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*Original Article*

# Chemical compositions and fatty acid profiles of ball sea cucumber (*Phyllophorella kohkutiensis*)

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## Abstract

Ball sea cucumber (*Phyllophorella kohkutiensis*) is an edible marine animal that is both consumed domestically in Thailand and exported to foreign countries. The chemical compositions (moisture, protein, ash, carbohydrate, lipid) and fatty acid (FA) profiles of fresh and dried samples, with and without tube feet (TF), were investigated. The fresh samples had a high moisture content (86.22 to 87.96%), while the dried samples showed high total protein (70.21 to 78.75%). All samples contained low levels of total lipids (0.01 to 0.10%). The dried samples with TF were found to have the highest total saturated FA with 63.75% of total fatty acid (TFA), while fresh samples without TF had the highest total monounsaturated FA at 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 at 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 composition, fatty acid profile, 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, the family Phyllophoridae, and living on muddy and sandy grounds. They are native species and geographically peculiar to Thailand, and can be found along the coasts of the Gulf of Thailand and the Andaman Sea (Panithanarak, 2022). In three coastal provinces of Thailand, namely Surat Thani, Satun, and Trat, ball sea cucumbers are used as food, especially in Koh Sarai sub-district of Satun province where the population density is only 0.07 persons per square kilometer (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, consisting of 40-60% protein (dry weight basis), and they 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 the 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 to the major consuming countries, such as China, Hong Kong, Japan, South Korea,

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and Singapore (Aydin, Sevgili, Tufan, Emre, & Köse, 2011; Bechtel, Oliveira, Demir, & Smiley, 2013; Choo, 2008). Previous studies have reported on the nutritional quality of some sea cucumber species, indicating that they have high protein contents, low lipid contents, and are rich in essential minerals, especially in calcium and zinc (Barzkar, Fariman, & Taheri, 2017; Bordbar, Anwar, & Saari, 2011; Liu *et al*., 2019), and in the natural products collagen, fatty acids, steroids, and polysaccharides. In addition, sea cucumbers are considered good sources 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 (Lee *et al*., 2009; Oliver, McGillicuddy, Phillips, Toomey, & Roche, 2010; Yu *et al*., 2016). Sea cucumbers can be marine animal resources with great economic value; however, information available on their chemical composition in relation to their nutritional value, and on their fatty acid profiles, is still limited. Therefore, the objectives of this study were to investigate the proximate composition, Ca and Zn contents, in ball sea cucumber *P. kohkutiensis*, and fatty acid profiles in the fresh and dried samples, both with and without the tube feet, to obtain information about the nutritional quality for human consumption, and for applications to local product development for added value.

## 2. Materials and Methods

### 2.1 Sample collection and preparation

#### 2.1.1 Ball sea cucumber sampling

The samples of ball sea cucumber (*Phyllophorella kohkutiensis* Heding & Panning, 1954) were collected from three stations at Koh Sarai, in Satun province, by dragging at depths 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.

#### 2.1.2 Sample preparation

In the laboratory, morphometric measurements were examined and the samples were classified by comparing with the sea cucumber identification guides of Clark and Rowe (1971), Heding and Panning (1954), and Putchakarn (1998). After that, the ball sea cucumber samples were divided into four lots for preparing fresh and dried samples with and without tube feet. The tube feet, which are black on the outer 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 drying the samples, they were allowed to dry under the sun exposed to air at the ambient temperature for 10 hours. The four types of prepared samples were subjected to analyses of proximate composition and fatty acid profile, while the fresh sample without tube feet was determined for calcium and zinc contents by the central laboratory.

### 2.2 Proximate composition, essential minerals, and fatty acids

#### 2.2.1 Proximate composition analysis

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

#### 2.2.2 Calcium and zinc contents

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

#### 2.2.3 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, the solvent was pooled, and then dried under nitrogen gas. Fatty acid 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. Then 5 ml of 5% sodium chloride, 5 ml of hexane, and 40 ml of 2% potassium bicarbonate were added, and the mix was filtered through anhydrous sodium sulfate and dried under nitrogen gas (Christie, 2003).


Figure 1. Fresh samples with (a), and without (b) the tube feet. Small pieces of fresh samples with (c), and without (d) the tube feet, of ball sea cucumber *Phyllophorella kohkutiensis*

Separation and identification of the fatty acids were carried out using 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), with helium as the carrier gas at 1.1 ml/min. Samples injected were 1 µl size, at the following conditions. The column temperature was 120ºC for 0.5 min, then thermal gradient to 170ºC was ramped at a rate of 5ºC $min^{-1}$, maintained for 10 min, then ramping from 170 to 190ºC at a rate of 3ºC $min^{-1}$, maintaining this for 15 min, and from 190 to 210ºC at a rate of 2ºC $min^{-1}$ and maintaining for 15 min. Injector and flame ionization detector temperatures were 240ºC and 260ºC, respectively. Fatty acid methyl esters were identified by comparison with known standard mixtures (Supelco 37- Component FAME Mix, Supelco, USA) and quantified by peak area percentage of total fatty acids.

#### 2.2.3 Data analysis

The experimental data are represented as means with standard deviations. Then, variances and comparisons of fatty acid contents between the groups were analyzed with one-way ANOVA and Duncan's test by using R software (Team, 2020). The significance level of $p < 0.05$ was used.

## 3. Results and Discussion

### 3.1 Proximate compositions of the ball sea cucumbers

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 had the highest moisture content (86.22 to 87.96%), whereas the dried samples had the highest percentages of total protein (70.21 to 78.75%). The moisture content of fresh samples ranged between 86.22% and 87.96%, while for dried samples it ranged between 5.81% and 6.50%. For the fresh samples with and without tube feet, the percent levels of protein, ash, carbohydrate, and lipid were in the ranges 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 those previously reported for various fresh sea cucumber species, ranging from 67.92% to 92.42%, and the proximate composition data for *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 moisture levels found in ball sea cucumbers were due to these marine animals holding high water contents (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 total protein levels higher than those reported for *Apostichopus japonicus* (3.40%), *Holothuria arenicola* (4.40%), and *Stichopus horrens* (3.47%) but lower than the protein contents observed for *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%), along with 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* had high protein contents, higher than those previously reported in various dried sea cucumber species that ranged 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).

### 3.2 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 levels of these essential elements were close to prior reporting for *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) was higher than for the sample in this current study. Calcium is one of the macro minerals that are 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 metabolic functions (Göçer, Olgunoglu, & Olgunoglu, 2018).

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

| Proximate content (%) | Sample type | | | |
|---|---|---|---|---|
| | 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

### 3.3 Fatty acid profiles in the ball sea cucumber

The profiles of fatty acids in the four types of ball sea cucumber samples differed significantly ($P<0.05$) as shown in Table 2. The results show the highest amounts of fatty acids in ball sea cucumber *P. kohkutiensis* for 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) in the range of 24.64 - 63.75% TFA, while the monounsaturated fatty acids (MUFAs) were in the range 16.76 - 23.80% TFA, and the polyunsaturated fatty acids (PUFAs) in the range 8.11 - 21.93% TFA, respectively. Previous studies have reported the dominant components in SFAs, MUFAs, and PUFAs as 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 the 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 the tube feet showed the highest total MUFAs (23.80% TFA) and total PUFAs (21.93% TFA). Regarding the PUFAs, arachidonic acid (ARA, C20:4n6), eicosapentaenoic acid (EPA, C20:5n3), and docosahexaenoic acid (DHA, C22:6n3) were found in the four types of ball sea cucumber samples. In addition, ARA presented as the main fatty acid of PUFAs in all samples, and it was 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), play 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). A 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, which 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 promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, and can interfere with normal brain development (Simopoulos, 2008).

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

| Fatty acid | Sample type | | | |
|---|---|---|---|---|
| | 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 | $2.29±0.06^{c}$ | $4.46±0.23^{b}$ | $4.14±0.05^{b}$ | $5.77±0.98^{a}$ |

Table 2. Continued

| Fatty acid | Sample type | | | |
|---|---|---|---|---|
| | Dried | | Fresh | |
| | with tube feet | without tube feet | with tube feet | without tube feet |
| 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.

## 4. Conclusions

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

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