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Compositional Characteristics and Antioxidant Properties of Fresh and Processed Sea Cucumber (*Cucumaria frondosa*)

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The antioxidant activity of fresh and rehydrated sea cucumber (*Cucumaria frondosa*) samples with/without internal organs was evaluated for the first time. In addition, their proximate, amino acid, and fatty acid compositions were examined. Rehydrated sea cucumber samples in distilled water were prepared from oven-dried products. All samples contained 83−90% moisture, but showed a significant difference among groups in their protein and lipid contents. Glutamic acid was the predominant amino acid in sea cucumber, followed by glycine and aspartic acid. Essential amino acids such as leucine and lysine were also present at high levels. The trend for free amino acid was different from that of total amino acids and varied among groups. Lipids in sea cucumber were dominated by eicosapentaenoic acid (EPA, C20:5n-3), ranging from 43.2 to 56.7% of the total fatty acids. Docosahexaenoic acid (DHA, C22:6n-3) was present at a much lower concentration of 2.0−5.8%. All sea cucumber samples exhibited radical scavenging property against 2,2′-azobis(2-aminopropane) dihydrochloride (AAPH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, with rehydrated samples, especially those with internal organs, possessing higher antioxidant activity than their fresh counterparts. No correlation existed between radical scavenging capacity and total phenolics content, suggesting that other components, in addition to phenolic compounds, contribute to the antioxidant activity of sea cucumber.

KEYWORDS: Sea cucumber; *Cucumaria frondosa*; composition; antioxidant activity

INTRODUCTION

Sea cucumbers are cylinder-shaped invertebrates that live in a variety of sea floor habitats from warm tropical waters to cold deep sea trenches. They are considered to be an important food in the Indo-Pacific region including The Philippines, Malaysia, Japan, Korea, and China. China is the largest sea cucumber producer worldwide with sea cucumber farming and ranching being a key part of its aquaculture industry (*1*). However, sea cucumbers are an underutilized fishery resource in the rest of the world, such as the United States and Canada. More recently, scientific evidence supporting their importance as nutraceuticals and functional foods has attracted growing interest from nutritionists and pharmacologists as well as the general public.

Sea cucumber, from a nutritional point of view, is an ideal tonic food with high nutritional value, as it contains a higher level of protein and a lower level of fat than most other foods (*1*). The body wall of sea cucumber, which consists of insoluble collagen, has been used as a nutrient supplement of hemotogenesis (*2*). Sea cucumber protein is rich in lysine, arginine, and tryptophan. The gelatin from sea cucumber is considered to be more valuable than other gelatins because of its characteristic amino acid composition, especially the essential amino acids (*1*).

In addition to their use as food, sea cucumbers are also used for medicinal purposes. In East Asia, sea cucumbers have long been used as a traditional medicine for the treatment of asthma, hypertension, rheumatism, anemia, and sinus congestion (*3*). They have also been reported as being effective in healing various external wounds, such as cuts and burns, and internal wounds, especially after clinical surgery, injury, or caesarian operation (*3*). The tissue repair ability of sea cucumber has been associated with its high eicosapentaenoic acid (EPA) content (*3*, *4*). Sea cucumbers are recognized as a good source of chondrotin sulfate, which is an arthritic pain reliever. Treating rheumatoid arthritis, osteoarthritis, and ankylosing spondylities with sea cucumber has been successfully established (*1*). Sea cucumbers also show activity in a number of physiological functions, including inhibition of lung and galactophore cancers (*5*), improving body immunity (*6*), and antiaggregation of platelet (*7*), among others. However, there is an absence of any information available on their antioxidant properties. Marine invertebrates, especially tropical invertebrates, are protected against oxidative stress caused by their chronic exposure to high levels of solar UV radiation and deleterious reactive oxygen species (ROS). This is possibly due to the presence of endogenous antioxidants and/or metabolites as well as the "UV-extremophilic" bacteria living in their tissues (*8*). This suggests that marine invertebrates such as sea cucumbers could serve as a potential source of antioxidants. Nevertheless, there appears

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to be an important gap in the existing literature on the antioxidant activity of sea cucumbers, which we intended to fill.

Edible sea cucumbers, body wall, or products made from internal organs such as gonads and intestines are usually sold directly to restaurants or processed in preserved forms such as smoked and dried products. Dried sea cucumbers are then rehydrated by soaking, which is required in preparation for cooking. However, the dehydration and rehydration processing may potentially affect the nutritional value of sea cucumber by causing changes in chemical composition as well as bioactivities, such as antioxidant activity. The purpose of this work, in addition to examining the antioxidant activity of sea cucumbers, was to investigate the effect of processing on the nutritional value of sea cucumbers (whole body and body wall) by comparing their compositional characteristics in both fresh and processed states. The evaluation of antioxidant effectiveness of the crude extracts of sea cucumbers, to the best of our knowledge, is the first report in the existing literature. This research also provides information on potential application of sea cucumbers and/or their extracts as a food additive or as a health-promoting commodity.

MATERIALS AND METHODS

Materials. Orange-footed sea cucumber (*Cucumaria frondosa*) samples were obtained from Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada. Phenyl isothiocyanate, norleucine, fluorescein sodium salt, 2,2′-azobis(2-aminopropane) dihydrochloride (AAPH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin−Ciocalteu's reagent, and gallic acid were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Trolox was purchased from Acros Organics (Morris Plains, NJ). Trifluoroacetic acid and all solvents used were obtained from Fisher Scientific Ltd. (Ottawa, ON, Canada). The solvents employed in this work were of ACS grade, pesticide grade, or HPLC grade.

Sample Preparation. Sea cucumber samples were divided into four groups, each consisting of five sea cucumbers ($n = 5$): fresh sea cucumber body wall with internal organs removed (F), fresh sea cucumber with internal organs (F + O), rehydrated sea cucumber body wall without internal organs (R), and rehydrated sea cucumber with internal organs (R + O). Rehydrated samples were prepared by dehydration in a forced-air oven (Fisher Isotemp 300, Fair Lawn, NJ) at 65 °C for 48 h followed by rehydration in distilled water at ambient temperature (22 °C) for 48 h. Fresh and processed samples from the same group were then pooled and homogenized for subsequent analyses. A portion of the homogenates was freeze-dried for amino acid analysis and antioxidant activity tests.

Proximate Composition. Proximate composition of fresh and processed sea cucumber samples was measured as follows. Moisture content was determined by oven-drying according to AOAC (*9*); ash by incineration in a muffle furnace at 550 °C for 24 h (*9*), crude protein by Kjeldahl method (*9*), and lipid by gravimetric determination following extraction, as described by Budge and Parrish (*10*); carbohydrates were calculated by difference.

Amino Acid Composition. Composition of total and free amino acid was determined by reversed-phase HPLC. Freeze-dried sea cucumber samples were hydrolyzed under nitrogen with 6 N HCl for 24 h at 110 °C for the subsequent analysis of total amino acids. Samples for free amino acid analysis were prepared by mixing with protein precipitant (0.5% trifluoroacetic acid in methanol) followed by centrifugation at 3000*g* for 5 min to remove proteins, as described by Bertolo et al. (*11*). Amino acids in the samples were then converted into phenyl isothiocyanate derivatives and analyzed by reversed-phase HPLC according to the method of Bidlingmeyer et al. (*12*). Individual amino acids were identified by comparing their retention times with those of authentic standards and quantified using norleucine as the internal standard.

Table 1. Proximate Composition of Fresh and Processed Sea Cucumber (Mean ± SD, $n = 5$)[a]

component (%)	F	F + O	R	R + O
moisture	87.4 ± 0.30 b	90.1 ± 0.21 a	84.0 ± 1.52 c	83.3 ± 0.45 c
protein	8.34 ± 0.50 c	5.11 ± 0.34 d	12.8 ± 1.57 a	10.9 ± 0.37 b
lipid	0.50 ± 0.06 c	0.70 ± 0.08 b	1.16 ± 0.07 a	1.27 ± 0.12 a
ash	2.97 ± 0.09 a	3.03 ± 0.20 a	0.67 ± 0.06 c	0.90 ± 0.01 b
carbohydrates	0.94 ± 0.46 b	1.19 ± 0.56 b	1.62 ± 0.57 b	3.92 ± 0.28 a

[a] Values in the same row with different letters are significantly different at $P < 0.05$. Abbreviations: F, fresh sea cucumber without internal organs; F + O, fresh sea cucumber with internal organs; R, rehydrated sea cucumber without internal organs; and R + O, rehydrated sea cucumber with internal organs.

Fatty Acid Composition. The fatty acid composition of sea cucumber lipid was analyzed by gas chromatography (GC). Fatty acids were converted to fatty acid methyl esters (FAMEs) using 6% sulfuric acid in methanol, according to the method of Hamam and Shahidi (*13*). The resultant FAMEs were analyzed using a Hewlett-Packard 5890 series II gas chromatograph (Agilent, Palo Alto, CA) equipped with a fused capillary column (Supelcowax-10, 30 m length, 0.25 mm diameter, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, ON, Canada). The temperatures of the injector and detector (FID) were both set at 250 °C. The oven temperature was programmed to increase from 220 to 240 °C at a rate of 30 °C/min. Ultrahigh-purity (UHP) helium was used as the carrier gas at a flow rate of 15 mL/min. Data were analyzed with Hewlett-Packard 3365 series II Chem Station software (Agilent). The FAMEs were identified by comparing their retention times with those of authentic standards. Results were expressed as weight percentage of each fatty acid in total fatty acids.

Preparation of Sea Cucumber Extracts. Extracts of sea cucumber were prepared using methanol as the extraction solvent as described below. Twenty milliliters of methanol was added to 1 g of freeze-dried sea cucumber sample and vortexed thoroughly. The mixture was then centrifuged at 2000*g* for 5 min. The supernatant was collected, and the residue was re-extracted twice with the same volume of methanol. The supernatants were combined, and the solvent was removed using a rotary evaporator. The extracts were redissolved in 4 mL of methanol for subsequent antioxidant activity tests.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay was carried out using a Fluostar Optima plate reader (BMG Labtech, Durham, NC) equipped with an incubator and two injector pumps. Fluorescein and AAPH were used as the probe and radical generator, respectively. The methanol extracts were diluted (10000 times) prior to the test. Twenty microliters of Trolox standard (6.25−50 μM) and sample solutions were added to each well, with phosphate buffer solution (PBS; 75 mM, pH 7.0) as a blank, followed by 200 μL of fluorescein (0.11 μM in PBS). The plate was incubated at 37 °C for 15 min, and the machine was programmed to inject 75 μL of AAPH (17.2 mg/mL in PBS) into the wells. The conditions used were as follows: 0.3 s position delay, 8 s orbital shaking before each cycle with 4 mm width, 210 s cycle time, and 25 cycles. Fluorescence was measured at an excitation wavelength of 485 nm and emission of 520 nm. A standard curve was plotted, and results were expressed as millimoles of Trolox equivalents (TE) per gram of dry sample.

DPPH Radical Scavenging Assay. The DPPH radical scavenging activity of sea cucumber extracts was determined according to the method of Hatano et al. (*14*). The methanol extracts were diluted 10 times prior to the test. One hundred microliters of Trolox standard and diluted sample solutions were added to 1.9 mL of methanolic DPPH (6 μM) and mixed thoroughly. The mixture was allowed to stand for 20 min at room temperature and the absorbance measured at 517 nm. A standard curve was obtained, and results were expressed as micromoles of Trolox equivalents (TE) per gram of dry sample.

Total Phenolics Content. The content of total phenolics in sea cucumber extracts was determined spectrometrically as described by Singleton and Rossi (*15*). The methanol extracts were diluted 10 times before the test. Folin−Ciocalteu's reagent (1 mL) was added to 1 mL of diluted samples and mixed thoroughly. To the mixture were added and mixed well 8 mL of sodium carbonate (75 g/L) and 10 mL of

Table 2. Total Amino Acids of Fresh and Processed Sea Cucumber (Milligrams per Gram of Dry Sample)[a]

amino acid	F	F + O	R	R + O
Val	19.7	16.8	27.7	26.1
Met	10.3	9.40	19.5	14.8
Ile	13.9	12.6	19.1	17.2
Leu	30.8	28.3	43.9	41.7
Phe	17.8	13.1	17.4	19.7
His	2.80	3.30	0.60	0.60
Thr	12.9	10.9	15.4	16.2
Lys	29.1	30.6	47.0	43.9
Asp	39.1	27.8	51.3	45.6
Glu	66.4	57.5	89.4	73.1
Ser	19.3	15.5	22.3	21.2
Gly	56.1	29.8	77.9	63.5
Ala	32.0	23.2	44.3	39.0
Arg	30.1	24.7	40.5	34.1
Pro	24.0	17.3	33.4	28.1
Tyr	15.6	13.4	21.4	19.5
Orn	5.70	1.60	5.70	12.8
EAA	137	125	191	180
NEAA	304	212	409	354
EAA/NEAA	0.45	0.59	0.47	0.51

[a] Values reported are averages of duplicate determinations. The coefficients of variation did not exceed 2%. Abbreviations: EAA, essential amino acids; NEAA, nonessential amino acids; those for samples are the same as in **Table 1**.

Table 3. Free Amino Acids of Fresh and Processed Sea Cucumber (Milligrams per Gram of Dry Sample)[a]

amino acid	F	F + O	R	R + O
Val	0.13	0.40	0.02	0.01
Met	0.38	0.63	0.10	0.31
Ile	0.08	0.22	0.04	0.20
Leu	0.18	0.48	0.10	0.46
Phe	0.07	0.08	0.00	0.08
His	1.07	1.25	0.48	0.28
Thr	0.06	0.25	0.05	0.42
Lys	0.14	0.54	0.04	0.27
Asp	1.68	2.83	0.13	0.45
Glu	0.22	0.57	0.07	0.07
Ser	0.22	0.46	0.03	0.09
Gly	0.56	2.02	0.11	0.29
Gln	0.06	0.13	0.01	0.13
Tau	1.43	2.29	0.08	0.19
Cit	0.00	0.00	0.00	0.10
Ala	1.12	2.18	0.10	0.57
Arg	1.47	2.03	0.31	0.11
Pro	0.15	0.52	0.02	0.10
Tyr	0.05	0.13	0.05	0.18
Orn	0.06	0.08	0.01	0.20
EAA	2.11	3.85	0.83	2.03
NEAA	7.14	13.5	0.95	2.49
EAA/NEAA	0.30	0.27	0.87	0.82

[a] Values are averages of duplicate determinations ± 0.01. Abbreviations: EAA, essential amino acids; NEAA, nonessential amino acids; those for samples are the same as in **Table 1**.

distilled water. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000*g* for 5 min, and the absorbance of the supernatant was read at 765 nm. A standard curve was obtained using various concentrations of gallic acid. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample.

RESULTS AND DISCUSSION

Proximate Composition. The proximate composition of fresh and processed sea cucumber is given in **Table 1**. The contents of major components, namely, moisture, protein, lipid, and ash, in samples tested were similar to those reported for other sea cucumber species, including *Parastichopus parvimensise*, *Parastichopus californicus* (*16*), *Acaudina molpadioides* (*1*), *Holothuria tubulosa* (*17*), and *Apostichopus japonicus* (*18*). Raw sea cucumber *H. tubuloasa* was reported to contain 86.7% water, 8.2% protein, and 0.2% lipid (*17*), which are quite close to the values from this work. In the present study, fresh sea cucumber with internal organs had higher moisture and fat contents but significantly ($P < 0.05$) lower protein content than those with organs removed. Rehydrated samples had lower moisture and ash levels but significantly ($P < 0.05$) higher protein and fat contents than their fresh counterparts. This suggests that the dehydration and rehydration process caused water and mineral loss and thus increased the protein and lipid content in the processed sea cucumber samples.

Amino Acid Composition. The composition of total amino acids of fresh and processed sea cucumber is shown in **Table 2**, and results are given on a dry weight basis. Seventeen amino acids were detected, including 8 essential amino acids (EAA) and 10 nonessential amino acids (NEAA). Glutamic acid dominated in all samples and ranged from 57.5 mg/g of dry sample in fresh sea cucumber with organs to 89.4 mg/g in rehydrated sea cucumber body wall. Glycine was the second most abundant amino acid present, followed by aspartic acid and alanine. The results are consistent with the published amino acid composition for sea cucumber *Holothuria insignis* (*1*). The EAA, such as leucine, lysine, and phenylalanine, were also present at high levels. A clear trend is obtained that the concentration of each individual amino acid increased after rehydration process and decreased when internal organs were included, both with the exception of histidine. This is probably because of the difference in their protein content, as the amino acid concentrations positively correlated with their protein contents (in dry matters, data not shown) among the groups. However, the ratio of EAA to NEAA, which reflects the quality of proteins, was higher in sea cucumber with internal organs than in those with organs removed.

Not only as the building block of proteins, amino acids also occur in the free form. Free amino acids (FAA) are very important taste-active substances and are responsible for the distinctive flavor and taste of many foods. FAA generally give rise to sweetness, bitterness, sourness, and umami taste (*19*, *20*), among others, and the characteristic taste of a food item is determined by the balance of all these tastes. Thus, the amount and composition of FAA can significantly influence the quality of numerous foods, especially marine foods. FAA in fresh and processed sea cucumber, which are independent from their proteins, differed from the total amino acids in compositional features. The major free amino acids varied among groups and altered from the total amino acids (**Table 3**). Furthermore, the concentration of individual free amino acid was higher in fresh sea cucumber than in the rehydrated counterparts and higher in samples with internal organs than in those without organs, which is opposite to that observed for total amino acid. It is indicated that internal organs of sea cucumbers contained higher levels of free amino acids than their body wall and that dehydration and rehydration caused loss of free amino acids in processed sea cucumber through leaching into the water.

Fatty Acid Composition. Table 4 summarizes the fatty acid composition of fresh and processed sea cucumber samples. The fatty acids C15:0, C16:1, C17:1, C18:0, C18:1, C20:1, C20:3, C20:5, C22:1, and C22:6 constituted the major fatty acids of sea cucumber lipids. Eicosapentaenoic acid (EPA, C20:5n-3) was the predominant fatty acid present in all samples, comprising 43.2−56.7% of the total fatty acids. EPA, a characteristic

Table 4. Fatty Acid Composition (Weight Percent) of Fresh and Processed Sea Cucumber (Mean ± SD, $n = 5$)[a]

fatty acid	F	F + O	R	R + O
14:0	1.88 ± 0.01 a	1.80 ± 0.03 a	1.42 ± 0.03 b	1.39 ± 0.08 b
ai-15:0[b]	2.18 ± 0.20 c	4.03 ± 0.48 b	3.92 ± 0.31 b	8.98 ± 0.32 a
16:0	2.33 ± 0.03 b	2.83 ± 0.34 a	2.14 ± 0.08 b	2.30 ± 0.09 b
16:1n-7	5.75 ± 0.03 c	7.36 ± 0.88 b	6.05 ± 0.28 c	11.3 ± 0.25 a
17:0	0.64 ± 0.00	0.35 ± 0.13	0.52 ± 0.11	0.67 ± 0.02
17:1n-7	3.87 ± 0.04 a	2.44 ± 0.98 b	3.44± 0.14 a	2.45 ± 0.04 b
16:4n-3	2.39 ± 0.07 bc	5.74 ± 2.25 a	3.67 ± 0.69 ab	1.31 ± 0.30 c
18:0	5.41 ± 0.02 a	4.20 ± 0.03 b	2.54 ± 0.09 c	1.51 ± 0.06 d
18:1n-9	2.43 ± 0.02 c	3.72 ± 0.01 a	2.60 ± 0.07 b	2.32 ± 0.05 d
18:1n-7	3.52 ± 0.02 a	3.37 ± 0.10 a	3.01 ± 0.10 b	3.36 ± 0.11 a
20:1n-9	4.00 ± 1.04 a	1.66 ± 0.14 b	1.85 ± 0.21 b	1.35 ± 0.24 c
20:3n-3	5.00 ± 0.15 b	2.54 ± 0.05 c	5.65 ± 0.19 a	4.05 ± 0.14 b
20:5n-3	46.1 ± 0.31 c	43.2 ± 0.96 d	56.7 ± 1.96 a	52.0 ± 1.89 b
22:0	1.95 ± 0.07 b	2.09 ± 0.04 ab	1.95 ± 0.04 b	2.28 ± 0.25 a
22:1n-9	2.25 ± 0.04	3.34 ± 1.30	nd[c]	nd
22:6n-3	4.96 ± 0.01 b	5.81 ± 0.62 a	2.28 ± 0.19 c	2.03 ± 0.07 c
others	5.37 ± 0.19 a	5.57 ± 0.57 a	2.29 ± 0.16 c	2.76 ± 0.09 b

[a] Values in the same row with different letters are significantly different at $P < 0.05$. Abbreviations: those for samples are the same as in **Table 1**. [b] Bacterial fatty acid. [c] Not detected.

fatty acid in fish oil, is believed to possess antithrombotic activity (*21*), and plays a part in blood clotting mechanism (*22*). The high EPA content is also associated with the tissue-repairing ability of sea cucumbers, as hypothesized by Fredalina et al. (*3*). In addition to EPA, docosahexaenoic acid (DHA, C22:6n-3) was also present, but at a much lower concentration of 2.0−5.8% of the total fatty acids. However, the concentrations of other polyunsaturated fatty acids (PUFA), which are commonly found in most plant and fish oils, such as C18:2, C18:3, and C20:4, were below the measurable level. Kasai (*23*) reported a very high monounsaturated fatty acids content and no PUFA in salted entrails of sea cucumber *S. japonicus*. The monounsaturated fatty acids found in lipids of *C. frondosa* in this study were C16:1, C17:1, C18:1, C20:1, and C22:1. The *ai*-C15:0 is a saturated fatty acid with a branched chain, and it is unusual in most natural fats while being predominant in bacteria (*24*); it is possibly a bacterial fatty acid produced by bacteria inhabiting within the sea cucumber. High levels of branched-chain fatty acids have been thought to be responsible for the potential role of sea cucumber in wound healing (*25*).

Distribution of major fatty acids varied depending on the tissue selected and processing status of the sea cucumbers under investigation. The body wall samples contained higher levels of C17:1, C18:0, C20:1, C20:3, and EPA and lower levels of *ai*-C15:0, C16:0, C16:1, C18:1, C22:1, and DHA than the whole body with internal organs in the fresh sea cucumbers. With respect to the processing status, rehydrated sea cucumber had significantly ($P < 0.05$) lower levels of C14:0, C18:0, C20:1, C22:1, and DHA than their fresh counterparts. Loss of these fatty acids might have occurred during the dehydration and rehydration process due to many possible factors, such as hydrolysis and/or oxidation. Percentages of *ai*-C15:0, C16:1, and EPA in total fatty acids were increased after rehydration, probably owing to the retaining or less loss of these fatty acids than the others.

Antioxidant Activity and Total Phenolics Content. Marine invertebrates, especially tropical marine invertebrates, are chronically exposed to high levels of solar-UV radiation and therefore suffer from oxidative stress. Moreover, unicellular algae residing in symbiosis within their tisues continuously release photosynthetic oxygen that works as an oxidation initiator (*26*). However, marine invertebrates are protected against oxidative stress, possibly by endogenous antioxidants and/or their metabolites. Active antioxidant substances found in marine invertebrates include vitamins E (*27*) and C (*28*), phenolic compounds (*29*), and carotenoids (*30*), among others. Sea cucumbers, although explored for numerous biological and physiological functions, have rarely been studied for their antioxidant property.

Table 5. Antioxidant Activity of Fresh and Processed Sea Cucumber[a]

sample	ORAC (mmol of Trolox equiv/g of dry sample)	DPPH (μmol of Trolox equiv/g of dry sample)	total phenolics (mg of gallic acid equiv/g of dry sample)
F	2.09 ± 0.09 c	4.51 ± 0.35 c	1.08 ± 0.00 a
F + O	2.36 ± 0.03 b	5.86 ± 0.34 b	1.00 ± 0.00 a
R	2.23 ± 0.12 bc	5.04 ± 0.16 c	0.30 ± 0.06 c
R + O	2.60 ± 0.04 a	7.48 ± 0.10 a	0.88 ± 0.06 b

[a] Values in the same column with different letters aere significantly different at $P < 0.05$. Abbreviations: those for samples are the same as in **Table 1**.

In this work, the antioxidant activity of the extracts from freeze-dried fresh and processed sea cucumber samples was determined as their scavenging capacity against certain free radicals. Trolox, a water-soluble analogue of α-tocopherol, was used as a control antioxidant. **Table 5** presents the scavenging ability of sea cucumber extracts against AAPH and DPPH radicals. All sample extracts exhibited an inhibitory effect against the formation of AAPH radical in the ORAC assay, with rehydrated sea cucumber with internal organs possessing the highest ORAC value (2.60 mmol of Trolox equivalents/g of dry sample). Samples with internal organs had a significantly ($P < 0.05$) higher AAPH scavenging capacity than those without organs, regardless of their processing status (fresh or rehydrated), suggesting that the internal organs of sea cucumber had higher antioxidant activity than its body wall. The ORAC value was significantly ($P < 0.05$) higher in rehydrated samples than in fresh ones when internal organs were included. However, the difference was not significant ($P > 0.05$) in sea cucumber body wall with organs removed. A similar trend was found in DPPH scavenging assay (**Table 5**), which indicated a strong correlation with results of the ORAC assay. The increase of antioxidant activity in rehydrated sea cucumber is possibly caused by loss of some water-soluble components during the rehydration process. Whereas sugars, among these components, have no or little effect on antioxidant property, some pro-oxidant effect may be rendered by certain salts and peptides.

The results of the two radical scavenging tests revealed that sea cucumber contained some active antioxidant substances, among which phenolic compounds are believed to play an important role. Phenolics are present abundantly in the plant kingdom and are considered to be effective natural antioxidants. Marine invertebrates appear to absorb phenolics of algal and/or microalgal origin, possibly from phytoplankton, which are a rich source of phenolic compounds including flavonoids, anthocyanins, anthocyanidins, and tannins. The content of total phenolics in fresh and processed sea cucumber extracts was measured, and the results are shown in **Table 5**. Fresh sea cucumbers had a significantly ($P < 0.05$) higher content of total phenolics than their rehydrated counterparts. Loss of phenolic compounds might have occurred during the rehydration process. Among rehydrated samples, those without internal organs had a significantly ($P < 0.05$) lower level of total phenolics than those with organs, whereas no significant ($P > 0.05$) difference existed among fresh samples. This is possibly due to the loss of phenolic compounds, which was higher in sea cucumber body

wall than in the internal organs during rehydration, probably owing to the higher exposure of body wall to the water environment. No correlation existed between total phenolics content and radical scavenging capacity. Other components, in addition to phenolic compounds, such as vitamin E, carotenoids, and some terpenoid metabolites, might have also contributed to the antioxidant activity of sea cucumber.

In summary, it was demonstrated that the proximate, fatty acid, and amino acid compositions of sea cucumbers (whole body and body wall) vary depending on their processing status. For the first time, sea cucumbers were found to possess an antioxidant property, and this was also affected by processing. Thus, sea cucumbers may be used as a potential food additive and/or for medicinal purposes. Further work is required to shed light on the exact chemical nature of the antioxidant components of sea cucumbers and to see whether dietary phenolics from seaweed are retained as such or as metabolites.

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