *H. atra* appear to have higher percentage of protein than the *H. scabra* from South Pacific Islands. [10]

The total free aminoacid and carbohydrate contents of *H. atra* and *H. scabra* have also been estimated by the usual method. The values of the total amount of substances extractable into methanol and the percentages of free aminoacids and carbohydrates present are given inTable 10.

Table 9

Protein contents of fresh and processed *Holothuria scabra* and *Holothuria atra.* (g/100g oven dried samples)

Species	Protein	
	Unprocessed	Processed
Holothuria scabra [I]	37.75	76.44
Holothuria scabra [II]	37.10	75.02
Holothuria scabra [IV]	36.69	73.31
Holothuria atra [I]	64.94	76.63
Holothuria atra [II]	63.85	76.25
Holothuria atra [III]	61.44	73.50
Holothuria scabra[10] [South pacific Islands]	–	59.12

Table 10. Percentage of free amino acids and carbohydrates

Species	Weight of animal/g	Total amount of material in methanol extract (%)	Free amino acids (%)	Carbohydrate (%)
Holothuria scabra (Mandaitivu)	200	3.15	0.33	0.58
Holothuria atra (Mandaitivu)	56	4.70	0.45	1.37

The carbohydrates present in both the species are in the polysaccharide form, as simple sugars were not detected by chromatographic analysis. Comparative two dimensional paper chromatographic analysis has shown that *H. atra* contains seventeen free aminoacids. Fifteen of them have been identified as aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, valine, methionine, histidine, lysine, phenylalanine, arginine, leucine and isoleucine. *H. scabra* contains fifteen aminoacids. Fourteen of these have been identified as aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine, threonine, valine, arginine, methinine, proline, phenylalanine, leucine and isoleucine. It is interesting to note that *H. atra* contains nine of the ten essential aminoacids whereas *H. scabra* contains only seven of them.

4. Conclusion

Processed *H. scabra* and *H. atra* from the coastal area of Northern Sri Lanka have a higher protein content [73–76%] than the processed *H. scabra* from the South Pacific Islands [59.12%]. Generally *H. scabra* is richer in alkali, alkaline earth and trace metals than *H. atra* whereas the reverse is true in the case of the elements iron and phosphorus. Both *H. scabra* and *H. atra* have similar Na/Ca ratio. But the K/Ca and Mg/Ca ratios of *H. scabra* are very much higher [> 60%] than those of *H. atra.* Thus it is possible to differentiate *H. scabra* and *H. atra* on the basis of the mineral constituents. Processing increases the protein content and the amounts of copper, manganese and zinc and decreases the alkali, alkaline earth and toxic elements.

Acknowledgement

The authors wish to thank Miss. P. Elanganayagam, Depratment of Zoology for providing samples of some holothurians and Messers. M. Ariyaratnam and N. Baskaran for technical assistance.

References

1. BALAKRISHNAN, V. & MAGESWARAN, R., (1983) *Proc. I. Chem. C* **12**, 13.

2. BARNES, R. D,(1974) *Invertebrate Zoology* (Third Edition), W.B. Saunders Company, 724.

3. ELENGANAYAGAM, P., (1983) M. Phil thesis.

4. ELANGANAYAGAM, P., GANESHALINGAM, V. K. & SACHITHANANTHAM, K. (1980) Proc. Sri Lanka Ass. Advmt. Sci. **36**, 62.

5. ELANGANAYAGAM, P., MAHENDRAN, M., KRISHNARAJAH, S.R., GANESHALINGAM, V. K. & SACHITHANANTHAM, K, (1982) *Proc. I. Chem. C.* **11**, 5.

6. JACKSON, M. L, (1973) *Soil Chemical Analysis*, Prentice-Hall of India Ltd., New Delhi pp. 151 – 154, 336, 389.

7. JACOBS, M. B, (1958) *The Chemical Analysis of Foods and Food Products*, D. Van. Nostrand Co. New York.

8. KAPPANNA, A. N. & RAO, V. S, (1962) *J. Sci. & Ind. Res.* **21** B 559.

9. MAGESWARAN, R. & SIVASUBRAMANIAM, S., (1984) *J. Natn. Sci. Coun.* Sri Lanka **12** (No. 2). 173.

10. SACHITHANANTHAM, K., (1973) South Pacific Islands, Beche de-mer fishery, Food and agriculture organisation of the United Nations; Rome.

11. SAHAI, D.N. (1974) *Technology*, 11 pp. 420 – 422 & Chenery, E. M., (1964) *The Analyst* **89** pp. 365 – 367.

12. SMITH, I. & SEAKINS, J. W. T., (1976) *Chromatographic and Electrophoretic Techniques* **(1)**, William Heinemann Medical Books Ltd., 84.

13. WILLARD, H. H., MERRITT, L. L. & DEAN, J. A., *Instrumental Methods of Analysis*, Van Nostrand Reinhold Company, 342.