

Proximate composition and fatty acids profiling of Seahorse originated from Simeulue, Aceh-Indonesia

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Abstract. The aim of this study was to determine the proximate composition, quantitative of fatty acids analysis as well as the content in the seahorse sample, *Hippocampus* sp which originated from Sibigo waters, Simeulue, Aceh, Indonesia. In this study, proximate composition and fatty acids of seahorse were determined. Based on the proximate analyses that had been conducted, results showed that protein was the most abundantly found in the seahorse (39.32±0.22%), orderly followed by carbohydrate (28.48±0.25%), ash (25.43±0.16%), moisture (6.29±0.13%) (dry weight basis) and fat (0.47±0.30%). Fatty acids profiling and analysis were also conducted by using the a GC-MS (Gas Chromatography-Mass Spectroscopy). The analysis result showed that sample possessed of saturated fatty acids in big size were palmitic acid, stearic acid, myristic acid, and lauric acid. While unsaturated fatty acids were arachidonic acid, linoleic acid and oleic acid. Traces of saturated fatty acids in small seahorse were found in the sample including palmitic acid, stearic acid, miristic acid and lauric acid, while unsaturated fatty acids were linoleic acid and oleic acid. The highest saturated fatty acid in seahorse was palmitic acid (60.67%), and unsaturated fatty acids were oleic acid (45.5%), arachidonic acid (25.08%) and linoleic acid (0.93%).

1 Introduction

Seahorses (*Hippocampus* sp.) are an outstanding marine species considering their reproductive patterns and other features. Owing to the iconic characteristics of these fishes, aquarium trade, and research efforts have increased in the last years. Types of seahorses found in Indonesia, among others, are the *Hippocampus barbouri*, *Hippocampus bargibanti*, *Hippocampus comes*, *Hippocampus histrix*, *Hippocampus kelloggi*, *Hippocampus kuda*, *Hippocampus spinosissimus*, *Hippocampus trimaculatus* and *Hippocampus* sp. [1]. The types of seahorses found in Sibigo waters, Simeulue, Aceh, Indonesia are *Hippocampus* sp.

Seahorses are superior fishes that has been over-exploited in nature owing to their use in several human activities, habitat loss, and the aquarium and antique trade, at the expense of its conservation [2]. Seahorses have been used in the discovery and development of traditional and allopathic medical treatments for a long time. Seahorses are efficacious as ingredients in traditional Chinese medicine for generations and are believed to strengthen stamina [3]. It is believed that seahorses have the potential to cure infertility, baldness, asthma and arthritis inflammation [4]. Apart from this, seahorses are also believed to have free radical scavenging effects in controlling the aging process [5]. The research result of Sanaye et al. [6], antioxidant activity of yellow seahorse, *Hippocampus kuda* was 24.04%.

The results of other studies stated that seahorses can be used as antifatigue with a cell proliferation activity value of 160% and able to inhibit oxidation activity with an IC₅₀ value of 43.8 ppm [7], inhibiting the growth of *E. coli* bacteria with an inhibition zone of 4 mm, and inhibiting the growth of the fungus *Aspergillus flavus* with an inhibitory zone of 2 mm. The many benefits of seahorses are owing to the presence of complex bioactive compounds [8]. Therefore, people often take advantage of seahorse as an alternative in natural medicine.

To being used in pharmaceuticals, other benefits of sea horses are as a food source of animal protein which is full of nutrition and very good for consumption by humans. Protein is a substance that important in the body. Amino acids are the main components that make up proteins has a metabolic function in the body and divided into two groups, namely amino acids essential and non-essential [9]. In addition to amino acids, seahorses also contain fatty acids, both saturated and unsaturated. The difference in the amount of content and the types of fatty acids present in seahorse are very instrumental in provide benefits to humans.

The nutritional content of sea horses other than protein, namely vitamins, minerals, carbohydrates, fiber, and others. The nutritional content of the seahorse has the potential as a dietary supplement for human health [10]. This research is related to proximate analysis and the content of fatty acids and the types contained in seahorses. This is because there is still a

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lack of information about the chemical characteristics of seahorses that are important to the community. The purpose of the study was to examine the proximate composition and fatty acids profiling of seahorse originated from Simeulue, Aceh-Indonesia.

2 Materials and methods

2.1 Materials

Seahorses (*Hippocampus* sp.) were collected from Sibigo waters, Simeulue, Aceh-Indonesia with a size of 3-10 cm to be used for this study. The sizes were divided into 2 group, small (3-5 cm) and large (6-10 cm). The seahorse sample was storage in an ice cooler box. This was to maintain low temperature and preserve the structure of the sample through the journey in bringing the sample to the laboratory. The sample was then thoroughly rinsed with distilled water for several times and oven-dried at temperature of 60 °C for 2 days, until the sample was completely dry. The dried sample was sorted and ground into powder form by using a high power grinder and then tightly-packed and stored at -18 °C cold room until further chemical analyses. All chemical analyses of the seahorses sample were carried out in triplicates to obtain more accurate results.

2.2 Methods

2.2.1 Moisture content analysis.

2 g sample was accurately weighed into the crucible and dried at 105 °C in a universal oven (Mettler) until constant sample weight was obtained [11].

2.2.2 Ash content analysis.

Dried seahorse sample that was obtained from the previous analysis was then burnt and ashed using a muffle furnace at 525 °C overnight. Sample was weighed to its nearest 0.001 g using an analytical balance [11].

2.2.3 Crude protein analysis.

Determination of crude protein content of seahorse sample was using semimicro Kjeldahl method. 0.5 g of dried seahorse sample was accurately weighed into 100mL Kjeldahl tube. 2 g of selenium and 25 mL of concentrated sulphuric acid (H₂SO₄) was carefully added into the digestion tubes and gently shaken. Destruction procedure was performed at 420 °C for approximately 120 minutes until clear blue/green solution was obtained and cooled for 30 minutes. Distillation procedure was then performed at 100 °C for 10 minutes. The distillate was titrated with 0.01 N HCl with a phenolphthalein as indicator until the color of the solution changes to pink. The percentage of crude protein was calculated by multiplying the percent of nitrogen, N found with a factor of 6.25 [11].

2.2.4 Fat content analysis.

The fat content of seahorse sample was extracted by using the Soxhlet method. 2 g of powder dried seahorse sample was weighed in filter paper and placed in the extraction thimble. The samples were extracted using a Soxhlet extractor by adding the 200 mL of hexane as a solvent in the boiling flask. Extraction process was run for 5 hours and the extracted fat was dried using a rotary evaporator. The boiling flask was dried in an oven at 105 °C for 1 hour and left to cool prior obtaining the final weight [11].

2.2.5 Carbohydrate content.

Carbohydrate content was calculated based on this calculation [% carbohydrate = 100%-%moisture+% ash+% crude protein+ % fat].

2.2.6 The lipids and fatty acids analysis.

The fatty acid content of seahorses identified from the chromatogram (peak) of the fatty acid methyl ester (FAME) fractionation using capillary gas chromatography (50 x 32 mm) SP2330. Methyl ester was obtained from the fat methylation process according to the modified British Standard Method, while the fat for methylation was extracted from the material by the modified Bligh–Dyer Method. Fatty acids were identified by comparing the peaks of the analysis with the peaks of standard fatty acids. Each sample of large and small seahorse was repeated twice with the method equipped with an Rxi-MS column, 0.25 m film thickness, capillary column and with 5973 MSD tissue mass spectrometer, operated in electron impact ionization mode (70 eV). Analysis GC-MS was performed in split mode (split ratio 1:50), using helium as the carrier gas (flow rate 1 mL/min). The injector temperature was set at 250 °C. The sample volume, injected 1 µL. The oven temperature was maintained at 150 °C for 2 minutes and then programmed at 5 °C/min to a final temperature of 280 °C, where it was maintained for 5 minutes, then waited until the separation of all fatty acids was completed [11].

3 Results and discussion

3.1 Proximate composition

Proximate analyses of the seahorse sample provided useful information on the composition of the seahorse (*Hippocampus* sp.) from Sibigo waters, Simeulue, Aceh-Indonesia. According to [12] the proximate content in the body of the seahorse can characterize the physiological and health conditions of the seahorse. In general, the body composition of the seahorse is obtained by proximate analysis, but measurements of physiological conditions can also be determined using a comparison of the standard weight and length relationship. The determined proximate compositions of seahorse are moisture content, ash content, crude protein, fat content and carbohydrate

content which had been done were tabulated as the following. Based on Table 1, shows that seahorse collected in Sibigo waters, Simeulue contained 39.32±0.22% of crude protein, 0.47±0.24% of fat, 25.43±0.16% of ash content, 6.29±0.13% of moisture content (powdered sample), and 28.48±0.25% of carbohydrate content. Seahorse sample contained quite a large amount of crude protein, ash and carbohydrate, but contained low fat.

Table 1. The proximate composition in seahorse from Simeulue.

Composition (dry weight basis)	Percentage (%) ± SD
Crude protein content	39.32±0.22
Fat content	0.47±0.30
Ash content	25.43±0.16
Carbohydrate content	28.48±0.25
Moisture content	6.29±0.13

The proximate composition of the seahorse was calculated based on dry weight. The moisture content of the sampled seahorse was 6.29±0.13. The moisture content contained in each type of seahorse was different which caused by several factors e.g the fishing season and the age of the species. [13] mentioned that the average moisture content in fresh seahorses was 65-76%. In addition, [14] stated that the moisture content in marine biota ranges from 50-85% depend on the type of species and body nutritional conditions. The season of fishing for some species that was suspected to be during the reproductive process, so that the species will loss of moisture content in their bodies.

The proximate composition of seahorses on a dry basis were 70.70% of protein, 1.71% of fat, 20.92% of ash and 38.15 (µg/g) of Zn mineral [12]. The nutritional content of seahorses can also be influenced by their living habitat and food sources. The protein content of the seahorse was 39.32±0.22% which was obtained from mixing several sexes of the seahorse that were sampled. [15] studied the quality of juvenile sea horse (*Hippocampus barbouri*), which fed with a modified diet, the crude protein content was 37.12-61.47%. The value of fat content in seahorses was 0. 0.47±0.30%. According to the results of research from [16-18], it is stated that the average value of fat content in the tuna meat is 0.51%. Fat is one of the excess energy stored by animals so that the amount of fat in the animal's body that is used as food is determined by the energy balance of the animal.

3.2 Fatty acids profiling

The fatty acids content of the seahorse (*Hippocampus sp*) were saturated and saturated fatty acids. The saturated fatty acids were pamic acid, stearic acid, myristic acid and behenic acid, while the unsaturated fatty acids were arachidonic acid and oleic acid. Table 2 and Tabel 3 shows the fatty acid composition, respectively.

Table 2. The fatty acids (FA) composition of big seahorses

No.	Kind of FA	Name of FA	Formula of Molecule	Amount (%)
1	Saturated FA	Tetradecanoic acid (Myristic acid)	C ₁₄ H ₂₈ O ₂	1.6
2		Hexadecanoic acid (Palimitic acid)	C ₁₆ H ₃₂ O ₂	25.2
3		Octadecanoic acid (Stearic acid)	C ₁₈ H ₃₆ O ₂	20.3
4		Docosanoic acid (Behenic acid)	C ₂₂ H ₄₄ O ₂	0.6
Total				47.7
1	Unsaturated FA	Eicosanoic acid (Aracidic acid)	C ₂₀ H ₄₀ O ₂	22.2
2		Oleic Acid	C ₁₈ H ₃₄ O ₂	30.2
Total				52.4

Table 3. The fatty acids (FA) composition of small seahorses

No.	Kind of FA	Name of FA	Formula of Molecule	Average
1	Saturated FA	Tetradecanoic acid (Myristic acid)	C ₁₄ H ₂₈ O ₂	0.7
2		Hexadecanoic acid (Palimitic acid)	C ₁₆ H ₃₂ O ₂	49.4
3		Octadecanoic acid (Stearic acid)	C ₁₈ H ₃₆ O ₂	37.3
4		Docosanoic acid (Behenic acid)	C ₂₂ H ₄₄ O ₂	0.7
Total				88.1
1	Unsaturated FA	Oleic Acid	C ₁₈ H ₃₄ O ₂	12.0
Total				12.0

The oil of seahorse was injected twice for each sample. It can be seen from the table that the transesterification has been successfully carried out. This is indicated by the presence of a chromatogram that has been identified. All sample of seahorse contain two kinds of fatty acids, namely saturated fatty acids and unsaturated fatty acids. For big size seahorses, 2 unsaturated fatty acids were identified, but for small seahorse only 1 unsaturated fatty acid that is oleic acid. The distribution of fatty acids differs from one species to another, and depends on various factors, such as season, temperature, growing place, fish species, age, sex and feeding habits [16].

Based on Table 2 and Tabel 3, the data obtained from the GC-MS analysis showed that the content of unsaturated fatty acids between large seahorses (52.4%) and small seahorses (12.0%) had quite a significant difference. The higher saturated fatty acid was palmitic acid both in large and small size, then followed by stearic acid. The fatty acid content of seahorses is the same as fish, which are both aquatic animals. The results of fatty acid analysis of oil carp (*Cyprinus carpio*), the

higher fatty acid was palmitic acid (C:16-0) of 17,15% [19].

The fatty acids contained in fish consist of saturated fatty acids (15-25%), unsaturated fatty acids (35-60%) and polyunsaturated fatty acids (25-40%) [20]. This happens because according to [21]. the quality of fish oil, especially its fatty acid composition, is influenced by season, fishing area, type of food, sexual maturity and age of fish. According to [22], Arachidonic acid (20:4n-6;AA) is also needed by larvae. Both types of fatty acids, namely AA and EPA, are substrates needed for the formation of eicosanoates which play a role in various physiological functions including ion regulation and egg maturity in female parents. The results of the study on the Arachidonic value of large seahorses of 44.3% which has an important role for the growth of seahorses.

The results of research conducted by [23] in Layur fish, myristic acid levels were only 0.24% of the total fatty acids in the sample. Meanwhile, the fat from mackerel and tuna is physically solid at room temperature because it has more saturated fatty acid composition than unsaturated fatty acids. This is in accordance with the results of research by [24] which shows that the composition of fatty acids is one of them influenced by species differences (species specific).

The results of the comparison test with other seahorses are the first on seahorses from Simeulu Waters while the comparison is on seahorse eggs (*Hippocampus barbouri*). The results of the seahorse eggs when the research was conducted showed that the seahorse eggs against repeated spawning the first treatment on Myristic acid as much as 0.53%, Palmitic acid as much as 26.49%, Stearic acid as much as 0.53% this is different from the analysis of the results of the study This was done because the research used seahorse samples, while in the comparison results using seahorse eggs spawned by seahorses. The content of seahorse eggs also depends on the large diameter of the eggs and the large number of mature eggs that will accommodate higher lipids which of course will have an impact on the high fatty acid content of the female seahorse (*Hippocampus barbouri*). While the results of the study the content of seahorse samples depends on the nutritional value or proximate.

4 Conclusion

This research can be concluded that the seahorse sample contains the nutritional composition and fatty acid composition of the large seahorse and small seahorse consisting of saturated fatty acids and unsaturated fatty acids. Saturated fatty acids contained in seahorses were Palmitic acid, Behenic acid, Myristic acid, and Stearic acid. Unsaturated fatty acids contained in seahorses were Oleic acid and Arachidonic acid.

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