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Protein profile, amino acids and taurine composition of sea slug (*Paromoionchis tumidus*) from Sumenep sea waters, Madura, Indonesia

HAFILUDIN[1,2,♥], SRI ANDAYANI[3], HARTATI KARTIKANINGSIH[3], MUHAMAD FIRDAUS[3]

[1]Fisheries and Marine Science Graduate Program, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Jl. Veteran Malang 65149, East Java, Indonesia

[2]Department of Fisheries and Marine Science, Faculty of Agriculture, Universitas Trunojoyo Madura. Jl. Raya Telang, Kamal, Bangkalan 69162, East Java, Indonesia. Tel.: +62-31-3011146, ♥email: hafiludin@trunojoyo.ac.id

[3]Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, University of Brawijaya. Jl. Veteran Malang 65149, East Java, Indonesia

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Abstract. *Hafiludin, Andayani S, Kartikaningsih H, Firdaus M. 2020. Protein profile, amino acids, and taurine composition of sea slug* (Paromoionchis tumidus) *from Sumenep sea waters, Madura, Indonesia. Biodiversitas 21: 2430-2436.* Sea slug (*Paromoionchis tumidus*) is one of the marine gastropods and inhabits the intertidal zone. Sea slug has long been used by coastal communities as food and medicine for breast cancer. Sea slugs are rich in nutritional content, a protein with the composition of essential amino acids, unsaturated fatty acids, and minerals, which are important as food and medicine in the future. The objective of the study was to investigate the composition of proximate and amino acids, protein profile, and taurine content of sea slug from the sea waters of Sumenep Madura. In this work, the sea slug collected from Sumenep was analyzed for protein, amino acids, and taurine. We compared the composition of proximate and amino acids before and after dried (fresh and dried), and part of the body of a sea slug (whole, muscle, viscera, and mucus) for the protein profiling and taurine content. The results showed that protein content reached 9.32% (fresh) or 60.82% (dried). The molecular weight of the protein was ranging from 22.53 kDa (viscera) to 49.61 kDa (viscera). The total amino acids were found at 6.14% (fresh) and 53.87% (dried). Furthermore, taurine was present at range from 53.568 µg/mL (viscera) to 158.784 µg/mL (whole). This experiment imparted a consistent result with previous studies regarding the richness of protein content in sea slug. Thus there is a wide opportunity for using it as taurine rich products in functional food and medicine.

Keywords: Nutritional, *Paromoionchis tumidus*, protein profiling, sea slug, taurine

INTRODUCTION

Marine mollusks may represent one of the extremely vast biodiversity, while in recent years, they have unequivocally demonstrated great importance related to its novel potent as a source of healthy foods (Khan and Liu 2019). Fish are well-known rich in protein (Priatni et al. 2018) and have a high rate of digestibility. For this reason, fish and other marine organisms are highly relevant to be converted into either food or feed for supporting human life (Mohanty 2015). Among nutritional composition in fish, protein seems to show the most appreciable component since its pivotal role for energy generation, growth, protein synthesis, and substrate for subsequent metabolic activities (Andersen et al. 2016). The chemical composition, including amino acids, of the marine organisms, may vary greatly, depending on species, size, sex, position in the fish, age, gonad maturity, climate, food source, and habitat condition (Saad and Alim 2015).

Generally, marine organisms, including fish and mollusks, are endowed with numerous nutritional components, including unsaturated fatty acids omega-3, essential amino acids, and protein (Tilami and Sampels 2018). Protein constitutes an important chemical component for the human diet, in the form of peptide and amino acid. Besides providing nutritious foods, the protein may also affect the sensory quality of the foods (Agustini et al. 2016). Thus, scientific evidence of fish biochemical composition is crucial.

Free amino acids are widely found in marine organisms. Among 20 amino acids, taurine may come with a great interest related to its biological actions (Klongnganchui and Muangthai 2016; Pyzłukasik et al. 2016). Taurine (2-aminoethanesulfonic acid) is a sulfur-containing amino acid (Ghosh and Sil 2015) with a distinctive chemical structure. It imparts imperative roles in many aspects such as physiological functions, regulation of calcium, stabilization of cellular membrane, regulation of blood pressure, and protection of endothelium cells. Taurine could stabilize cellular membrane, moderate the amount of intracellular calcium, as well as induce osmoregulation and detoxification. In addition, the taurine was reported to have therapeutic (Schaffer and Kim 2018), antioxidants (Ibrahim et al. 2020), and anti-inflammatory effects (Liu et al. 2017). Although taurine can be formed through endogenous synthesis from methionine and cysteine with the presence of vitamin B6, the main source of taurine is diet, particularly through seafood. Therefore, there is a need for further exploration of taurine from marine organisms such as sea slug.

Sea slug (*Paromoionchis tumidus*) belongs to gastropods and is widely distributed in many parts of the

world (Bula et al. 2017). They are found worldwide, but species and genetic diversity are highest in the tropical Indo-West Pacific, where they colonized a variety of microhabitats, especially in mangroves (Goulding et al. 2018). The gastropod often exists with one shell or shell-less, tentacle, and two reproductive organs (Galan et al. 2015). The shell-less sea slug was present in a great amount in several sites of Madura, including coastal water in Pamekasan (Nurjanah et al. 2012) and Sumenep. Based on empirical evidence of local communities in Pamekasan and Sumenep, the sea slug is consumed as food and medicine. The muscle of sea slug was processed for salad, side-dish, and soup. The study aimed to determine changes in proximate content, protein profiles, amino acids, and taurine in the fresh and dried form of sea slug collected from Sumenep sea waters, Madura.

MATERIALS AND METHODS

Collection and preparation of sample

Sample preparations have been carried out to separate the mantle or meat, viscera, and mucus of sea slug (*Paromoionchis tumidus*). Briefly, the animals were captured from Talango sea waters in Sumenep, Madura, Indonesia (Figure 1), then transported immediately to the laboratory using plastic containers covered with wet clothes to maintain RH, enabling to keep them alive. After sacrificed, the muscle, mucus, and visceral were collected separately, then each pulverized using an electric blender. The juice was freeze-dried to produce the powder. The resulting powder was stored in cold storage for further analyses.

Proximate analysis

Proximate composition (moisture, ash, protein, fat) of sea slug was assessed according to the method of AOAC 2012. The Determination of crude fiber was performed according to method No. IK.LP-04.13-LT1.0 of IPB laboratory.

Assessment of amino acid

HPLC (High-Performance Liquid Chromatography) Varian-940-LC was used for the assessment of amino acids. A total of 0.2 g sample mixture was added with 5 mL of HCl 6 M. Subsequently, it was dried at 100°C for 24 h, mixed with methanol, sodium acetate, and trimethylamine (2:2:1), and evaporated using nitrogen gases. The dried sample was re-purified using methanol, thiocyanate acid, and trimethylamine (3:3:4), then dissolved in 10 ml of acetonitrile 60%, left for 20 min. The Sample was screened before injected into HPLC apparatus. The analysis condition was run at 27°C, rate of 1 ml/min, and pressure of 3000 psi. Mobile phase, i.e. acetonitrile 60% and buffer phosphate 0,1 M was used, while absorbance was measured at a wavelength of 256 nm.

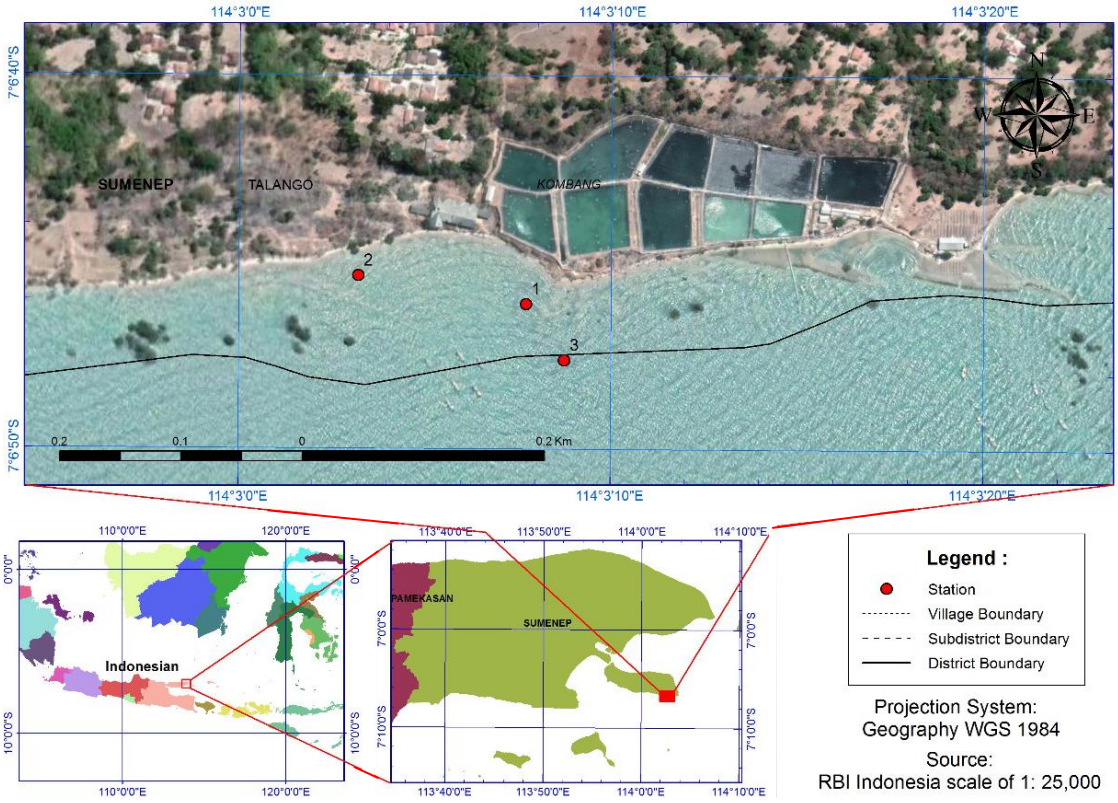

Figure 1. Location of sampling sea slug (*Paromoionchis tumidus*) at Talango sea waters, Sumenep, Madura, Indonesia: point 1 (7º6'46.12"S, 114º3'07.34"E), point 2 (7º6'45.37"S, 114º3'02.83"E); and point 3 (7º6'46.62"S, 114º3'08.37"E)

Extraction of crude protein

Protein extraction was conducted according to the method in marine gastropods (Chen et al. 2017). Sea slug (30 g) was crushed with addition of 30 ml of phosphate buffer saline (PBS) pH 7.8 at 1:1, then ultrasonic-homogenized at 12 × 30 sec. The homogenized sample was centrifuged at 10000 rpm for 30 min to collect the supernatant. The supernatant was added with ammonium sulfate 80% and centrifuged at 10000 rpm for 30 min. The filtrate was added with buffer tris HCl (pH 8), then kept at -20°C for further analysis.

Electrophoresis of protein

The experiment of electrophoresis was carried out according to the method in marine organisms (Chakraborty et al. 2010). The electrophoresis procedure used to buffer (non-denaturing) and Sodium Dodecyl Sulfate-SDS (denaturing) gels. The separation gel was prepared at a concentration of 12.5%, consisting of acrylamide, Tris HCl, SDS, distilled water, and N,N,N',N'-Tetramethyl-ethylenediamine (TEMED). After mixing all these components, the mixture was loaded into gel mold, allowing it to solidify. A 5% stacking gel was made from acrylamide, Tris HCl, SDS, distilled water, and TEMED. After placing the appropriate comb, the stacking gel was poured into the gel mold. Separation of marker and sample solution was performed at 0.4 A, 120V for 100 min. After completion of electrophoresis, the gel was stained using coomassie blue, and finally washed using destaining solution.

Extraction and isolation of taurine

The procedure of taurine extraction referred to previous protocols (Lee et al. 2018). A 100 g of sea slug was washed for removal of mud and unwanted materials. The muscle of the animal was crushed using a blender, transferred into a beaker glass, added with water at 1:1 (w/v), and heated at 90°C for 8-10 min. The liquid was filtered using a plain-woven textile (calico). Centrifugation (5000 rpm, 15 min) was used to collect the filtrate, then freeze-dried. The dried sample was stored at freezer (-4°C) for further use.

The dried sample (2 mg) was thoroughly mixed with 20% (w/v) of sulfosalicylic acid placed on ice, then centrifuged at 28000 rpm for 2 min at 3°C. The supernatant was collected using a pipette, added to 0.4 M borate buffer, and vortexed. The sample was vortexed and re-centrifuged to collect supernatant (taurine). The taurine isolate was stored at -80°C for further analysis.

Detection of taurine

Analysis using HPLC was performed according to previous protocols (Omer et al. 2018). The analysis condition was set as follows: acetonitrile and 0.1% tricholoroacetate acid at 30:70 (v/v) as a mobile phase with a flow rate of 0.1 mL min^{-1}, injection volume 20 µL, detection by fluorescence detector at a wavelength of 470 nm.

RESULTS AND DISCUSSION

Characteristics of Sea Slug (*Paromoionchis tumidus*)

The sea slug captured from Sumenep sea waters was the genus *Paromoionchis tumidus* (Figure 2). The total of sample collected in February 2019 was 415 samples and 502 samples in Mei 2019. The sea slug (*P. tumidus*) has an average length of 3.86 ± 0.66 cm and a weight of 6.86 ± 2.43 g in February 2019, while in May 2019 the average length of 4.00 ± 0.69 cm and weight 7.63 ± 2.70 g.

Proximate content

Table 1 exhibits a difference in the chemical composition of Sea slug (*Paromoionchis tumidus*) collected from three regions in Indonesia. Our experiment found that the protein content of sea slug reached 9.32% (fresh) and 60.82% (dried), while the amount of fat was lower, i.e., 0.34% and 2.31%, respectively.

Protein profile

The protein profile of sea slug (*Paromoionchis tumidus)* collected from Sumenep sea waters was presented in Figure 3 and Table 2. The results showed that the molecular weight of protein isolated from sea slug ranged between 22.53-49.61 kDa. Total band protein from sea slug at least in the mucus (lane 4) of 1 band and the most in the muscle (lane 5) of 6 bands.

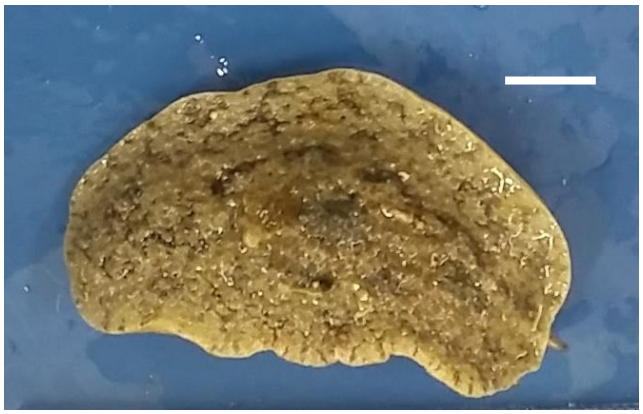

Figure 2. Sea slug (*Paromoionchis tumidus*) collected from Sumenep sea waters, Madura, Indonesia. Bar = 1 cm

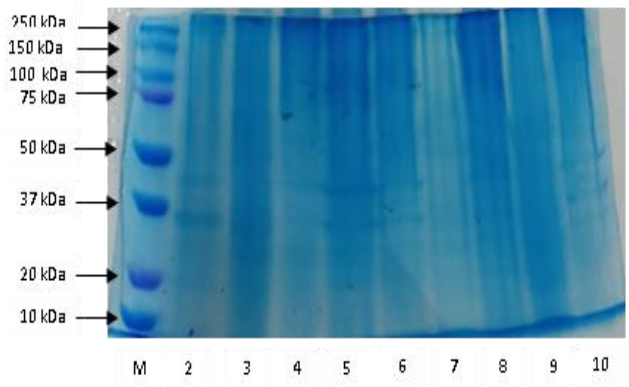

Figure 3. Molecular weight profile of protein isolated from sea slug (Paromoionchis tumidus). M (marker); 2,5,6,8,10 (muscle); 3,7,9 (viscera); 4 (mucus)

Table 1. Comparison of chemical composition (%) in sea slug collected from three different sites: Sumenep (current study), Pamekasan, and Cirebon

Components	Sea slug (*Paromoionchis tumi*dus) from Sumenep Present study		Sea slug (*Discodori*s sp.) from Pamekasan (Hafiluddin et al. 2011)		Sea slug (*Discodoris* sp.) from Cirebon (Putri et al. 2013)
	Fresh	Dried	Fresh	Dried	
Moisture	85.86	7.44	83.00	11.17	78.44
Ash	1.61	19.68	1.87	17.96	3.17
Protein	9.32	60.82	12.31	45.13	15.66
Fat	0.34	2.31	0.44	2.67	0.10
Carbohydrate	2.87	9.75	2.38	23.07	2.65
Crude fiber	0.31	3.25	-	-	-

Table 2. Profile of protein in sea slug (*Paromoionchis tumidus*) collected from Sumenep sea waters

Lane	Total Bands	Distance migrated (cm)	Gel length (cm)	Rf	Mr (kDa)	Log Mr
2	4	1.85	4.3	0.4302326	42.56	1.6289740
		2.30	4.3	0.5348837	35.23	1.5469097
		2.55	4.3	0.5930233	31.69	1.5009166
		2.90	4.3	0.6744186	26.54	1.4238833
3	4	1.60	4.3	0.3720930	48.23	1.6833328
		2.20	4.3	0.5116279	36.69	1.5645509
		2.40	4.3	0.5581395	33.81	1.5290019
		2.70	4.3	0.6279070	29.54	1.4703703
4	1	1.90	4.3	0.4418605	41.61	1.6191847
5	6	2.00	4.3	0.4651163	39.84	1.6003575
		2.20	4.3	0.5116279	36.69	1.5645509
		2.35	4.3	0.5465116	34.52	1.5380098
		2.60	4.3	0.6046512	30.98	1.4910631
		2.70	4.3	0.6279070	29.54	1.4703703
		2.90	4.3	0.6744186	26.54	1.4238833
6	4	2.00	4.3	0.4651163	39.84	1.6003575
		2.30	4.3	0.5348837	35.23	1.5469097
		2.60	4.3	0.6046512	30.98	1.4910631
		2.90	5.3	0.5471698	34.47	1.5375032
7	4	1.60	4.3	0.3720930	48.23	1.6833328
		2.40	4.3	0.5581395	33.81	1.5290019
		2.70	4.3	0.6279070	29.54	1.4703703
		3.00	4.3	0.6976744	24.97	1.3974272
8	4	1.95	4.3	0.4534884	40.71	1.6096596
		2.35	4.3	0.5465116	34.52	1.5380098
		2.60	4.3	0.6046512	30.98	1.4910631
		3.00	4.3	0.6976744	24.97	1.3974272
9	5	1.55	4.3	0.3604651	49.61	1.6955764
		1.95	4.3	0.4534884	40.71	1.6096596
		2.45	4.3	0.5697674	33.10	1.5198446
		2.60	4.3	0.6046512	30.98	1.4910631
		3.15	4.3	0.7325581	22.53	1.3527967
10	4	1.90	4.3	0.4418605	41.61	1.6191847
		2.10	4.3	0.4883721	38.22	1.5822566
		2.25	4.3	0.5232558	35.95	1.5557429
		2.55	4.3	0.5930233	31.69	1.5009166

Table 2. Comparison of amino acids (%) in sea slug (*Paromoionchis tumidus*) collected from Sumenep and Pamekasan sea waters

Amino acids	Sea slug (*Paromoionchis tumidus*) from Sumenep Present study		Sea slug (*Discodoris* sp.) from Pamekasan (Hafiluddin et al. 2011)	Sea slug (*Discodoris* sp.) from Cirebon (Putri et al. 2013)
	Fresh	Dried		
Aspartic acid	0.80	6.45	0.91	0.89
Threonine	0.28	2.17	0.52	0.33
Serine	0.31	2.51	0.55	0.40
Glutamate	1.12	9.50	2.19	1.51
Glycine	0.48	7.74	0.22	0.65
Alanine	0.39	3.71	0.39	0.54
Valine	0.33	2.50	0.81	0.38
Methionine	0.08	0.38	0.28	0.19
Isoleucine	0.29	2.28	0.43	0.36
Leucine	0.52	3.96	1.42	0.59
Tyrosine	0.16	1.24	0.50	0.28
Phenylalanine	0.31	2.23	0.36	0.32
Histidine	0.15	1.10	0.35	0.12
Lysine	0.31	2.39	1.40	0.47
Arginine	0.62	5.72	0.46	0.74
Total	**6.14**	**53.87**	**10.79**	**7.77**

Composition of amino acids

The results demonstrated that the composition of amino acids in sea slug captured from Sumenep, then compared with that captured from Pamekasan (Table 3). Totally, the sample comprised of amino acids, both essential and non-essential, reaching 6.14% in the fresh sample and 53.87% in the dried sample. The highest quantity of amino acids in the fresh and dried sample was glutamate, i.e., 1.12% and 9.50%, respectively; on the contrary, the lowest one was methionine, i.e., 0.08% and 0.38%, respectively**.**

Taurine quantity

The results revealed that the taurine content of sea slug from Sumenep sea waters reached 53.568-158.784 µg/mL (Table 4). The highest and lowest amount of taurine was found in whole (158.784 µg/mL) and viscera (53.568 µg/mL), respectively. This confirms that the amount of taurine in sea slug differs remarkably with different parts of the body. Muscle showed the highest content of taurine, while the lowest one was found in viscera.

Table 3. Content of taurine in sea slug (*Paromoionchis tumidus*) collected from Sumenep sea waters

Parts of the body	Fresh (µg/mL)	Boiled (µg/mL)	Freeze-dried (µg/mL)
Whole	158.784	109.489	90.836
Muscle	129.276	103.018	99.920
Viscera	53.568	138.187	108.207
Mucus	84.867	81.949	81.608

Discussion

Sea slug (*Paromoionchis tumidus*) captured from Sumenep sea waters has a dark brown color resembling mud, soft body, dorsal bumps with black spots, and tentacles (Figure 2). These sea slugs were found in the coastal and intertidal zone. Differences in skin color and character were found to be different in some other families of Onchidiidae. Adaptation to various environments and habitats cause these sea slugs to differ from others (Xu et al. 2018). Some sea slugs Onchiididae have been reported to have black, brown (light brown) skin color and some white spots (Dayrat et al. 2017), and they are found in intertidal areas with mangrove habitat (Goulding et al. 2018).

Chemical composition in an organism was needed as a reference in compiling intake patterns in the body. This information was important as a basic ingredient in development in the field of food. Sea slug was rich in nutrients such as protein, essential amino acids, unsaturated fatty acids, and mineral content and have been used as food (Hafiluddin et al. 2011). The chemical content of sea slug in this study (Table 1) showed that the protein from dried samples (60.82%) was the highest content compared to fat, carbohydrates and fiber, as well as when compared to the protein content of the Pamekasan area (45.13%), but the protein content in the fresh samples are lower than in other regions. We reported that the protein quantity isolated from sea slug (*Paromoionchis tumidus*) tended to be similar to that isolated from *Perronia virruculata* (59.42±1.82%), with the fat content of 5.73±0.98% (Solanki et al. 2017). Furthermore, the protein quantity in sea slug from Sumenep was recorded higher than that in other gastropods, such as *B. zeylanica* (41.53%) (Jayalakshmi 2016), *Laevistrombus turturella* (44.66%) (Rasyid and Dody 2018), while the fat content was also lower than other gastropods. This suggests that amount of protein and fat may differ due to the type and origin of the gastropods. The difference may come from ecological factors, including weather and availability of nutritions in their habitats, the chemical composition difference in species, habitat, and environment (Saad and Alim 2015).

Protein profiles were part of proteomics that can explain biological events. Quantitative evaluation of protein levels can show unique expression (sick vs. healthy, treated vs. untreated, experimental vs. control) when the protein of a cell is compared to other cells, one technique for measuring protein profiles was gel electrophoresis (Şanli-Mohamed et al. 2011). In this study, the protein profiles on sea slug (*P. tumidus*) from Sumenep sea waters ranged from 22.53-49.61 kDa (Figure 3 and Table 2). The range was rather similar in comparison with sea slug *Armina babai*, i.e., 13-72 kDa (Ramya et al. 2014) as well as compared with gastropods *Thais*, i.e., 20-32 kDa (Ali et al. 2018). The most abundant distribution of protein was found at muscle (lane 5), with the molecular weight of 26.54-39.84 kDa, while the molecular weight of protein from mucus (lane 4) was recorded at 41.61 kDa. However, in general, protein levels in sea slugs (*P. tumidus*) are distributed in 4 levels. The results showed a difference compared to the molecular weight of protein extracted from molluscan ink, ranging between 62-249 kDa (Vennila et al. 2011).

Marine organisms have been considered functional foods for humans due to their high levels of amino acids, one of which was marine gastropods (Ragi et al. 2016). The results demonstrated that the composition of amino acids of sea slug captured from Sumenep sea waters, then compared with that captured from Pamekasan (Table 3). Totally, the sample comprised of amino acids, both essential and non-essential, reaching 6.14% in the fresh sample and 53.87% in the dried sample. The highest quantity of amino acids in the fresh and dried sample was glutamate, i.e., 1.12% and 9.50%, respectively; on the contrary, the lowest one was methionine, i.e., 0.08% and 0.38%, respectively. As presented in Table 3, there is a meaningful difference between total amino acid in sea slug from Pamekasan, Sumenep, and Cirebon. The fresh sample of sea slug from Pamekasan contained 10.79% of amino acids, in which the lowest and greatest quantity of amino acid was glycine 0.22% and glutamate 2.19%, respectively (Hafiluddin et al. 2011). The difference also occurs with the amino acid content of sea slug from Cirebon sea waters, with a total amino acid 7.77% (Putri et al. 2013). The greatest and lowest quantity of amino acid was glutamate and histidine. Generally, the total amino acid of sea slug from Sumenep sea waters was lower than in other regions. The differences in chemical composition, including amino acids in certain species, depending on the habitat environment, food availability, and metabolism. Moreover, we reported that total amount of amino acids in sea slug was lower than that in other gastropods, such as *Tibia curta* with the highest portion of arginine up to 32.85% (Ragi et al. 2016), *Strombus luhuanus* with the highest portion of glutamate up to 2.67%, and *Lamis lambis* up to 2.82% (Leiwakabessy and Lewerissa 2017). Additionally, glutamate was also found to be the most abundant amino acids in *Thais* (*T. bufo, T. hippocastanum, and T. rudolphi*) (Ali et al. 2018).

Fish was consumed worldwide, and it contains some health beneficial components such as omega-3, fatty acid, protein, vitamin D, and including the amino acid taurine (Klongnganchui and Muangthai 2016). The results revealed that the taurine content of sea slug from Sumenep sea waters reached 53.568-158.784 µg/mL (Table 4). The highest and lowest amount of taurine was found in whole (158.784 µg/mL) and viscera (53.568 µg/mL), respectively. This confirms that the amount of taurine in sea slug differs remarkably with different parts of the body (Table 4 and Figure 3). Muscle showed the highest content of taurine, while the lowest one was found in viscera. This result was

similar to other studies on fish (Suseno et al. 2014), the quantity of taurine isolated from escolar fish was found higher in the flesh (44.201 mg/100 g) than in viscera (43.915 mg/100 g). The results of other studies indicate that the differences in the content of taurine in freshwater and seawater fish (Klongnganchui and Muangthai 2016).

In addition, the processing condition is also significant in altering the amount of taurine content. The reduction of taurine occurred in the boiled and freeze-dried sample for muscle and mucus, with the exception of viscera. An increase in the amount of taurine in the viscera in boiled was possible because there was an addition of taurine found in sea slug food, namely algae, taurine is abundant in algae (Marles et al. 2010). We believed that the loss of such amino acid resulted from heat exposure (boiling) and freeze-drying condition. Taurine is present as a free amino acid, which makes it highly susceptible to high temperature (Pasqualone et al. 2003). Taurine content in escolar fish (*Lepidocybium flavobrunneum*) was higher with the use of lower extraction temperature (Suseno et al. 2014).

Experimental data revealed the protein abundance of sea slug (*Paromoionchis tumidus*) from Sumenep sea waters, which was present in an appreciable quantity of 9.32% (fresh) and 60.82%(dried) with a molecular weight of 22.53-49.61 kDa. The content of amino acids was observed at a total amount of 6.14% (fresh) and 53.87% (dried), in which glutamate showed the most abundant amino acid. In addition, taurine was present at an amount of 53.568-158.784 µg/mL. Noticeably, the taurine content could be different, as affected by parts of the body and extraction conditions.

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