Chemical, Physical and Microbiological Analysis of the Smoked Bat Milk (Holothuria fuscogilva) and Pineapple Sea Cucumber (Thelenota ananas)

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Research Article

Chemical, Physical and Microbiological Analysis of the Smoked Bat Milk (*Holothuria fuscogilva*) and Pineapple Sea Cucumber (*Thelenota ananas*)

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Abstract

Objective: This study was designed to analyze the chemical, physical, microbiological quality of smoked sea cucumbers. **Materials and Methods:** Amino acid in sea cucumbers was analyzed using HPLC method. Polycyclic Aromatic Hydrocarbons (PAH) was analyzed using gas chromatography. The microbiological analysis of smoked bat milk sea cucumber and smoked pineapple sea cucumber was performed. **Results:** Both types of smoked sea cucumbers have 8 components of essential amino acids (Histidine, Threonine, Arginine, Methionine, Valine, l-leucine, Leucine, and Lysine) and 7 components of non-essential amino acids (Aspartic acid, Glutamic acid, Serine, Glycine, Alanine, Tyrosine, and Phenylalanine). The most abundant amino acids is Alanine. The total amino acids are 36.56% in bat milk sea cucumber and 28.22% in smoked pineapple sea cucumber. The pathogen analysis of *Escherichia coli* for the two types of smoked sea cucumber showed the same value (<3 MPN g^{-1}). Also, *Staphylococcus aureus* showed the same value as well as the results of salmonella analysis for both types of smoked sea cucumbers were negative. High-quality Polycyclic Aromatic Hydrocarbons (PAHs) compounds were found in smoked bat milk sea cucumbers and low-quality in pineapple sea cucumbers. The highest total plate count was found in pineapple sea cucumber (2.1 log CFU g^{-1}) while lowest in the bat milk sea cucumber (1.3 log CFU g^{-1}). **Conclusion:** This study found that both types of sea cucumber (smoked) meets the requirements of Indonesian National Standards.

Key words: Amino acids, sea cucumbers, polycyclic aromatic hydrocarbons, total plate count, pathogenic bacteria

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the Ministry of Indonesian Marine and Fisheries the production of sea cucumbers in Indonesia was 5.428 tons in 2014 with an average increase of 1.08% from 2004 to 2014[1]. Up to now, sea cucumbers are only processed in the dry and smoked form. They are exported with relatively little added value compared to sea cucumber extracts for supplements. Proper coaching from relevant agencies are needed to improve the quality of processed smoked dried sea cucumber so that the value of processed products can be increased as the processed products from other countries.

Sea cucumber smoking is generally done traditionally using hot smoke that generate from burning wood inside the smoke house. The sea cucumber processors generally use any type of wood as their smoke fuel, according to the availability of wood in the surrounding environment. Chemical compounds of wood smoke are generally in the form of phenol (which act as antioxidants), organic acids, alcohols, carbonyls, hydrocarbons and nitrogen compounds such as nitrous oxide, aldehydes, ketones, esters, ether, which stick to the surface and then penetrate into the meat[2]. Sea cucumber smoking is an effective method of preservation and processing that has been widely used by the community in North Sulawesi. Sea cucumbers are a commodity of fishery products which are known to be easily deteriorate. The process of deterioration cannot be totally stopped but slow down the spoilage by processing and preserving. Smoking is one of the preservation and processing techniques[3]. According to Girard[4] smoking of sea cucumbers and other foodstuffs extend the shelf life of the product, and enhance the flavor, appearance and taste. Previous studies conducted by Stolyhwo and Sikorski[5] and Visciano *et al.*[6] have shown that smoking is a preservation method that not only increases shelf life but also gives the desired taste and color to smoked products due to the presence of phenol and carbonyl compounds. Sea cucumbers are processed through the penetration of volatile compounds produced by the burning of wood, the anti-bacterial activity of wood smoke produce specific taste, color and aroma as well as extend the shelf life of the processed sea cucumbers. Sea cucumbers have the potential to be used as food, in most European and Asian countries (Hong Kong, Singapore, South Korea, Malaysia, Japan, America) it is mainly exported in dry form. The main sea cucumber exporters in the international market are Indonesia, the Philippines, New Caledonia, Maldives, India and Sri Lanka. The sea cucumbers that have a high market value are *Holothuria scraba*, *Holothuria nobilis*, *Actinopyga echinites*, *Actinopyga miliaris*, *Bohadschia argus* and *Theleonata ananas*[7]. In dry conditions, sea cucumber contain moisture content (8.9%), protein content (82%), fat content (1.7%), ash content (8.6%) and carbohydrate (4.8%).

The Indonesian sea cucumber export is increasing every year. Based on Ferdouse's report[8] in 2000 Indonesia was the largest producer of sea cucumber and its export reach to 2,500 tons. Nearly 50% of the production is consumed in China, Hongkong and Malaysia. The increasing demand for Asian markets is driving increased efforts to exploit sea cucumbers in various producing countries. North Sulawesi is one of the potential producers of sea cucumbers in Indonesia. Since sea cucumber fishing activities are increasing every year, it is fear that it will cause over exploitation which can lead to scarcity of sea cucumber resources. This can also affect the existing sea cucumber business therefore, comprehensive research on sea cucumber should be carried out. Sea cucumbers also contain vitamin B12, thiamine, riboflavin, minerals, phosphate, iron, arsenic, iodine, calcium, magnesium and copper[9]. Sea cucumbers are foods that contain lots of nutrients but the calorific value is lower than fish, mollusks and squid[10]. The purpose of this study was to investigate the final quality of smoked sea cucumber products so that it can be ready for export.

MATERIALS AND METHODS

Experimental site: Smoked sea cucumber samples were obtained from Darunu village, Wori District, North Minahasa Regency. The experiment lasted for 3 months.

Materials and research tools: Two types of smoked sea cucumber were used in this study. The chemicals used were NaOH, K_2CO_3, NaBr, KI, NaCl, KCl and $BaCl_2$ (Merck Darnstad Germany) and the tools used were Ohaus analytical scales; Burette; Oven; Test tube; Desiccator; Soxhlet; Drop pipettes; etc.

Experimental design: This study used a completely randomized design (CRD) consisting of two treatments and three replications.

Parameters measured: Proximate analysis (moisture content, protein content, fat content and ash content) was performed following the method of AOAC[11]. Amino acid in products was analyzed using HPLC method. The microbiological analysis was performed to determine the total viable count based on Indonesian National Standard Fisheries (INS).

Statistical analysis: Data were analyzed using one way ANOVA. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 20.0 for windows (SPSS Inc., Chicago, IL, USA). Values were expressed as Means±SD (standard deviation). Values of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Proximate analysis: Sea cucumbers are preserved using heat and smoke from hardwood. Smoke contains phenol compounds and formaldehyde, each of which is bactericidal (kills bacteria). The combination of these two compounds is also used as a fungicide which form a binding layer on the surface of sea cucumbers. Heating process also kill microbes and decreases moisture content. Items contain low moisture content are not easily destroyed by microbes. Based on the results of the analysis it can be seen that the moisture content of the smoked sea cucumber ranged from 31.62-40.7%.

Low moisture content has a positive impact on the long durability of sea cucumber. The drier a product, the longer is its durability[12]. Moisture content also affects the texture of the final product. If the moisture content is too high then the texture of the dried sea cucumber becomes flabby and not compact and thus affect the consumer acceptability[13]. Drying including smoking process can eliminate water content of food. Winarno[14] explained that the long drying time reduced the moisture content of the food. Results of smoked bat milk sea cucumbers and pineapple sea cucumber can be seen in Table 1.

The highest protein content (46.66%) was found in smoked sea cucumber, while the lowest (39.17%) was in smoked pineapple sea cucumber. Like other fishery products, sea cucumbers also contain high protein. Protein is an important food substance for the body because it functions as a source of energy, building and regulating substances[15]. Smoking is a method used for the hydrolysis of protein, the protein can be converted into value added products through enzymatic hydrolysis, which is widely used to improve the nutritional properties of proteins. The enzymatic hydrolysis process of sea cucumber improved its economic value, biomedical application and antioxidant properties[16].

The highest fat content (2.11%) was found in smoked pineapple sea cucumber while the lowest (1.31%) was found in the smoked bat milk sea cucumber. The dried sea cucumbers have relatively low fat content (1.7 g/100) compared to other sea cucumbers but high level of omega-3 fatty acids[17]. Fat is a source of energy that is more effective than carbohydrates and proteins (9:4) and improve joint lubrication[18]. Martoyo *et al.*[19] stated that differences in nutrient content in sea cucumbers are due to differences in species and biological conditions of sea cucumbers, these differences can also be caused by the availability of food in the waters and the types of sea cucumbers themselves.

Analysis of data showed that the smoked bat milk sea cucumber has the lowest (3.43%) while the pineapple sea cucumbers has the highest ash content (4.4%). The higher the ash content contained in a food, the more mineral content produced. Sea cucumbers contain mineral contents such as chromium, ferum, cadmium, manganese, nickel, cobalt and zinc[20]. Method of difference was used to determine the carbohydrate contents in food. Results showed that the lowest carbohydrate was found in smoked bat milk sea cucumber (2.41%), while the highest was found in smoked pineapple sea cucumbers (3.58%).

Amino acid in smoked sea cucumber: Analysis of amino acid is very important, because the protein quality of a food is largely determined by the amino acid content it contains[21]. Nutritional amino acids are divided into 2 groups, namely non-essential amino acids and essential amino acids. Non-essential amino acids are amino acids that can be provided by the body through a complex biosynthetic process of nitrogen compounds found in food and essential amino acids are amino acids that cannot be synthesized by the body. The qualities of food protein are determined by the availability of amino acids that can be absorbed by the body. The results of amino acid testing of both types of sea cucumbers can be seen in Table 2. Table 2 shows the results of the analysis of amino acid in smoked bat milk and pineapple sea cucumber.

The results showed that the smoked sea cucumber contained 15 components of essential amino acids namely histidine, threonine, arginine, methionine, valine, l-leucine, leucine and lysine. In addition, there are components of

Table 1: Results of proximate analysis

	Mean±standard deviation				
Samples/treatments	Moisture	Protein	Fat	Ash	Carbohydrate
Bat milk sea cucumber (smoked)	31.62±0.02	46.66±0.03	1.31±0.04	3.43±0.36	2.31±0.03
Pineapple sea cucumber (smoked)	40.70±0.04	39.17±0.02	2.11±0.03	4.40±0.16	3.58±0.02

Table 2: Analysis of amino acid in smoked bat milk sea cucumber and smoked pineapple sea cucumber

Type of amino acid	Smoked bat milk sea cucumber	Smoked pineapple sea cucumber
Aspartic acid	4.30±0.01	3.65±0.03
Glutamic acid	3.52±0.03	3.72±0.01
Serine	1.22±0.01	1.44±0.02
Glycine	2.07±0.04	0.26±0.05
Alanine	5.11±0.02	4.10±0.03
Tyrosine	2.25±0.02	1.32±0.04
Phenylalanine	2.73±0.04	2.39±0.03
Arginine	1.63±0.05	2.32±0.04
Histidine	1.33±0.01	0.48±0.01
Threonine	2.42±0 .05	2.74±0.03
Methionine	1.37±0.03	1.18±0.02
Valine	2.98±0.01	1.44±0.02
Isoleucine	1.88±0.03	0.77±0.04
Leucine	2.27±0.04	2.11±0.03
Lysine	1.48±0.05	0.30±0.02
Amino acid total	36.56±0.04	28.22±0.02

non-essential amino acid such as aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine and phenylalanine. The most abundant amino acid is alanine which is 5.11 in smoked sea cucumber and 4.10 in smoked pineapple sea cucumber. The results showed that the sample of smoked sea cucumber and smoked pineapple sea cucumber contained 8 components of essential amino acids namely histidine, threonine, arginine, methionine, valine, l-leucine, leucine and lysine. In addition, there are 7 components of non-essential amino acids such as aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine and phenylalanine. Table 2 shows that the total amino acid in smoked bat milk sea cucumber was higher (36.56%) than that of the smoked pineapple sea cucumber which was 28.22%. A glutamic acid is a charged (polar) amino acid together with aspartic acid. This can be seen from the low isoelectric point, where the acid has tendency to capture electrons (acidic)[20]. In addition, glutamate can be produced by the human body so it is not considered as essential. In general, vegetable protein contains more amino acids such as arginine, glycine and alanine, while animal protein contains more lysine and methionine.

Previous studies noted that the amino acids, lysine and methionine tended to increase cholesterol levels, whereas arginine showed the opposite effect. Methionine is a precursor of homocysteine which is a risk factor. That's why the animal foods are more hypercholesterolemia than vegetable foods. Other studies have also shown that all essential amino acids except arginine have the potential to be hypercholesterolemic, while lysine and methionine shows more chances of hypercholesterolemia.

Amino acids are the basic constituent of protein and can be produced by the body for metabolic purposes and are found in all foods that contain protein[14]. Amino acids are classified as essential and non-essential amino acids. Glutamic acid is classified as a non-essential amino acid. Glutamic acid is a constituent of protein found in a variety of vegetables, fruit, meat, fish and breast milk. Animal protein contains 11-22% glutamic acid while vegetable protein contains 40% glutamic acid. Glutamic acid is the most important non-essential amino acid and used as a flavor enhancer[22]. It also improves the taste of meat[23]. The flavoring effect of glutamate come with its free form, where it is not bound to other amino acids in protein[24]. Glutamic acid plays an important role in the synthesis of amino acids because glutamic acid is an effective non-specific nitrogen source[25].

Protein is commonly found in animal and plant products. Proteins are polymers of approximately 20 different types of amino acids that are connected by peptide bonds. Amino acids with peptide bonds will form the primary structure of proteins. Amino acids are divided into two groups, namely non-essential and essential amino acids. A total of 12 types of non-essential amino acids are produced by the body, while 8 essential amino acids must be obtained through food. Non-essential amino acids produced by the body include tyrosine, cysteine, serine, proline, glycine, glutamic acid, aspartic acid, arginine, alanine, histidine, glutamine and asparagine. Essential amino acids that are not produced by the body include tryptophan, threonine, methionine, lysine, leucine, isoleucine, phenylalanine and valine. To meet the protein requirements of an organism, the additional essential amino acids are required which are obtained from food or feed consumed.

Protein deficiency leads to various health problems. Additional animal and vegetable protein increase HB levels in patients with anemia. At least, fifteen essential amino acids must be available in food, namely: phenylalanine, tyrosine, isoleucine, lysine, methionine, cystine, threonine, valine, tryptophan, arginine, histidine, glycine, serine, asparagine and

Table 3: Microbiological quality of smoked sea cucumber and smoke pineapple sea cucumber

Parameter	Bat milk sea cucumber (smoked)	Pineapple sea cucumber (smoked)	Unit
Total plate count	2.04×10^{1}	1.30×10^{2}	Colony g^{-1}
Escherichia coli	<3	<3	MPN g^{-1}
Staphylococcus aureus	0	0	-
Salmonella	Negative	Negative	-

Table 4: Analysis of PAH compounds in bat milk and pineapple sea cucumbers

Test parameters	Bat milk sea cucumbers (smoked)	Detection limit	Pineapple sea cucumbers (smoked)	Detection limit	Unit
Naphthalene	<0.87	0.87	<0.87	0.87	mg kg^{-1}
Acenaphthene	<0.10	0.10	<0.10	0.10	mg kg^{-1}
Phenanthrene	0.72	-	<0.20	0.20	mg kg^{-1}
Pyrene	<0.70	0.70	<0.76	0.76	mg kg^{-1}
Benzo(a)anthracene	1.23	-	<2.26	2.26	mg kg^{-1}
Benzo(a)pyrene	<2.83	2.83	<2.83	2.83	mg kg^{-1}

proline. The nutritional quality of a food protein is determined by the suitability between the type and amount of amino acids in food and the type and amount of amino acids needed for the development of analysis methods[26].

Microbiological quality: The microbiological quality of bat milk and pineapple sea cucumber (smoked) are presented in Table 3. The total plate count in bat milk sea cucumbers (smoked) was 2.04×10^{1} colonies g^{-1} while in pineapple sea cucumbers (smoked) was 1.30×10^{2} colonies g^{-1}. The lowest total plate count was observed in pineapple sea cucumber (smoked) and the highest was observed in the bat milk sea cucumber (smoked). The *Escherichia coli* for the both types of smoked sea cucumber were the same (<3 MPN g^{-1}). The same value (0) was also found for *Staphylococcus aureus*. The results of salmonella analysis for both types of smoked sea cucumbers were negative.

Total plate count (TPC) are the total microbial colonies that grow on food or on finished products. The TPC limit for foodstuffs is 10^{6}CFU g^{-1}. Growing colonies show the number of all microorganisms in the sample such as bacteria, mold and yeast[27]. The TPC was low in bat milk and pineapple sea cucumber (smoked), it was due to good and hygienic curing process. Furthermore, the number of samples detected for E. coli and salmonella show zero contamination in both types of smoked sea cucumbers. Escherichia coli is a strain of coliform that is gam-negative, non-spherical, facultatively anaerobic, rod-shaped and can ferment lactose by producing acids and gases at 35°C for 48 h. Poor sanitation and hygiene can contaminate food during processing. The transmission of disease can be prevented by raising awareness and understanding among workers about the importance of sanitation. *Staphylococcus aureus* is a gram-positive bacterium that has a higher tolerance than those of the other pathogenic bacteria. *Staphylococcus aureus* bacteria lives on the surface of the skin, nails and human respiratory tract. Processed products that undergo a heating process are easily contaminated by these bacteria through the hands of the processor, during storage, temperature that suits the optimum tolerance can cause the growth of these bacteria. Salmonella is a gram-negative, non-spherical and facultatively anaerobic bacterium, mostly motile. Animal food and waste water are known as good media for the growth of these bacteria. It colonizes the gastrointestinal tract and transmitted during transportation of raw materials and processing.

Polycyclic aromatic hydrocarbons (PAH): PAH analysis was conducted to determine the quality of bat milk and pineapple sea cucumbers (smoked). The results of the analysis of PAH compounds using gas chromatography are presented in Table 4.

Analysis of PAH compounds in bat milk and pineapple sea cucumbers: The compounds identified in smoked bat milk sea cucumbers and pineapple sea cucumbers were: naphthalene, acenaphthene, phenanthrene, pyrene, benzo (a) anthracene and benzo(a)pyrene.

Polycyclic aromatic hydrocarbons (PAH) compounds in both types of smoked sea cucumber: PAH compounds in smoked bat milk sea cucumbers with values of each were naphthalene (<0.87), acenaphthene (<0.10), phenanthrene (0.72), pyrene (<0.70), benzo(a)anthracene (1.23), Benzo(a)pyrene (<2.83). PAH compounds in smoked pineapple sea cucumbers with values of each were: naphthalene (<0.87), acenaphthene (<0.10), phenanthrene (<0.20), pyrene (<0.76), benzo(a)anthracene (<2.26), benzo(a)pyrene (<2.83). The detected PAH compounds were caused by reactions that occur in the smoke component. Low levels of compounds in both types of smoked sea cucumbers are thought to be due to the component of smoke and sea cucumber meat itself which is able to inhibit the formation of PAH compounds. Based on the standards of the European Commission Regulation, the amount of PAH compounds is still below the quality standard thus still said to be harmless[28]. The PAH in smoke depends on the heat source (coal, wood and gas), the temperature, the intensity of the fire in combustion and the compounds formed during combustion. The combustion temperature during the smoking process is very critical factor where PAHs are formed in the incomplete combustion process (for example burning wood, coal or charcoal, oil). PAH can be formed in 3 ways, namely through high temperatures (700°C), pyrolysis of organic compounds at low to moderate temperatures (100-150°C) and re-formation of organic compounds by microorganisms[29].

Akpan *et al.*[30] reported a strong relationship between sea cucumber fat and PAH compounds specifically, PAH compounds stored in sea cucumber fat tissue. When the fat in the meat is roasted, large amounts of fat drops fall on the embers in the presence of high temperatures. PAHs with 4, 5 and 6 rings are more carcinogenic than PAHs with simpler or even larger ring systems and configuration of angles tends to be more carcinogenic than PAHs with linear ring systems. Low molecular weight PAHs such as naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene and anthracene possess 2-3 rings which are not classified as highly carcinogenic compounds[31].

CONCLUSION

Both types of smoked sea cucumber contain moisture (31.62%) protein (46.66%) fat (1.31%) ash (3.43%) and carbohydrate content (2.41%). Overall the smoked sea cucumbers meat the quality standards set by the Indonesian National Standard. In addition, there are 8 components of essential amino acids and 7 components of non-essential amino acids. The most abundant amino acids is Alanine. The total plate count in smoked sea cucumbers was 2.04×10^1 colonies g^{-1} while in smoked pineapple sea cucumbers was 1.30×10^2 colonies g^{-1}. The total plate count was the lowest in smoked pineapple sea cucumber and the highest in the smoked sea cucumber. The *Escherichia coli* for the both types of smoked sea cucumber were the same (<3 MPN g^{-1}). The same value (0) was also found for *Staphylococcus aureus*. The results of salmonella analysis for both types of smoked sea cucumbers were negative.

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