

PROXIMATE COMPOSITION AND MACRO AND MICRONUTRIENTS ANALYSIS OF *STICHOPUS CHLORONOTUS* (SEA CUCUMBER)

Krizelle C. Pasig, Joval C. Afalla,
Luzviminda U. Rivero and Lourdes R. Simpol
Chemistry Department
Ateneo de Davao University
E. Jacinto St., Davao City 8016, Philippines
letlet@addu.edu.ph

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Abstract - The sea cucumber species *Stichopus chloronotus* grown in BFAR-XI Marine Sanctuary in Barangay Bato, Sta. Cruz, Davao del Sur, was studied with respect to its moisture, ash, crude fat and crude protein composition, and macro and micro nutrient contents. The main purpose of the study was to provide a nutrient profile of *S. chloronotus*, which could be utilized by the Bureau of Fisheries and Aquatic Resources (BFAR) in its effort to promote a stable sea cucumber industry in the Philippines.

The study showed that *S. chloronotus* grown in this area contains significant amounts of moisture, ash, fat, protein, phosphorous, sodium, potassium, calcium, magnesium, iron, and zinc.

INTRODUCTION

Sea cucumber (*Stichopus chloronotus*) (Figure 1), locally referred to as “bat” (Madrid, 2001) and commercially known as “beche de mer” or “trepang” (Akamine, 2002), is a marine animal found in coastal waters and ocean floors throughout the world. They are considered relatives of starfish and sea urchins. Most of these marine species, which belong to the class Holotheroidea and under the family Stichopodidae, are less than a foot long and resemble cucumbers in shape (Hickmann,

1993). The smallest species are less than three cm in length and *Stichopus* from the Philippines may attain a length of 1 m and a diameter of 24 cm (Rupert and Barnes, 1994). Sea cucumbers feed on algae that they sift from sand on the seabed. They stretch out their branched tentacles and sweep them on the bottom surface, gathering particulate materials on the tentacular surfaces that are then stuffed into the mouth opening (Rupert and Barnes, 1994). Sea cucumbers are relatively sluggish animals. They move along by means of muscular contractions of the body, much like those of a caterpillar, and with the aid of the tube feet that press against the bottom (Levinton, 1995).

Importance of Sea Cucumber

Of the 1,200 Holothurian species known today, there are at least 24 commercially exploited species in the Philippines. Of those identified, an appreciable portion belongs to the genera *Stichopus*. Although trepang is produced throughout the Philippines, Zamboanga City and Puerto Princesa City are the largest trepang-producing areas (Akamine, 2002). Sea cucumbers are perceived to have great economic potential.

In the modern medical field, they serve as valuable source of substances for natural



Figure 1. Sea cucumber (*S. chloronotus*).

health products. Some species produce toxins of medical importance and are of interest to pharmaceutical firms (Kerr, 2000). Russian, Japanese, and Chinese studies reveal that sea cucumbers contain saponins (triterpene glycosides), compounds that are structurally similar to the active constituents of ginseng and ganoderma (<http://www.itmonline.org/arts/seacucumber.htm>, July 2, 2005). According to the analysis by principles of traditional Chinese medicine, the sea cucumber nourishes the blood, tonifies kidney, and moistens dryness especially of the intestines. Some of its common uses include treating weakness, impotence, and muscular disorders attributed to old age (<http://www.itmonline.org/jintu/dermasea.htm>, July 10, 2005). Compounds so far isolated from sea cucumber exhibit antimicrobial activity or act as anti-inflammatory agents and anticoagulants (Kerr, 2000). Chinese studies indicate anticancer properties of both the sea cucumber saponins and polysaccharides (<http://www.itmonline.org/arts/seacuke.htm>, July 10, 2005).

Sea cucumber is an important ingredient in oriental gourmet food. Chinese cooks have revered them since ancient times. Nutritionally, sea cucumber is an ideal tonic food. It is higher in protein (at 55%) than many other foods except egg whites (at 99%), and it is lower in fat than most foods (less than 2 %). (http://www.itmonline.org/arts/sea_cuke.htm, July 10, 2005).

The processing of the sea cucumber involves four major steps: cleaning, cooking, smoke drying, and sun drying. Major export destinations of processed sea cucumbers from the Philippines are China, Hong Kong and Singapore (<http://www.spc.org.nc/coastfish/News/BDM/13/03.Schoppe.html>, August 1, 2005). Sea cucumbers also form the basis of a multimillion-dollar industry that processes the body wall for sale as beche-de-mer or trepang (Kerr, 2000). Generally, larger trepangs with a thick body wall are preferred in the market. Trepang from Puerto Princesa City is sold at PhP 1,000-2,000 per kg (Akamine, 2002; Fernandez, 2005).

The sea cucumber has been commercially exploited over a wide tropical and subtropical range for centuries. The increasing depletion of the natural stocks of high-commercial value

species has become apparent. Their sizes have become smaller and collections have moved to deeper waters (<http://www.spc.org.nc/coastfish/News/BDM/13/03.Schoppe.html>, August 1, 2000). This depletion of the natural stocks has prompted the Bureau of Fisheries and Aquatic Resources (BFAR) Region XI to study the mariculture of sea cucumber. As a resource enhancement program, mariculture ensures that the locally occurring organisms with economic value, such as sea cucumbers, can be allowed to grow to sexual maturity before they are harvested while retaining some reproductive individuals to replenish stocks (Estepa and Menez, 1998). The project aims to restore the depleted natural stock of sea cucumber in the inter-tidal zone (Fernandez, 2005). They have established seabed pen for grow out culture of *S. chloronotus* at the shallow water within the marine sanctuary located at the coastal waters of Barangay Bato, Sta. Cruz, Davao del Sur.

As a supplemental livelihood, BFAR-XI sea cucumber mariculture also aims to provide sustainable food and income for the communities, and is an entry point into a promising industry that can enhance the country's economy. The Department of Agriculture through BFAR is currently conducting a study on the grow-out culture of sea cucumbers that aims to restock as well as to develop the propagation and utilization of *S. chloronotus* (Fernandez, 2005).

This study seeks to determine the proximate analysis of sea cucumber in terms moisture, ash, crude fat, and crude protein, and the concentrations of macronutrients (R, Na, K, Ca, and Mg) and micronutrients (Fe and Zn). The study was limited to the samples grown in BFAR-XI marine sanctuary at Barangay Bato, Sta. Cruz, Davao del Sur.

METHODS AND MATERIALS

Sampling and preparation

Two sampling batches were conducted at the marine sanctuary of BFAR XI in Barangay Bato, Sta. Cruz, Davao del Sur. Two mature samples, weighing 0.5-1 kg each, were gathered randomly by hand underwater, placed in a container and transported immediately to the laboratory. They were kept at low temperature

during transport to retain freshness and prevent moisture loss.

The samples were eviscerated and cleaned by removing the inedible body parts. After cutting into small pieces, the samples were drained by suspending them in a strainer for 10 min and were analyzed for moisture content. The dried residue was utilized for the rest of the analyses.

Moisture content

The moisture content was determined following the procedure in AOAC Official Method 950.46, Volume II, 39.1.02. The previously prepared sample was weighed into a porcelain dish previously heated to constant weight at 100 ± 2 °C. The dish was placed uncovered in the oven at 100-102 °C for 16-18 h. Subsequently, the dish was cooled and weighed. This procedure was repeated, with shorter heating time, until constant weight was obtained. The moisture content was calculated as follows:

$$\text{Moisture (\%)} = (\text{weight of moisture} / \text{weight sample}) \times 100 \%$$

Ash content

The ash content was determined following the procedure in AOAC Official Method 938.08, Volume II, 35.1.14. Two g of the dried sample was accurately weighed into a porcelain crucible that has been previously heated to constant weight at 525 °C. The sample was completely charred on a hot plate, placed in the furnace and the temperature was slowly raised to 525 °C. Ashing was initially done for three h and continued until white ash and constant weight were obtained. The ash content was calculated as follows:

$$\text{Ash (\%)} = (\text{Weight of ash} / \text{Weight of sample}) \times 100 \%$$

Crude fat content using Soxhlet Method

Fat content was determined following the procedure in AOAC Official Method 948.16 Volume II 35.1.25. Two grams of dried, ground sample were accurately weighed into an extraction thimble, which was then subjected to Soxhlet extraction with 100 mL petroleum

ether for 5 hr. The petroleum ether extract was then evaporated to dryness. The flask containing the fat residue was dried in an oven at 100 °C for 5 minutes. The procedure was repeated until constant weight was obtained. The fat content was calculated as follows:

$$\text{Crude Fat (\%)} = (\text{weight of extracted fat} / \text{Weight of sample}) \times 100 \%$$

Determination of crude protein content using Macro Kjeldahl Method

Crude protein content was determined following the procedure in AOAC Official Method 981.10 Volume II 39.1.19. One gram of the dried, ground sample was weighed accurately and was transferred to an 800-mL Kjeldahl flask. Seven grams of potassium sulfate and 0.1 g of copper sulfate were added into the flask followed by 12 mL of concentrated H_2SO_4 . The flask was heated until foaming has ceased and the solid contents were completely dissolved. The liquid sample was boiled for 2 hr, or until the loss of the light blue-green color. A steam distillation apparatus was set up and the tip of the condenser receiver was immersed in 100 mL of 0.1 M HCl with 4 drops of phenolphthalein indicator solution in a 500-mL receiver flask. The cooled sample in the Kjeldahl flask was diluted to about 300 mL with distilled water and fresh boiling chips were added. Fifteen min after heating has commenced; 10 mL of 10 M NaOH was added carefully into the Kjeldahl flask. Distillation was continued until 150 mL of the distillate was collected in the receiver flask. The collected distillate was titrated with standard 0.1 M NaOH. A blank test was also conducted. The crude protein value was calculated from total nitrogen using the general factor, 6.25, for meat products. The amount of protein was calculated as follows:

$$\text{Crude Protein (\%)} = [(\text{amount HCl reacted} \times 0.014 \times 6.25) / \text{weight sample}] \times 100 \%$$

Determination of P content

Phosphorus content was determined following the procedure in AOAC Official Method 991.27 Volume II 39.1.12.

a. Preparation of the Sample Solution

Two g of dried, ground sample were weighed accurately into a porcelain crucible and the sample was ashed in a furnace at 500 °C. The resulting ash was cooled, treated with 5-10 mL of 6 M HCl and evaporated to dryness. Fifteen mL of 3 M HCl were added, and the acidified sample was heated until the first sign of boiling. The sample was then cooled and filtered into a 100-mL volumetric flask, retaining as much of the solids as possible in the crucible. Ten mL of 3 M HCl were added to the residue. The residue was heated to boiling, cooled and filtered. The filtrate was combined into same volumetric flask. Finally, the filtrate was diluted to the mark with distilled water. A corresponding blank solution was also prepared. To determine the percent recovery of the method, a spiked sample was prepared by adding 10 mL of 500 ppm standard phosphorus working solution.

b. Preparation of standard phosphorus solutions

Four standard solutions with concentrations ranging from 0.1 ppm to 1.5 ppm were prepared to establish a calibration curve. These were prepared from a working standard solution of 10 ppm concentration.

c. Determination of the phosphorus concentration

A 10-mL aliquot of the sample solution was transferred into a 100-mL volumetric flask and was diluted to the mark with distilled water. Another 10-mL aliquot was transferred into a 50-mL volumetric flask and also diluted to mark with distilled water. To these solutions were added 2 mL of ammonium molybdate reagent. Immediately before spectroscopic measurement, two drops of SnCl₂ solution were added to each solution and mixed thoroughly. Spike, blank and standard solutions were similarly analyzed. The concentration of phosphorous was determined at 698 nm using a UV-Vis Spectrometer (Perkin Elmer Lambda 25). The concentration of phosphorous was calculated as follows:

$$\text{ppm (P)} = [\text{UV-Vis reading} \times (\text{L}/1000 \text{ mL}) \times \text{dilution factor}] / (\text{weight of sample in kg})$$

Determination of Na, K, Ca, Mg, Fe, and Zn content using AAS

a. Preparation of Sample (Dry Ashing Method)

Two g of dried, ground sample were accurately weighed into a porcelain crucible, and ashed in a furnace at 500 °C. The resulting ash was cooled, treated with two mL of concentrated HNO₃ and mixed thoroughly. The acidified sample was evaporated to dryness, and then heated in a furnace for 1 hr at 500 °C. The sample was cooled and treated again with HNO₃ to obtain a practically carbon-free ash. The ash was dissolved in 10 mL of 3 M HCl and heated on a warm hot plate. The resulting mixture was filtered into a 100-mL volumetric flask and was diluted to the mark with distilled water. A portion of this solution was set aside for Fe and Zn analysis.

From the solution in (a), a 10-mL aliquot from the prepared sample solution was diluted into a 50-mL volumetric flask with 0.1 M HNO₃. This was set aside for K analysis.

From the solution in (a), 10-mL aliquot was transferred to a 50-mL volumetric flask. Two mL of 2% SrCl₂ were added and the solution was diluted to the mark with 0.1 M HNO₃. This was set aside for Ca and Mg analyses.

From the solution in (a), a 2-mL aliquot was transferred into a 50-mL volumetric flask and was diluted to the mark with 0.1 M HNO₃. From this solution, a 10-mL aliquot was further transferred to a 50-mL volumetric flask and was diluted to the mark with acidified distilled water. This was set aside for Na analysis.

b. Blank, Spike, and Standard Solutions

A corresponding blank was prepared by following the same procedure above. The blank solution for Ca and Mg analysis was added with two mL of 2% SrCl₂.

The spike solutions were prepared by adding 25.00 mL of 5000 ppm Na, 5.00 mL each of 1000 ppm K, Ca and Mg, 1.00 mL of 100 ppm Fe, and 0.20 mL of 100 ppm Zn solutions into the sample prior to ashing.

Three standard solutions were prepared for each analysis to establish the calibration curve for each parameter. For the analysis of Na and Zn, the standard solutions had the concentrations of 0.1 ppm, 0.5 ppm, and 1.0

ppm. For the analysis of Ca and K, the standard solutions were 1.0 ppm, 3.0 ppm, and 5.0 ppm. For Fe, the standard solutions were 1.0 ppm, 3.0 ppm, and 6.0 ppm. For Mg, the concentrations of standards were 0.1 ppm, 0.3 ppm, and 0.5 ppm. The standard solutions for Ca and Mg contained 2 mL of 2% SrCl₂. All these were prepared from the 10 ppm working solutions of Na, K, Ca, Mg, Fe and Zn by applying the principle of dilution. These were all analyzed using the Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 100).

RESULTS AND DISCUSSION

In general, the concentration of each analysis was reported as the mean of the two batch samples. The proximate analysis is summarized in Table 1.

Table 1. Proximate composition.

Composition	% (mean of means) ± SD**
Moisture	93.11 ± 0.21
Ash	26.41 ± 0.62
Fat	1.23 ± 0.14
Protein	57.40 ± 2.00

* Ash fat and protein values are in % dry basis, moisture value in % wet basis.

** 2 batches of sampling at 3 trials per batch.

The amount of moisture in *S. chloronotus* at 93.11% is higher than the values obtained for *S. californicus* at 80.70% (Nobmann, 1993) and *S. japonicus* at 91.30% (Tanikawa *et al.*, 1955). The amount of ash from *S. chloronotus* samples was found to be 26.41%, which is relatively higher compared with 14.51% for *S. californicus* (Nobmann, 1993) and 20.50% for *S. japonicus* (Tanikawa, 1955).

S. chloronotus has the lowest fat content at 1.23%. *S. californicus* was reported to have 2.07% fat (Nobmann, 1993) while *S. japonicus* has 5.06% (Tanikawa *et al.*, 1955).

Protein content of *S. chloronotus* was found to be 57.4%, which is lower compared with that of *S. californicus* with 67.36% (Nobmann, 1993). It is however higher than *S. japonicus* with 42.38% (Tanikawa *et al.*, 1955).

The macronutrients and micronutrients analyses are presented in Tables 2 and 3.

Table 2. Macronutrient composition.

Composition	ppm (mean of means) ± SD**
Phosphorus	278.0 ± 8.7
Sodium	44.0 × 10 ³ ± 3.0 × 10 ³
Potassium	10.76 × 10 ³ ± 0.56 × 10 ³
Calcium	4830 ± 120
Magnesium	2690 ± 190

** 2 batches of sampling at 3 trials per batch.

Table 3. Micronutrient composition.

Composition	ppm (mean of means) ± SD**
Iron	37.1 ± 1.5
Zinc	32.5 ± 1.7

** 2 batches of sampling at 3 trials per batch.

S. chloronotus showed much higher Ca and Fe content (4830 and 37.1 ppm, respectively) than *S. californicus* (1554 and 31.1 ppm) (Nobmann, 1993).

Based on the results, it is recommended that further studies be conducted on other nutrients in *S. chloronotus* in order to obtain a more comprehensive nutritional profile, as well as the effect of the environmental factors on its nutritional content.

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