Original Article

Chemical Composition and Fatty Acid Profiles of Ball Sea Cucumber (*Phyllophorella kohkutiensis*)

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Abstract

Ball sea cucumber (*Phyllophorella kohkutiensis*) is an edible consumed domestically and exported to foreign countries. The chemical composition (moisture, protein, ash, carbohydrate, lipid) and fatty acid (FA) profiles of the fresh and dried samples with and without tube feet (TF) were investigated. The fresh samples had high moisture content (86.22 to 87.96%), while dried samples showed the highest total protein (70.21 to 78.75%). All samples contained low total lipids (0.01 to 0.10%). The dried samples with TF were found the highest total saturated FA with 63.75% total fatty acid (TFA), while fresh samples without TF had the highest total monounsaturated FA with 23.80% TFA, and total polyunsaturated FA with 21.93% TFA, including Arachidonic acid (ARA), Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA). The essential minerals, calcium (Ca) and zinc (Zn) were 73.38 and 0.047 mg/100 g, respectively. The ball sea cucumber *P. kohkutiensis* provides good nutritional quality for human consumption.

Keywords: ball sea cucumber, proximate compositions, fatty acid profiles, calcium and zinc

1. Introduction

Ball sea cucumbers (*Phyllophorella kohkutiensis* Heding and Panning, 1954) are marine invertebrates with spherical bodies belonging to the phylum Echinodermata, family Phyllophoridae which live on muddy and sandy grounds. They are native species and geologically peculiar to Thailand that can be found along the coasts of the Gulf of Thailand, and the Andaman Sea (Panithanarak, 2022). In three coastal provinces of Thailand including Surat Thani, Satun, and Trat, ball sea cucumbers are used as food, especially in Koh Sarai Sub-district, Satun province where a population density is 0.07 persons per square meter (Kasiroek, 2022). Basically, echinoderms have a water vascular system, usually with tube feet, which helps them with breathing, feeding, and movement in their habitat. The body walls of sea cucumbers are the major edible portion of them consist of 40-60% protein (dry weight basis) which are covered with black tube feet ball as found in *P. kohkutiensis*, the edible sea cucumbers can be eaten in fresh or dried form as well as with- or without tube feet (Wang, Tian, Chang, Xue, & Li, 2020). Besides, various sea cucumber species including the genus *Holothuria*, *Actinopyga*, *Bohadschia*, and *Stichopus*, have been consumed in Asian countries and can be exported in dried, fresh or frozen form in major consuming countries such as China, Hong Kong, Japan, South Korea, and Singapore (Bechtel, Oliveira, Demir, & Smiley, 2013; Aydin, Sevgili, Tufan, Emre, & Köse, 2011; Choo, 2008). Previous studies have been published on the nutritional quality of some sea cucumber species indicating they contain high protein contents, low lipid contents and are rich in essential minerals, especially calcium, and zinc (Liu *et al.*, 2019; Barzkar, Fariman, & Taheri, 2017; Bordbar, Anwar, & Saari, 2011), and natural products such as collagen, fatty acids, steroids, and polysaccharides. In addition, sea cucumbers are considered a source of fatty acids, especially N-6 and N-3 highly unsaturated fatty acids (HUFAs) such as ARA (Arachidonic acid; C20:4n6), EPA (Eicosapentaenoic acid; C20:5n3) and DHA (Docosahexaenoic acid; C22:6n3) which play an important role in inflammatory response control through eicosanoid production (Yu *et al.*, 2016; Oliver, McGillicuddy, Phillips, Toomey, & Roche, 2010; Lee *et al.*, 2009). Sea cucumbers can be marine animal resources with great economic value; however, the chemical composition related to their nutritional value and fatty acid profiles are still limited. Therefore, the objectives of this study were to investigate the proximate composition, Ca and Zinc content of ball sea cucumber *P. kohkutiensis*, and fatty acid profiles in the fresh and dried samples including with and without tube feet to obtain information about the nutritional quality of it for human consumption and application in value added of local products development.

2. Materials and Methods

Sample collection and preparation

Ball sea cucumber sampling

The samples of ball sea cucumber (*Phyllophorella kohkutiensis* Heding & Panning, 1954) were collected from three stations at Koh Sarai, Satun province by dragging at a depth of 5-15 meters. The samples were soaked in seawater and internal organs were removed. Then, they were kept on ice for 24 hours and taken to the Institute of Marine Science's Laboratory.

Sample preparation

In the laboratory, morphometric measurements were examined and classified by comparing with the references according to the sea cucumber identification guides of

Heding & Panning (1954) (Clark & Rowe, 1971; Putchakarn, 1998). After that, ball sea cucumber samples were divided into four types of them including fresh and dried samples with and without tube feet. The tube feet which are the outer black of their body wall were carefully scraped off, then cleaned under running water. The whole-body walls with and without tube feet were cut into small pieces. For the dry process, samples were allowed to dry by sun exposure and air at the environment temperature for 10 hours. Four types of sample preparation were used for proximate compositions and fatty acid analysis, while the fresh sample without tube feet was estimated calcium and zinc content by the central laboratory.

Proximate composition, essential minerals, and fatty acids analysis

Proximate composition analysis

Total protein was analyzed by the Kjeldahl method in which the content of the crude protein was estimated as the content of the nitrogen (N)×6.25. Total lipid content was analyzed by Gravimetric Method (AOAC, 2000) using the Soxhlet extraction technique. Additionally, moisture (AOAC, 2000) and ash (Muffle furnace; AOAC, 2000) were also analyzed. The nitrogen-free extract was calculated by following the formula as NFE = 100 - moisture - ash - protein - lipid

Calcium and Zinc contents analysis

The estimation of macro (Ca) and micro (Zn) contents were analyzed using the inductively coupled plasma (ICP) technique with the following AOAC (2019) 984.27 and AOAC (2019) 999.10 method by the central laboratory.

Lipid extraction and fatty acids analysis

Total lipids were extracted from 0.5 g of a dry sample and 10 g of a wet sample according to Bligh & Dyer (1959). All samples were dissolved in chloroform (containing

BHT 0.1 ppm) : methanol (containing BHT 0.1 ppm) in a ratio of 2:1, pooled the solvent, and dried under nitrogen gas. Fatty acids methyl esters were prepared by acid-catalyzed transmethylation of total lipids (10 ml of 1% sulphuric acid in methanol and placed in an oven at 50ºC for 16 hours. Added 5 ml of 5% sodium chloride, 5 ml of hexane, and 40 ml of 2% potassium bicarbonate and then filtered through anhydrous sodium sulfate and dried under nitrogen gas (Christie, 2003).

Separation and identification of fatty acids were carried out and analyzed in Agilent Technologies GC7820A equipped with a FAME WAX, USA fused silica capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness), using helium as carrier gas at 1.1 ml/min. Samples were injected with 1 µl at the following conditions. The column temperature was 120ºC during 0.5 min, the thermal gradient to 170ºC at a rate of 5ºC min^{-1}, maintained for 10 min, 170 to 190ºC at a rate of 3ºC min^{-1}, maintained for 15 min, 190 to 210ºC at a rate of 2ºC min^{-1} and maintained for 15 min. Injector and flame ionization detector temperatures were 240ºC and 260ºC, respectively. Fatty acids methyl esters were identified by comparison with known standard mixtures (Supelco 37-Component FAME Mix, Supelco, USA) and quantified by area percent of total fatty acid.

Data analysis

The experimental data were calculated and represented as the mean and standard deviation. Then, variance and comparisons of fatty acid content between the groups were analyzed with One way ANOVA with Duncan's test by using R (Team, 2020). The significance level of $p < 0.05$ was used.

3. Results and Discussion

Proximate composition in the ball sea cucumber

Proximate compositions (moisture, protein, ash, carbohydrate, lipid) of the fresh and dried samples with and without tube feet are shown in Table 1. The fresh samples contained the highest moisture content (86.22 to 87.96%), whereas dried samples had the highest percentage of total protein (70.21 to 78.75%). The moisture content of fresh samples ranged between 86.22% and 87.96%, while dried samples ranged between 5.81% and 6.50%, respectively. For the fresh samples with and without tube feet, the percent levels of protein, ash, carbohydrate, and lipid were in the range of 8.55 - 11.16, 1.47 - 2.62, 0.84 - 1.14, and 0.01 - 0.04, respectively. Fresh samples of ball sea cucumber *P. kohkutiensis* had high moisture contents which were similar to reported data found in various fresh sea cucumber species ranging from 67.92% to 92.42%, and proximate compositional data of *P. kohkutiensis* was close to contents of moisture (85.24%), protein (7.88%), ash (5.13%), and lipid (0.09%) in *Holothuria mammata* (Ardiansyah, Bayu, Wulandari, & Putra, 2022). The high values of moisture found in ball sea cucumbers were due to marine animals comprising high water content (Sales *et al.*, 2021). The protein content in fresh samples without tube feet (11.16%) was higher than in fresh samples with tube feet (8.55%). Fresh samples of ball sea cucumber *P. kohkutiensis* had percentages of total protein, which were higher than those reported in *Apostichopus japonicus* (3.40%), *Holothuria arenicola* (4.40%), and *Stichopus horrens* (3.47%) but were lower than the protein content observed in *H. parva* (17.61%), *Bohadschia marmorate* (43.23%), and *S. chloronotus* (57.93%) (Ardiansyah, Bayu, Wulandari, & Putra, 2022; Barzkar, Fariman, & Taheri, 2017; Omran, 2013). The dried samples with and without tube feet had high contents of protein (70.21 to 78.75%), ash (14.29 to 23.62%), carbohydrate (0.26 to 0.37%), and very low content of lipids (0.08 to 0.10%). Dried samples of ball sea cucumber *P. kohkutiensis* contained high protein contents which were higher than those previous reports in various dried sea cucumber species ranging from 36.99% to 63.30% (Ardiansyah, Bayu, Wulandari, & Putra, 2022; Wen, Hu, & Fan, 2010). Mostly, sea cucumbers have a high nutritional quality which is valuable because of their high protein content and low amount of lipids; however, their proximate compositions are varied depending on species, seasonal variations, environmental factors, feeding behavior, and geographic location (Ardiansyah, Bayu, Wulandari, & Putra, 2022).

Ca and Zn contents in the ball sea cucumber

The macro (Ca) and micro (Zn) contents in the fresh sample without tube feet were 73.38 and 0.047 mg/100 g, respectively. Both values of essential elements were close to prior reporting in *H. Arenicola* which contained 83.25 mg/100 g calcium and 0.04 mg/100 g zinc (Barzkar, Fariman, & Taheri, 2017). However, the calcium content in *H. sanctori* (656.73 mg/100 g) had higher than the sample in this study. Calcium is one of the macro minerals that have crucial for the growth and maintenance of bones, teeth, and muscles. Zinc is one of the most important trace elements involved in immune system activity and metabolism function (Göçer, Olgunoglu, & Olgunoglu, 2018).

Fatty acid profiles in the ball sea cucumber

The profile of fatty acids in four types of ball sea cucumber samples was significantly different ($P<0.05$) which is shown in Table 2. The result showed the highest amount of fatty acids in ball sea cucumber *P. kohkutiensis* was lauric acid (C12:0), palmitic acid (C16:0), steric acid (C18:0), oleic acid (C18:1n9), eicosenoic acid (C20:1n9), and arachidonic acid (C20:4n6), representing the saturated fatty acid (SFAs) was in the range of 24.64 - 63.75% TFA, the monounsaturated fatty acids (MUFAs) was in the range of 16.76 - 23.80% TFA, and the polyunsaturated fatty acids (PUFAs) was in the range of 8.11 - 21.93% TFA, respectively. Previous studies have reported the most dominant component in SFAs, MUFAs, and PUFAs are palmitic acid, eicosenoic acid, and arachidonic acid, respectively (Ardiansyah, Bayu, Wulandari, & Putra, 2022). Moreover, the sample without tube feet provided the essential fatty acids, whereas the sample with tube feet delivered the saturated fatty acids, especially lauric acid which can be used for acne treatment as a natural antibiotic against *Propionibacterium acnes* (Nakatsuji *et al.*, 2009). The finding is due to tube feet being composed of lipid membranes with a high content of saturated fatty acids. The highest total SFAs was markedly found in dried samples with tube feet (63.75% TFA), while fresh samples without tube feet showed the highest total MUFAs (23.80% TFA) and total PUFAs (21.93% TFA). For PUFAs, arachidonic acid (ARA, C20:4n6), eicosapentaenoic acid (EPA, C20:5n3), and docosahexaenoic acid (DHA, C22:6n3) were found in four types of ball sea cucumber samples. In addition, ARA content presented as the main fatty acid of PUFAs in all samples, had higher than EPA content which was similar to prior studies reported in *H. poli* and *H. mammata* (Aydin, Sevgili, Tufan, Emre, & Köse, 2011), *Thelenota anax* and *Bohadschia marmorata* (Nishanthan, Kumara, Croos, Prasada, & Dissanayake, 2018). The omega-6 PUFA, ARA or its precursor linoleic acid (LA) plays a structural and functional role in cell membranes involving the nervous system, skeletal muscle, and immune system. (Tallima, & Ridi, 2018). EPA and DHA belong to the omega-3 PUFA which play a key role in the prevention and treatment of coronary artery disease, diabetes, hypertension, arthritis, and other inflammatory and autoimmune disorders (Rogero *et al.*, 2020). The balanced omega-6/omega-3 ratio is a critical factor for health throughout the life cycle and the proper ratio for omega-6/omega-3 is 3:1 to 4:1 could prevent the pathogenesis of many diseases (Simopoulos, 2016). These ratios were found in the dry ball sea cucumber with tube feet (5.50:1.88). A target of omega-6/omega-

3 is 1:1 to 2:1 and is considered adequate which appears to be consistent with studies on the evolutionary aspects of diet, neurodevelopment, and genetics. The ratio of 2:1 of omega-6/omega-3 appeared in the fresh ball sea cucumber without tube feet (14.33:6.72), the dry ball sea cucumber without tube feet (10.07:4.65), and the fresh ball sea cucumber with tube feet (10.77:5.09). In addition, excessive amounts of omega-6 PUFA and a very high omega-6 to omega-3 ratio, can be promoted as pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, and interfere with normal brain development (Simopoulos, 2008).

4. Conclusions

The nutritional composition study of edible ball sea cucumber (*P. kohkutiensis*) by quantitative analysis found high protein and low lipid content, especially fresh ball sea cucumber without tube feet found high levels of omega-3 fatty acids, EPA and DHA, and essential minerals including calcium and zinc which be suitable for human consumption in term of functional food. However, the dried ball sea cucumber with tube feet provided high content of lauric acid used as a natural antibiotic in the cosmetics industry.

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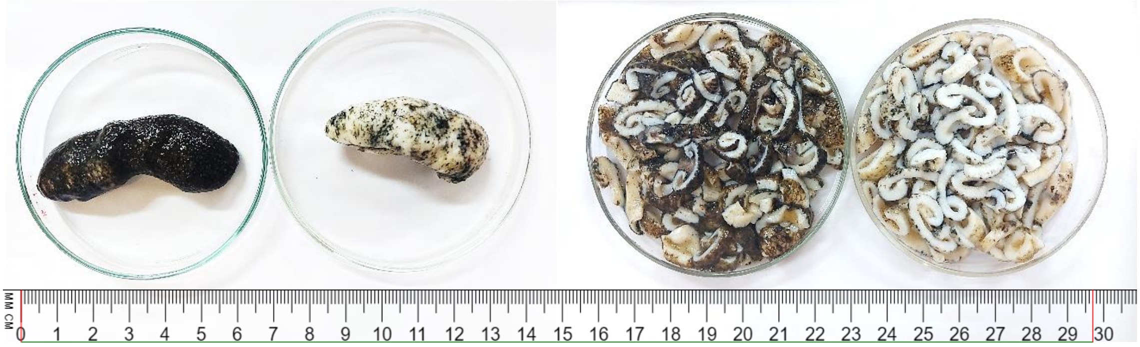
Figure



(a) (b) (c) (d)

Figure 1 the fresh sample including with (a) and without (b) tube feet; small pieces of fresh sample including with (c) and without (d) tube feet of ball sea cucumber *Phyllophorella kohkutiensis*

Table

Table 1 Proximate composition (%w/w ± standard deviation) of ball sea cucumber *Phyllophorella kohkutiensis*

Proximate Content (%)	Sample types			
	Dried		Fresh	
	with tube feet	without tube feet	with tube feet	without tube feet
Moisture	5.81 ± 0.56	6.50 ± 0.06	87.96 ± 0.55	86.22 ± 0.33
Ash	23.62 ± 1.08	14.29 ± 0.38	2.62 ± 0.29	1.47 ± 0.10
Lipid	0.10 ± 0.01	0.08 ± 0.00	0.04 ± 0.01	0.01± 0.00
Protein	70.21± 1.39	78.75 ± 0.50	8.55 ± 0.40	11.16 ± 0.41
Carbohydrate	0.26 ± 0.07	0.37 ± 0.17	0.84 ± 0.16	1.14 ± 0.02

Note: Carbohydrate (nitrogen-free extract, NFE) = 100 - moisture - ash - protein - lipid

Table 2 The profile of fatty acids in the four types of ball sea cucumber samples

Fatty acids	Sample types			
	Dried		Fresh	
	with tube feet	without tube feet	with tube feet	without tube feet
C6:0	nd	0.23±0.02[b]	0.76±0.01[a]	nd
C8:0	nd	nd	nd	nd
C10:0	0.33±0.00[b]	nd	1.13±0.01[a]	0.45±0.31[b]
C11:0	nd	nd	nd	nd
C12:0	22.42±0.22[a]	1.15±0.12[d]	7.27±0.08[b]	2.13±0.21[c]
C13:0	nd	nd	nd	nd
C14:0	15.50±0.16[a]	3.60±0.13[c]	5.85±0.06[b]	2.94±0.30[d]
C15:0	0.44±0.01[a]	0.86±0.08[b]	1.59±0.02[c]	0.89±0.10[b]
C16:0	12.52±0.10[a]	9.22±0.64[b]	7.33±0.04[c]	5.43±0.55[d]
C17:0	2.17±0.04[b]	6.70±0.61[a]	0.51±0.10[c]	0.50±0.10[c]
C18:0	7.21±0.08[b]	8.08±0.55[a]	5.86±0.03[c]	5.74±0.54[c]
C20:0	1.17±0.02[c]	2.56±0.21[a]	1.99±0.01[b]	2.33±0.21[a]
C21:0	0.64±0.01[d]	1.44±0.11[b]	1.21±0.01[c]	1.65±0.14[a]
C22:0	0.90±0.09[c]	1.95±0.14[a]	1.51±0.03[b]	1.64±0.17[b]
C23:0	0.28±0.00[c]	0.68±0.10[a]	0.46±0.00[b]	0.55±0.05[b]
C24:0	0.18±0.01[c]	0.34±0.02[ab]	0.22±0.01b[c]	0.40±0.14[a]
Total SFAs	63.75±0.68[a]	36.60±2.56[b]	35.70±0.16[b]	24.64±2.36[c]
C14:1	nd	nd	0.15±0.00[b]	0.17±0.00[a]
C15:1	nd	nd	0.16±0.10[a]	0.46±0.48[a]
C16:1n7	1.73±0.01[c]	3.25±0.27[a]	3.34±0.09[a]	2.58±0.25[b]
C17:1	0.28±0.02[c]	0.69±0.02[b]	0.69±0.21[b]	1.09±0.19[a]
C18:1n9 (c+t)	7.98±0.03[a]	3.65±0.31[b]	3.76±0.02[b]	3.48±0.32[b]
C20:1n9	4.32±0.14[c]	8.76±0.70[b]	7.95±0.13[b]	10.24±1.01[a]
C22:1n9	nd	nd	nd	nd
C24:1n9	2.29±0.06[c]	4.46±0.23[b]	4.14±0.05[b]	5.77±0.98[a]
Total MUFAs	16.76±0.30[c]	20.47±1.12[b]	20.19±0.55[b]	23.80±0.08[a]
C18:2n6 t	1.04±0.01[b]	0.75±0.04[d]	0.89±0.03[c]	1.19±0.13[a]
C18:2n6 c	0.31±0.03[b]	0.56±0.03[a]	0.20±0.01[c]	0.31±0.04[b]
C18:3n6	0.33±0.01[b]	0.74±0.06[a]	0.31±0.32[b]	0.81±0.07[a]

C18:3n3	0.15±0.01[b]	0.44±0.04[a]	nd	nd
C20:2	0.57±0.21[a]	1.36±1.14[a]	0.51±0.03[a]	0.59±0.07[a]
C20:3n6	nd	nd	nd	nd
C20:4n6 (ARA)	3.83±0.03[d]	7.93±0.48[c]	9.23±0.01[b]	11.70±1.08[a]
C20:3n3	nd	0.63±0.24[a]	0.16±0.00[b]	0.20±0.04[b]
C20:5n3 (EPA)	1.49±0.11[d]	3.07±0.15[c]	4.34±0.00[b]	5.60±0.47[a]
C22:2	0.15±0.00[b]	nd	0.07±0.10[ab]	0.28±0.03[a]
C22:6n3 (DHA)	0.24±0.03[b]	0.49±0.03[b]	0.58±0.01[ab]	0.92±0.36[a]
Total PUFAs	8.11±0.26[c]	16.26±0.41[b]	16.43±0.38[b]	21.93±1.63[a]
Σn3	1.88	4.65	5.09	6.72
Σn6	5.50	10.07	10.77	14.33
Σn3/ Σn6	0.34	0.46	0.47	0.47
Σn6/ Σn3	2.92	2.17	2.12	2.13

Note: Data are the mean values of three replicates ± standard deviation. Means within the same row without a common lowercase letter differ significantly ($p<0.05$) nd= not detected, SFAs = saturated fatty acids, MUFAs= monounsaturated fatty acids, PUFAs= polyunsaturated fatty acids.

The Answer Sheet

Submission ID: **SJST-2022-00052**
Title: **Chemical Composition and Fatty Acid Profiles of Ball Sea Cucumber (*Phyllophorella kohkutiensis*)**

The modification list is shown below:

Comments / Questions	Modifications
Abstract	
What is TFA in Line 20?	It is a % total fatty acid (TFA). I wrote in a full name for the first time, then used an abbreviation after that. Please see lines 17-18.
It would be better to inform the finding of this study according to the key words of the title. Since chemical composition is started first, this should mention how much water content? and the rest, total solid, is divided into which component. Then, what the matter with lipid in ball cucumber and finally wrap up with lipid profile in your samples.	I revised the contents of the abstract with the flow of keywords in the title as well. Please see lines 11-23.
Line: 15-17, It is a bit confusing with the different essential fatty acids, assessed by four different methods. What are those methods and how different?	
Please check the unit of minerals Ca and Zn in Line 24 "mg/kg wet wt."	I checked and used the unit of minerals Ca and Zn with "mg/ 100 g". Please see line 21.
Introduction	
Line 61: If this research is applicable, more consumption of this animal resource would be increased. How come this work will provide sustainable utilization? Please clarify?	I revised the objectives and results of this study, then provided suitable utilization that has a possibility related to this research data. Please see lines 27-60, with the highlight of new contents and references.
It is not sound to the readers that only chemical composition and fatty acid provide strong scientific evidence to utilize this material as medical health food utilizations?	
Materials and Methods	
Drying method was not scientifically performed. Why does it have to be sun drying? Please provide the justification.	I decided to use sun drying in process because local people always let the ball sea cucumber drying in this way before exporting to foreign countries. They are the dried samples that we need to know about the nutritional values for human consumption and application in value added of local products development.
The sample preparation methods are not clear.	I agreed with you, then rewrote it step-by-step for clarification. Please see lines 70-80.
Line: 72-75: Which parts of cucumber are used for performing the chemical composition? Whole, without black skin?	I revised the sample preparation and already rewrote, please see lines 72-76.
Line 80-85: What is the main methodology for this section?	Please see lines 70-72.

Results and Discussion	
Please discuss proximate analysis and mineral content separately	Yes, I did it. Please see lines 118-157.
Please inform the reader that there are many varieties or species of ball cucumber. Then, the results from this study can be compared to the previous one	Yes, I revised the results in this study with comparing previous studies that reported in the chemical composition, fatty acid profiles, and mineral content of various sea cucumber species.
Is it trustable to claim that sea cucumbers are useful for medicine and high nutrition? As the current MS, this bioresource is just normal biomaterial containing a potential for being food products	I revised and provided suitable utilization that has a possibility related to this research data. Please see lines 22-23; 59-60; 200-205.
There must be several mineral content in this biomaterial. Why only Ca and Zn focused? How about others?	In fact, several elements and essential minerals that are interested in studying and can be found in marine animals. I decided to study calcium content in ball sea cucumber because it has calcite ($CaCO_3$) which is a component of ossicle embedding in its body wall, so Ca content is very high. Besides, I chose zinc as representing of minor or trace mineral with expectation in potential benefit of property about anti-inflammation, reduction in oxidative stress and maintaining a healthy immune system related to the epidermic of Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) (Dhawan, Emran, & Choudhary, 2022; Prasad, Malysa, Bepler, Fribley, & Bao, 2022; Li, Kong, Chen, Liu, & Zhang, 2018).
Conclusions	
Based on the results, there is no information indicating the potential of using this material for medical application	Please see lines 200-205 and 170-171.
Table	
It is better to separate the proximate analysis data out of mineral contents	Yes, I did it.
Table 2, The head of the table should be modified. Here is an example	Yes. I changed the new head of Table 2, and Table 1 too.
Figure	
Figure 1: There are 3 sub figures here. What are they? It must be divided into sub figures A, B and indicated what are they? Why do you have to put this one in this figure (Kasiroek, 2022)? It should be figures taken by this experiment	Yes. I put new pictures and description of samples in Figure 1 as follow.