

A study on the nutrient substances of sea cucumber *Stichopus variegatus* flour using vacuum oven

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Abstract

The commercially cultured *Stichopus variegatus*'s flesh from South Lampung, Indonesia was dried using a vacuum oven and grinded to make a sea cucumber flour sizing of 60 mesh. The nutrient component was analyzed for proximate, amino acid profile, and fatty acid profile. The result showed that *Stichopus variegatus* flour contained $34.33 \pm 0.10\%$ protein and $1.08 \pm 0.01\%$ fat. The amino acid of flour consisted of essential and non-essential amino acids with glycine ($4.99 \pm 0.01\%$) and lysine ($5.79 \pm 0.01\%$) as the highest ones. The lipid of the sea cucumber flour contained palmitic acid ($3.51 \pm 0.02\%$) and stearic acid ($2.16 \pm 0.03\%$) as the highest ones. Meanwhile, DHA that contained arachidonic acid as the highest of all fatty acids was detected only in a fresh sea cucumber. The nutrient substances of sea cucumber flesh to flour decreased, the sea cucumber flour still are potential source to be develop as functional food.

Keywords

Flour

Nutrient component

Stichopus variegatus

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Introduction

Sea cucumbers are potential marine commodity in the subsector of fisheries that can be exported in the dried form. They can also be frozen, cooked-dried, cooked-salted and cooked-salted-dried products. Bordbar *et al.* (2011) mentioned that sea cucumbers have many kinds of nutriments, and they can be made into tonic products, due to high protein as proven by Haider *et al.* (2015). So that, they has been developed to be functional food for food industry and pharmacy

Sea cucumbers are well-known as functional food because they have a beneficial effect on human health. Bordbar *et al.* (2011) mentioned that several papers published in the last two decades found that sea cucumber extracts exert medicinal effects as wound healing promoter, anticancer, and have immunomodulatory properties. In contrast, Bruckner (2005) mentioned that there are only few papers regarding to nutrient substances being used in the geographical classification of aquatic food material. In fact, the local environmental factors have influenced the nutrient substances especially the water environment as mentioned by Liu *et al.* (2010)

Stichopus variegatus also can be used as protein source in food. Saito *et al.* (2002) determined that the major edible portion is the body wall mainly

consisting of collagen and mucopolysaccharides. High concentration of collagen in the body wall recommends significantly higher value for nutraceutical or pharmaceutical application as proven by Liu *et al.* (2010). Amoo *et al.* (2006) said that its protein content is higher than the raw material, and its moisture becomes lower than the fresh flesh. Recent study of Hamaguchi *et al.* (2010) stated that two differently processed tissue of *Cucumaria frondosa* products were source of bioactive ingredients.

A few studies on sea cucumbers in Indonesia have been conducted, but there is a scarce information regarding to *Stichopus variegatus*, especially that is commercially harvested in South Lampung, Indonesia. There is a few information exists relating to *Stichopus variegatus* sea cucumber species of Indonesia in terms of their nutrient composition and the effect of processing. In this study, the processing of flour sea cucumbers was using a vacuum oven. A vacuum oven was suggested more economical and applicable scale up for industry than freeze drying. So, this study aimed to analyze the nutrient substances of fresh flesh and flour of *Stichopus variegatus* from Lampung, Indonesia as preliminary step and base line data for the development of functional food product or further research field based on the sea cucumber flour using a vacuum oven.

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Materials and Methods

Flour preparation

Fresh sea cucumber (*Stichopus variegatus*) were purchased alive from local fisherman in South Lampung, Indonesia during February – June 2014. Thirty randomly selected sea cucumbers, were measured to obtain sample with 600 grams in weight and 25 cm in length. The internal organs of sea cucumber were removed then washed with sea water. Samples were placed in the ice bucket and transported to laboratory at the Department of Food Science and Technology, Bogor Agriculture University. Then, fresh flesh of sea cucumber was cut, dried with vacuum oven (50°C, 65 cmHg, 4h), and milled to obtain 60-mesh flour. All flour samples were blended and packaged in frozen at -20°C until used for analysis. An analysis of nutrient composition of fresh flesh and flour were conducted.

Proximate analysis

Proximate analysis was conducted according to standard procedure from the Association of Official Analytical Chemist (AOAC, 2005). Moisture content analysis was conducted by drying the sample in an oven at 102-105°C and dried until reached a constant weight. Ash content was determined by incinerating 5 g of the sample at 600°C for 6 h. Crude fat content was analyzed by Soxhlet extraction method using n-hexane as solvent. Nitrogen was determined using Kjeldahl method and the quantity of protein was calculated by multiplying the percentage of nitrogen content by the conversion factor of 6.25. All analyses were repeated three times. Results were expressed as mean values \pm standard deviation (SD).

Amino acid analysis

Amino acid analysis was determined according to AOAC method using high performance liquid chromatography (AOAC, 2005). Samples were hydrolyzed with 5 mL 6N HCl for 22 h at 110°C and derivatized using Alpha Amino Butiric Acid (AABA) prior to injection onto HPLC system (AccQtag column (3.9 x 150 mm; used for separation purposes)) maintained at 37°C. The mobile phase consisting of acetonitrile 60% – AccqTag Eluent A was flushed through the column at flow rate of 1 mL/min using a linear gradient system. The fluorescence absorption detector at a wavelength of 260 nm was employed to monitor amino acids. The identification of unknown amino acids was based on the comparison of their retention times with pure standards, whereas standard calibration curves were used for quantification purpose. Amino acid

standards (Thermo Scientific) used were aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, methionine, valine, phenylalanine, isoleucine, leucine, proline and lysine. All results are expressed as means and with standard errors of three separate contents.

Fatty acid analysis

Fatty acid analysis were conducted according to AOAC (2005). Lipid obtained from soxhlet destruction were transmethylated by refluxing the fractions in alkalized (sodium hydroxide) methanol for 20 min at 80°C. Samples were cooled and converted into fatty acid methyl esters (FAME) using 16% boron trifluoride in methanol. Internal standard was added to aid the identification of FAME. FAME were separated and quantified by Shimadzu Gas Chromatograph (GC) 17A (Shimadzu Corporation, Kyoto, Japan) fitted with a Flame Ionisation Detector (FID). Gas chromatography (GC) was performed with an Agilent Technologies 6890 gas chromatograph equipped with an HP-5 cross-linked cyanopropyl methylsil capillary column (60 m \times 0.25 mm I.D., 0.25 μ m film thickness). Helium was the carrier gas. Oven temperature was programmed from 190°C to 230°C, gradient temperature was performed by 10°C/min, and gas flow was 20°C/min for N₂ and 30°C/min for H₂. Peak area was quantified and expressed as percentage of total fatty acids. All standards used in the identification of peaks were purchased from Supelco® (Bellefonte, PA). All results are expressed as means and with standard errors of three separate contents.

Results and Discussion

Proximate analysis

Stichopus variegatus that decreased mainly in length and weight during washing and preparation before transportation was processed into flour. It yielded 5.12 \pm 1.03% of its initial weight through processing into dried form. This results was similar to Purcell *et al.* (2009) studied that *Stichopus herrmanni* in which it decreased much more in length and lost weight (around 96.7 percent) during processing into beche-de-mer. For a vacuum oven processing, the depreciation weights was estimated 27.77 \pm 14.54% of fresh flesh to flour form. Table 1 showed that the main features of sea cucumber were high quantity of protein and very low fat content. The protein content of 2.65 g/100g wet weight determined in this study was similar to Forghani *et al.* (2012) result using other species of sea cucumbers namely *Stichopus horrens*.

Table 1. Proximate composition of *Stichopus variegatus* (% w/w, dry weight)

Parameters	Fresh flesh	Flour
Moisture	93.36 ± 0.02	6.27 ± 0.01
Ash	1.50 ± 0.63	4.34 ± 0.14
Protein	39.80 ± 0.02	34.33 ± 0.10
Fat	3.39 ± 0.33	1.08 ± 0.01

In this study, *Stichopus variegatus* flour showed 34.33 ± 0.10% protein. The nutrient component between fresh flesh and flour were different because of the processing effect. In addition, the transporting process has taken a long time (± 12 h) to laboratory so that it suspected lysis process results in a water soluble nutrient components reduced (Pickering and Newton 1990) after it was caught. The ash and fat contents determined in the present study were different from previous studies reported for *Parastichopus parvimensis*, *Parastichopus californicus*, *Acaudina molpadioides*, *Holothuria tubulos*, and *Apostichopus japonicas* sea cucumbers as proven by Sicuro *et al.* (2012); Bechtel *et al.* (2013). The ash contents of sea cucumbers found in this study were 1.50 ± 0.63% and 4.34 ± 0.14% in dry weight (Table 1) due to the formation of cuticle and ossicles that composed of silica and calcium carbonate. Smiley (1994); Fechter (1969) told that the ash content was influenced not only geographical aquatic but also the species of sea cucumber. Haider *et al.* (2015) studied that the ash content of *Actinopyga mauritiana* was 31.81 ± 0.34% in dry weight.

Our study determined that the moisture content of the *Stichopus variegatus* was 93.36 ± 0.02% (wet weight) as described in Table 1, and this value was higher than the moisture content of *P. Californicus* muscle bands (84.5 ± 0.1%, wet weight) as mentioned by Bechtel *et al.* (2013). In this study, sea cucumbers were caught alive, immediately cleaned and froze then processed into dried products by vacuum oven at 50°C for four hours, which then caused chemical degradation of the tissues. Similar results were also determined in Chang-Lee *et al.* (1989) study that sea cucumber that being processed for 24 h post-mortem and dried in a gravity-convection oven at 60°C for 36 h showed tissue degradation but no significant.

Amino acid profile analysis

The consumption of sea cucumber is more related to its high protein and low fat content. Sea cucumber sometimes can be a tonic food, it reflects in many articles (Chen, 2003; Wen *et al.* 2010; Haider *et al.* 2015). High protein and low fat values in this species were found similar as determined in the case of another study (Ridzwan *et al.* 2014; Haider *et al.*

Table 2. Amino acid composition of *Stichopus variegatus* (%w/w, dry weight)

Amino acids in g/100g, dry weight	Fresh flesh	Flour
Acid amino group		
Aspartic acid	2.11 ± 0.01	2.59 ± 0.01
Glutamic acid	2.56 ± 0.01	4.71 ± 0.01
Polar amino group		
Serine	2.26 ± 0.02	0.93 ± 0.01
Glycine	8.73 ± 0.01	4.99 ± 0.01
Threonine	0.90 ± 0.01	1.31 ± 0.02
Basic amino group		
Arginine	2.26 ± 0.02	2.33 ± 0.02
Lysine	7.68 ± 0.03	5.79 ± 0.01
Histidine	0.60 ± 0.01	0.29 ± 0.02
Hydrophobic amino group		
Alanine	3.01 ± 0.01	2.61 ± 0.02
Methionine	0.30 ± 0.01	0.51 ± 0.02
Valine	1.05 ± 0.02	1.10 ± 0.03
Phenylalanine	1.05 ± 0.02	0.86 ± 0.02
Isoleucine	1.05 ± 0.01	0.86 ± 0.03
Leucine	1.05 ± 0.01	1.16 ± 0.02
Proline	4.67 ± 0.01	2.61 ± 0.01

2015).

The amino acid profile of *S. variegatus* is presented in Table 2. The content of lysine as one of essential amino acids, was significantly higher in fresh flesh and flour of *Stichopus variegatus*. This value of *S. variegatus* was as lower as *S. herrmanni* that was reported by Wen *et al.* (2010). The amino acid values in *Stichopus variegatus* flesh was at lower side as compared to other species undertaken for research (Fangguo, 1997; Haider *et al.* 2015). That differences could be because of the region specific like discussed earlier. The total amino acids of this sea cucumber included in ranged 33.32 to 54.13 g/100g, dry weight as proven by Wen *et al.* (2010).

The results showed that the amino acid profile was different between fresh flesh and flour of *Stichopus variegatus*, because the bioseparation reaction of transamination and reamination might occur during the flour processing until amino acid analysis (Pickering and Newton 1990). However, both of them have higher hydrophobic amino group than polar amino group. The literature study showed that the presence of hydrophobic amino acid residues is associated with ACE inhibitor activity as mentioned (Ching-Mars, 2006; Ryan *et al.* 2011).

This profile suggests that the presence of hydrophobic amino in the flour can act as an ACE inhibitor sequences. The hydrophobicity of the protein or peptide is very important for accessibility to the target hydrophobic and increases the affinity and reactivity of protein into the cell membranes of living cells as determined (Chen *et al.* 1996; Alema'n *et al.* 2011). The high flexibility of protein or peptide is required for scavenging free radicals, which is also recommended as an antioxidant.

Table 3. Amino acid scoring patterns for toddlers, children, adolescents and adults (amended values from the 2007 WHO/FAO/UNU report)

	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val		
Tissue amino acid pattern (mg/g protein) ¹	27	35	75	73	35	73	42	12	49		
Maintenance amino acid pattern (mg/g protein) ²	15	30	59	45	22	38	23	6	39		
Protein requirements (g/kg/d)											
Age (yr)	Maintenance	Growth ³	Amino acid requirements (mg/kg/d) ⁴								
0-5	0.66	0.46	22	36	73	63	31	59	35	9.5	48
1-2	0.66	0.20	15	27	54	44	22	40	24	6	36
3-10	0.66	0.07	12	22	44	35	17	30	18	4.8	29
11-14	0.66	0.07	12	22	44	35	17	30	18	4.8	29
15-18	0.66	0.04	11	21	42	33	16	28	17	4.4	28
>18	0.66	0.00	10	20	39	30	15	25	15	4.0	26
Scoring pattern mg/kg protein requirements ⁵											
0-5			20	32	66	57	27	52	31	8.5	43
1-2			18	31	63	52	25	46	27	7	41
3-10			16	30	61	48	23	41	25	6.6	40
11-14			16	30	61	48	23	41	25	6.6	40
15-18			16	30	60	47	23	40	24	6.3	40
>18			15	30	59	45	22	38	23	6.0	39

His, histidine; Ile, isoleucine; Leu, leucine; SAA, sulphur amino acids; AAA, aromatic amino acid; Thr, threonine; Trp, tryptophan; Val, valine.

¹amino acid composition of whole-body protein

²adult maintenance pattern

³calculated as average values for the age range: growth adjusted for protein utilization of 58%

⁴sum of amino acids contained in the dietary requirement for maintenance (maintenance protein x the adult scoring pattern) and growth (tissue deposition adjusted for a 58% dietary efficiency of utilization x the tissue pattern)

⁵amino acid requirements/protein requirements for the selected age groups. Noted that these values, some of which are slightly amended from the 2007 report, are the correctly calculated values. In the published report, the value for the SAA requirements for children aged 3-10 is incorrect (18mg/kg/d) as are the SAA patterns for infants preschool and school children up to 10 (28,26 and 24 mg/g protein).

The amino acids or peptide are part of protein that can act as an antioxidant. Alema'n *et al.* (2011) stated the antioxidant activity of protein is affected by the profile amino acid, sequences amino acid, molecular weight and amino acid component. In this study, the highest amino acid components of the flour were lysine ($5.79 \pm 0.01\%$), glycine ($4.99 \pm 0.01\%$) and glutamic acid ($4.71 \pm 0.01\%$), respectively. Histidine and methionine were the lowest amino acids, while Wen *et al.* (2010) found three limiting essential amino acids in eight dried sea cucumber products investigated to be histidine, lysine, and methionine. Even though amino acid profiles from sea cucumber flesh to flour decreased, it can be utilized as ingredient for functional foods based on FAO/WHO/UNU (Table 3) discussed earlier.

Protein is required for maintenance and growth in human body. The requirements of leucine and lysine are higher than other amino acids. Lysine is a limiting amino acid found in the smallest quantity in particular food, mainly in cereal grains, but it is plentiful in most legumes. Consequently, meals that combine cereal grains and flour of *S.variegatus* sea cucumber could provide complete protein in the diets of by choice or by

requirements for vegetarian. L-lysine is an essential building block for all protein in the body (Lehninger *et al.* 2000). In this study, histidine and methionine were described as the limiting amino acids. Essential amino acids that usually action a limiting capacity are lysine, methionine, threonine, and tryptophan. The results of this study were similar to Nurjanah (2008), whereas Nurjanah's results pointed out that the sand sea cucumber (*Holothuria scabra* J) contains almost all essential amino acids except histidine. Other sea cucumber species, *Actinopyga* and *Thelenota*, contained no phenylalanine (Wen *et al.* 2010). Proline, glycine, lysine, and glutamic acid were high in the flour of sea cucumbers but methionine was low in fresh flesh ($0.30 \pm 0.01\%$ dry weight).

This study results were quite similar to Wen *et al.* (2010), who already observed that sea cucumbers have much lower lysine-to-arginine ratios than those found in many other seafood products including fish as proven (Zuraini *et al.* 2006; Zhao *et al.* 2010), and shrimp (Inhamuns *et al.* 2009). Capillas *et al.* (2002) stated that the percentage of amino acid could diversify among live organisms because of geographical differences, species, age

Table 4. Fatty acid composition of *Stichopus variegatus* (%w/w, dry weight)

Fatty acid	Flour	Fresh Flesh
Lauric acid (C12:0)	0.05 ± 0.01	0.19 ± 0.01
Myristic acid (C14:0)	0.72 ± 0.02	4.43 ± 0.03
Pentadecanoic acid (15:0)	0.16 ± 0.02	0.89 ± 0.02
Palmitic acid (C16:0)	3.51 ± 0.02	22.48 ± 0.01
Heptadecanoic acid (C17:0)	0.33 ± 0.02	1.96 ± 0.01
Stearic acid (C18:0)	2.16 ± 0.03	19.89 ± 0.02
Palmitoleic acid (C16:1)	1.09 ± 0.01	6.59 ± 0.01
Oleic acid (C18:n9)	1.95 ± 0.02	20.58 ± 0.03
Eicosenoic acid (C20:1)	0.07 ± 0.01	0.76 ± 0.02
Linoleic acid (C18:2 n6)	0.74 ± 0.02	3.86 ± 0.02
Linolenic acid (C18:3 n3)	ND	0.13 ± 0.01
Arachidonic acid (C20:4n6)	0.63 ± 0.02	46.30 ± 0.02
EPA (C20:5 n3)	0.04 ± 0.02	3.42 ± 0.04
DHA (C22:6 n3)	ND	0.32 ± 0.02

*ND (Not Detected)

and physiological condition. In addition, arginine, glutamic acid, alanine and glycine are free amino acids responsible for the formation of flavour. Thus, the quality of marine foods can be influenced significantly by the composition of free amino acids. Glutamate, glycine and a number of hydrophobic amino acids; methionine, phenylalanine, leucine, alanine can be useful as immunomodulators and anticancer as described by Bordbar *et al.* (2011).

Glycine and glutamic acid are essential components for glutathione synthesis which can stimulate the activation and proliferation of NK cells. Arginine might stimulate immune cells to promote T-cell activation and proliferation as mentioned by Bordbar *et al.* (2011). Therefore, amino acid components of sea cucumbers involved in the regulation of immune function.

However, lysine in sea cucumbers tends to be easier to get browning due to Maillard reaction. Lehninger *et al.* (2000) stated that the Maillard reaction can be caused by the pH, water content, oxygen, metals, sulfur dioxide, and inhibitor compounds. The temperature is the critical point for the protein content because a half of it is lost by flour processing. One of the most recent breakthroughs in degradation, conversion, and resistance to protein hydrolysis was the temperature as mentioned by Pickering and Newton (1990). Proteins from sea cucumber body wall are rich in glycine, glutamic acid and arginine. The protein and amino acid composition of the diet could affect serum cholesterol profile as determined (Crim and Munro, 1994).

The previous studies of Crim and Munro (1994) have shown that glycine could reduce the serum total cholesterol level. Most (70%) of the body wall of sea cucumber protein consists of collagen as mentioned by Saito *et al.* (2002). Glycine, proline and hydroxyproline are the components of collagen. Collagen is the main structural protein of various

connective tissues. It can be formed as gelatin, which is used in many foods, including flavored gelatin desserts, pharmaceutical, cosmetic, also photography industries. In this study, lysine, glutamic acid, and glycine were high in the flour of sea cucumbers.

Fatty acid profile

The profile and levels of fatty acids are presented in Table 4. The fatty acid profiles were dominated by polyunsaturated fatty acids (PUFA). In this study (*S. variegatus*), the major fatty acids (FA) were palmitic acid, stearic acid and oleic acid that were different from Fredalina *et al.* (1999). Palmitic acid (C16:0), stearic (C18:0), palmitoleic (C16:1) were also found in some abyssal echinoderms. In general, Cakli *et al.* (2009) showed that sea cucumber that lives as the bottom sediment feeder organisms, should contain high levels of branched chain fatty acids (FA). For their potential role in wound healing and eicosapentaenoic (EPA), they could be associated with the ability to initiate tissue repair since EPA is involved in prostaglandin inhibition as mentioned (Prato *et al.*, 2010).

In *S. variegatus*, there was trace of arachidonic acid (46.30 ± 0.02%) that is a major FA in almost all tropical species. Lower content of EPA in *S. variegatus* could be related to the period of study that was conducted not during the reproduction and ovarian maturation period. This fact needed further investigation, considering that the effect of seasonal change in sea cucumber body composition has been reported (Dong *et al.* 2006). Orban *et al.* (2002) pointed that the body chemical composition of sessile animals majority depends on the food resources available and the season of harvest. In addition, Fuentes *et al.* (2009) also stated that the breeding or collection sites could influence the proximate composition of other echinoderms.

The sea cucumber flour studied in this work had lower amounts of SFA, MUFA and PUFA. This discrepancy might be causable to numerous factors, in which feed and ambient temperature are the most important ones. Moreover, the differences were reasonable due to the processing treatment of dried sea cucumber product. The sea cucumbers were gutted and dried as soon as they have been caught, as mentioned by Bruckner (2005). While polyunsaturated fatty acids were the predominant fatty acid class in freeze-dried muscle bands as mentioned by Zhong *et al.* (2007). The other hand, the most abundant class of fatty acids in freeze-dried body wall were monounsaturated fatty acids (Zhong *et al.* 2007).

A previous study confirmed that heating

process has showed considerable effect on fatty acid composition in food as mentioned (Cakli *et al.* 2009). The fat content of fresh flesh sea cucumbers consisted of saturated and unsaturated fatty acids. Fat content might decrease due to the possibility of the oxidation process and autooxidation during processing as mentioned (Cakli *et al.* 2009). The fat oxidation reaction is affected by unsaturated fatty acid, configuration of the double bond, the degree of esterification, catalyst, oxygen, and temperature.

The presence of oxygen acts as a trigger for the oxidation reaction, as well as high temperature storage that can initiate autooxidation reactions as mentioned (deMan, 2008). Processed dried sea cucumber is potential to be utilized as a functional food based on the total amino acid and unsaturated fatty acid compositions. Processed sea cucumbers is also suitable for diabetics because its insulin-stimulating amino acid level is quite high. Moreover, it has low level of saturated fatty acids that is suitable for diseases associated with fat, such as heart disease and atherosclerosis. The fatty acid composition of *S. variegatus* sea cucumber was very interesting even though the EPA values were much lower compared to other studies as proven by Zhong *et al.* (2007).

An arachidonic acid was the principal n-6 PUFA in *S. variegatus*, as reported by Valentine and Valentine (2010). Many research were reported for abyssal sea cucumber species whereas high values were reported for tropical species has a similar contents of fatty acids. Arachidonic acids (AA) plays a key role in growth, and wound healing. It will interfere with blood clotting process and attach to endothelial cells during wound healing due to its antithrombotic activity (Prato *et al.* 2010). The unsaturated fatty acids like EPA and DHA are associated with decreased risk of coronary heart disease and cancer.

In latest studies, EPA and DHA are major n-3 PUFA in fresh and frozen sea cucumber as mentioned (Svetashev, 1991; Drazen, 2008). The loss of DHA is reasonable in the processing treatment of dried sea cucumber products, such as drying with vacuum dryer. The long-chain PUFAs such EPA and DHA are considered to be highly susceptible to oxidation during heating and other culinary treatments as mentioned (Kulas and Ackman, 2001).

Zhong *et al.* (2007) determined that DHA content ranged from 2% to 6% in *C. frondosa*. Kaneniwa *et al.* (1986) reported in a separate study of the fatty acids in *Cucumaria* sp., that its EPA and DHA contents were 36.9% and 1.1% (w/w), respectively. For eight sea cucumber dried products derived from the Western Central Pacific, Wen *et al.* (2010) found that EPA content ranging from 0.3% to 3.9% total

fatty acids. Interestingly, this study reported that DHA was not detected in any sea cucumber flour. Similar results from Ridzwan *et al.* (2014) that DHA was also not detected in *Stichopus horrens*, although all species of sea cucumber from his research showed low percentages of EPA using methanol solvent for extract of lipid. Conversely, Fredalina *et al.* (1999) also determined that the EPA and DHA were detected in the crude extracts made from *Stichopus chloronotus*. Of particular interest is the AA content in *S. variegatus* (Table 4), which was the principal n-6 polyunsaturated fatty acid was detected.

The different sampling locations, kind of solvent extractions, and environment factors influences for determining the composition fatty acid in lipid extracts of sea cucumbers as postulated by Ridzwan *et al.* (2014). The distribution and occurrence of certain fatty acids is unique to this *S. variegatus*, due to representative of the fatty acid composition for organism which lived in temperate-polar marine. This is, to the best of our knowledge, the first study documenting the fatty acid profile of *S. variegatus*.

Conclusion

This preliminary study found that sea cucumber (*Stichopus variegatus*) contained essential nutrient component for human health with high in protein, but low in lipid content. Both fresh flesh and flour portions of this sea cucumber comprised different profiles of amino acid and fatty acid at varying concentrations. The flour processing using vacuum oven reduced nutrient content in sea cucumbers, but several amino acids were increased compared to the fresh flesh. The nutrient substances of this sea cucumber can be useful as nutrients for better human health or for the development of functional food.

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