

Chemical Composition, Fatty Acid Profile, And Amino Acid Profile Of Sahoaki (*Tripneustes Gratilla*) Gonads From Sangihe Island, Indonesia

Ni Wayan Suriani

Department of Science Education, Universitas Negeri Manado, 95618 Minahasa, Indonesia

*Corresponding Author:

Email: niwayansuriani@unima.ac.id

Abstract.

Sahoaki (Tripneustes gratilla), abundant in Sangihe Island, is a food ingredient by local people. This study aims to determine the chemical composition, fatty acid profile (bioactive content of omega-3, omega-6, and omega-9 fatty acids), and amino acid profile of the sahoaki gonads from Sangihe Island, Indonesia. The fatty acid profile was tested using the Gas Chromatography method (GC 7890B), while the amino acid profile was tested using Liquid chromatography-mass spectrometry. The results of research on fresh sahoaki gonads showed that the chemical composition: water content (84.24 and 85.99)%, fat (3.77 and 2.67)%, protein (10.12 and 9.34)%, carbohydrates (0.08 and 0.05)%, and ash (3.19 and 2.95)%. Fatty acid profile: there are 14 saturated fatty acid profiles, seven monounsaturated fatty acid profiles, nine polyunsaturated fatty acid profiles, the total content of omega-3 (19.46 and 15.95)%, omega-6 (16.25 and 16, 39 %), and omega-9 (12.94 and 13.94)%. Seven amino acid profiles were detected, consisting of five essential amino acids and two non-essential amino acids. Sahoaki gonads have good nutritional value because they contain high omega-3, Omega-6, and Omega-9 fatty acids and are equipped with essential amino acids, which have the potential as functional food ingredients.

Keywords: Sahoaki; sea urchins; *Tripneustes gratilla*; omega-3 fatty acids and functional food.

I. INTRODUCTION

Indonesia is a country that has a long coastline of about 108,000 km and has been crowned as the largest archipelagic country in the world [1, 2]. It makes the existence of marine fauna in Indonesia very diverse, including animal echinoderms that dominate coastal waters. Sea urchins have significant economic value, especially as highly nutritious food and export commodities. In addition, sea urchins have an important role in marine ecology, acting as a controller for microalgae populations and a determining factor for the abundance of shallow-water marine plants [3]. Feather gonads can be consumed and have high economic value [4]. Sea urchin gonads are an export commodity to countries such as Japan, China, Korea, Canada, France, Russia, and the United States [5-7]. The selling price of sea urchins in Japan ranges from 50 to 500 US dollars per kilogram [8]. One type of sea urchin often consumed and traded is *Tripneustes gratilla*. The great potential of the marine sector in Indonesia still needs to be optimized in line with the application of the blue economy concept, one of which is the processing industry based on marine resources. One of the biological resources with a high level of species diversity is the sea urchin; there are around 950 sea urchins spread throughout the world [9], while in Indonesia, as many as 84 species come from 48 clans and 21 tribes [7, 10]. Sea urchin groups are one of the inhabitants of seagrass beds and coral reefs [6, 11-14]—these animals like the habitat clear on a rigid substrate. Sea urchins (Echinoidea) are invertebrate animals often found in rocky and sandy tidal areas [15].

One of the three species of sea urchins in the genus *Tripneustes* is the *Tripneustes gratilla*; the other two are the *Tripneustes ventricosus* and the *Tripneustes depressus*. Oceans all over the world are home to this species, including the West Pacific, East Africa, the Southern Islands, Australia, and Southern Japan [16]. Previous studies have shown that *Tripneustes gratilla* is abundant in Indonesian waters [17-26]. *Tripneustes gratilla* is generally characterized by a dark color, the upper surface of the body is slightly curved, and the lower body is flat; the shell structure is tough, calcareous, and filled with white spines mixed with orange; the pedicle is white with a dark or black base [27]. *Tripneustes gratilla* is a sea urchin with various colors that symbolize environmental adaptability. [19]. *Tripneustes gratilla* makes seagrass meadows the best habitat for growth and protection from waves, predator attacks, and sunburn by trying to cover itself with pieces of algae, seagrass, or coral fragments [28]. Food availability in an area will affect the food

consumption pattern of the people there [29]. Communities in an area use the results of nature to meet their food needs [30]. People in Sangihe Islands Regency used abundant *Tripneustes gratilla* as a food source. Sahoaki is a local term for the people in Sangihe Islands Regency to refer to *Tripneustes gratilla* (see Figure 1). Communities in the Sangihe Islands Regency use sahoaki gonads (see Figure 1) as food ingredients to be processed into daily side dishes.

The most prominent characteristic of how they consume sahoaki is mixing the sahoaki gonads into the porridge given to the baby. They acknowledge that sea urchin gonads taste good when consumed but are unaware of their nutritional value. The characteristics of the gonads of sea urchins that have a pleasing taste are seen from the color (yellow and red) and texture (dense and smooth) of the gonads [31]. Containing 18 amino acids, B complex vitamins, vitamin A, minerals, and omega-3 and omega-6 unsaturated fatty acids, sea urchin gonads can be used as a food source [32]. A previous study [33] discovered that sea urchin gonads contained 15 types of detectable amino acids, 8 of which were essential and 7 of which were non-essential. Additionally, methionine, valine, phenylalanine, isoleucine, leucine, threonine, lysine, and histidine are the essential amino acids found in the three gonads of the sea urchin. However, non-essential amino acids like arginine, aspartate, glutamate, serine, glycine, alanine, and tyrosine were examined. Glutamate is the most abundant amino acid in protein foods and the brain's most abundant free amino acid [34]. Glutamate plays a crucial role in normal brain function [35]. Glycine can lower stomach acidity, stimulate the release of growth hormone, and aid in wound healing and muscle development [36]. The quality of protein depends on the completeness of its essential amino acid levels. Omega-3 unsaturated fatty acids are effective at lowering human cholesterol levels and are also found in sea urchins. Lipids, proteins, and carbohydrates are a group of biological macromolecules that are very important for living things [37]. Thus, the human body requires the gonads of sea urchins as an additional nutrient-dense food source.



Fig 1. Sahoaki (*Tripneustes gratilla*): body (left) and gonads (right)

The abundance of sahoaki (*Tripneustes gratilla*) on the coast of the Sangihe Islands and its role as a food ingredient by the local community is the basis for the importance of researching more about the nutritional value of gonad sahoaki. In addition, there has yet to be a published research article specifically looking at the nutritional value of sahoaki in the Sangihe Islands Regency. Therefore, our study aims to determine the chemical composition (water content, fat, protein, carbohydrates, and ash), fatty acid profile (contain bioactive fatty acids omega-3, omega-6, and omega-9), and profile gonad amino acid sahoaki at two different locations in Sangihe Islands Regency, North Sulawesi Province, Indonesia. The results of this study are helpful for the community as information about the nutritional value of sahoaki gonads and as a recommendation for making sahoaki gonads an essential ingredient for baby porridge formulations to create a functional food product that is safe and healthy.

II. METHODS

Materials

The raw materials for analysis are sahoaki (*Tripneustes gratilla*) gonads taken from two locations, namely on the Lapango coast and Manalu coast in Sangihe Islands Regency, North Sulawesi Province, Indonesia. The chemicals used for analysis include distilled water, HCl, NaCl, Na₂SO₄, NaOH, CuSO₄, and K₂SO₄.

Instrumentation

The equipment for research consists of a set of distillation apparatus, soxhlet extraction tube, Kjeldahl flask, biuret for titration, electric heater, oven, analytical balance, Gas Chromatography (GC 7890B), and LCMS (Liquid chromatography-mass spectrometry).

Procedure

The research was designed experimentally with the exploratory, descriptive research design used. Chemical composition analysis refers to [38]. Fatty acid profiles (Saturated Fatty Acid (SFA), Mono Unsaturated Fatty Acid (MUFA), Polyunsaturated Fatty Acid (PUFA), omega-3, omega-6, and omega-9) were tested using the Gas Chromatography method (GC 7890B). On the other hand, Amino acid profiles were tested using LCMS (Liquid chromatography-mass spectrometry). We carried out the testing site for chemical composition, fatty acid profile, and amino acid profile at the Integrated Research and Testing Laboratory (LPPT) at Gadjah Mada University, Yogyakarta, Indonesia.

III. RESULT AND DISCUSSION

The Chemical Composition

The chemical composition of the gonads of sahoaki was determined by proximate analysis. The analysis included moisture, fat, protein, carbohydrates, and ash content. The results of the analysis carried out on fresh gonads from two locations on the coast of the Sangihe Islands are shown in Table 1. The analysis of the chemical composition of the two locations showed a slight difference, which was probably caused by environmental factors where the sea urchin *Tripneustes gratilla* lived.

Table 1. Chemical Composition of Sahoaki (*Tripneustes gratilla*) Gonads

Chemical Composition (%)	Lapango Coast	Manalu Coast
Water	84.24	85.99
Fat	3.77	2.67
Proteins	10.12	9.34
Carbohydrates	0.08	0.05
Ash	3.19	2.95

This study's results are similar to the research conducted [39]. The chemical composition of the gonads *Tripneustes gratilla* is 73.55% moisture, 3.42% ash, 2.76% fat, 10.68% protein, and 8.25% carbohydrates. However, there are some differences with research conducted by [31] on fresh gonads of Diadema setosum from Ambon Island waters; the moisture content was 73.76% - 84.13%, ash 0.20% - 2.12%, fat 3.47% - 5.81%, protein 5.40% - 17.69%, carbohydrates 2.11% - 7.50%. The chemical composition can vary between species, individuals within a species, and parts of an individual. These variations can be caused by several factors, including age, metabolic rate, movement activity, food, and conditions before and after the egg-laying season. Type, age, habitat, nutrition, and water conditions significantly affect the nutritional composition of sea urchin gonads [31]. The water content in the gonads of the sea urchin *Tripneustes gratilla* studied at two coastal locations on the Sangihe Islands was 84.24% and 85.99%.

These results have a higher water content than the results of previous studies [39], namely the gonad water content of the sea urchin *Tripneustes gratilla* of 73.55% and [33] namely 66.86% - 77.24%, but not different from other studies [31] fresh gonads of Diadema setosum from Ambon Island waters obtained a water content in the range of 73.76% - 84.13%. The high water content is caused by the habitat of sea urchins, which spend their whole lives in the waters. Differences in chemical composition can occur due to the season when caught, reproductive phase, sex, and environmental or water conditions [40]. These differences reflect the original form of the material consumed, such as plankton, as well as the result of the

metabolism of the material consumed. The gonad fat content of the sea urchin *Tripneustes gratilla* in this study was 3.77% and 2.67%. This result was higher than the gonadal fat content of fresh *Diadema setosum*, which was studied from Martafons waters at 3.47% and Waai at 0.85% and lower in Sopapei waters at 5.81% [31], and did not differ much from the lipid content in *Tripneustes gratilla* studied by Hasan [39] which only reached 2.76%. The fat content in the gonads of sea urchins *D. setosum*, *E. calamaris*, and *E. diadema* were 6.89%, 5.71%, and 3.65%, respectively [33]. Differences in fat content in the gonads of sea urchins are thought to be due to the different gametogenesis phases in each species. Besides that, the diet of the organism itself also influences it.

The age of the species and the degree of gonad maturity can both have an impact on differences in fat content [41]. The gonad protein levels of Sahoaki sea urchins of the type *Tripneustes gratilla*, studied at two coastal locations of the Sangihe Islands, were 10.12% and 9.34%. These results were lower than previous studies [33], which stated that the gonadal protein levels of sea urchins *D. setosum*, *E. calamaris*, and *E. diadema* were 12.60%, 11.40%, and 13.20%, respectively. However, this is similar to the protein content of the *Tripneustes gratilla*, which is 10.68% [39]. In another study [42] on the gonads of edible sea urchins, *Paracentrotus vilidus*, from several regions, namely Oristano, Alghero, Cagliari, and Sassari, the protein content of the four samples was 10.96%, 11.64%, 12.20%, and 10.60%. The protein content in the gonad samples of the studied sea urchins did not differ much from the protein content in *Tripneustes gratilla* and was in the same range as *Paracentrotus vilidus*. The difference in protein levels is likely due to the different types of sea urchins and aquatic habitats. Also, factors of type, age, size, and environmental or habitat conditions affect protein content [33]. One of the factors that affect the fat content in the gonads of sea urchins is food. There are two food sources for sea urchins: non-planktonic, which does not come from plankton but comes from the parent egg yolk, and planktonic, which comes from phytoplankton and zooplankton [43]. Another factor that affects the high-fat content is the size of the gonads. The gonads of sea urchins that are large in size contain proportionately fatter. High-fat content tends to produce a large gonad volume to be used as an energy reserve for development [44, 45]. It may be related to the abundance of food available in their habitat.

Carbohydrate levels in the gonads of sahoaki (*Tripneustes gratilla*) studied in this study were obtained by different methods, namely 0.08% and 0.05%. This result is deficient compared to a study conducted by Hasan [39], who obtained a gonadal carbohydrate content of *Tripneustes gratilla* of 8.25%, and Afifudin [33] who found a carbohydrate content of 11.58% in *D. cytosum*, 4.90 % in *E. calamaris*, and 3.83% in *E. diadema*. Also very different compared to the *Diadema setosum* gonads studied by Tupan & Silaban [31] from Martafons, Sopapei, and Waai Ambon waters, respectively 3.80%, 2.11%, and 7.50%. Sea urchins have three important biochemical components: carbohydrates, fats, and proteins [43]. Sea urchins use these three components as energy sources and structural components during egg development. The gonad ash content of the sea urchin *Tripneustes gratilla* in this study was 3.19% and 2.95%. The results of this study were almost the same as research conducted by Hasan [39], that the mineral/ash content in the gonads of *Tripneustes gratilla* was 3.42%. However, it is higher when compared to a study conducted by Afifudin [33] on the gonads of sea urchins *D. setosum*, *E. calamaris*, and *E. diadema*, respectively 2.09%, 1.74%, and 2.10%. Each species' ash content differs due to several factors. Metals derived from food and the environment will accumulate in the body at different levels, so there is a difference in the amount of ash found in each species [41]. It is likely because each organism can absorb metals differently. The mineral content of the organisms in it can also be affected by environmental factors like water quality and food availability. The mineral content of the organisms that live in it can be affected by environmental factors like water quality and food availability [33].

The Fatty Acids Profile

The results showed that the gonads of the sea urchin *Tripneustes gratilla* from two coastal locations are similar but different. The composition contains 14 types of saturated fatty acids, SFA (saturated fatty acid), butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), stearic acid (C:18), arachidic acid (C:20), heneicosanoic acid (C21:0), behenic acid (C22:0), tricosanoic (C23:0), and lignoceric acid (C24:0).

Also, seven monounsaturated fatty acids MUFA (Monounsaturated Fatty Acid) myristoleic acid (C14:1 ω -5), palmitoleic acid (C18:1 ω -7), elaidic acid (C18:1 ω -9), oleic acid (C18:1 ω -9), eicosanoic acid (C20:1 ω -9), erucic acid (C22:1 ω -9), and nervonic acid (C24:1 ω -9). Also, 9 PUFA (Polyunsaturated Fatty Acid): linoleic acid (C18:2 ω -6), linolelaidic acid (C18:2 ω -6), eicosadienoic acid (C20:2 ω -6), arachidonic acid (C20:4 ω -6), γ linolenic acid (C18:3 ω -6), linolenic acid (C18:3 ω -3), eicosatrienoic acid (C20:3 ω -3), eicosapentaenoic acid (EPA) (C20:5 ω -3), and docosahexaenoic acid (DHA) (C22:6 ω -3). The same type of food could have caused the equation of fatty acids from two gonad sample locations in the form of algae, seagrass beds, and various kinds of marine animals. The overall composition of fatty acids is shown in Table 2.

Table 2. The Fatty Acid Profile of Sahoaki (*Tripterus gratilla*) Gonads

Fatty Acid Composition	% Relative	
	Lapango Coast	Manalu Coast
Butyric acid (C4:0)	<0.1	<0.1
Caproic Acid (C6:0)	<0.1	<0.1
Caprylic Acid (C8:0)	<0.1	<0.1
Capric Acid (C10:0)	<0.1	<0.1
Lauric Acid (C12:0)	<0.1	<0.1
Myristic acid (C14:0)	9.91	10.71
Pentadecanoic Acid (C15:0)	0.81	0.78
Palmitic acid (C16:0)	27.52	28.97
Stearic Acid (C18:0)	5.34	5.22
Arachidic Acid (C20:0)	1.73	1.54
Heneicosanoic Acid (C21:0)	2.75	2.78
Behenic Acid (C22:0)	1.82	1.8
Tricosanoic Acid (C23:0)	0.57	0.97
Lignoseric Acid (C24:0)	0.74	0.6
Total Saturated Fatty Acid (SFA)	51.19	53.37
Myristoleic Acid (C14:1 ω -5)	0.27	0.24
Palmitoleic Acid (C18:1 ω -7)	2.74	3.04
Elaidic Acid (C18:1 ω -9)	0.32	0.35
Oleic acid (C18:1 ω -9)	3.23	3.61
Eicosanoic Acid (C20:1 ω -9)	4.15	3.69
Erucic Acid (C22:1 ω -9)	1.4	1.71
Nervonic Acid (C24:1 ω -9)	0.83	1.3
Total Omega-9	12.94	13.94
Total Mono Unsaturated Fatty Acid (MUFA)	12.94	13.94
Linoleic Acid (C18:2 ω -6)	1.17	1.19
Linolelaidic Acid (C18:2 ω -6)	1.59	1.46
Eicosadienoic Acid (C20:2 ω -6)	2.61	2.14
Arachidonic Acid (C20:4 ω -6)	9.77	7.62
γ Linolenic Acid (C18:3 ω -6)	1.11	0.97
Linolenic Acid (C18:3 ω -3)	7.61	7.52
Eicosatrienoic Acid (C20:3 ω -3)	0.55	0.35
Eicosapentaenoic Acid (EPA) (C20:5 ω -3)	10.72	10.63
Docosahexaenoic Acid (DHA) (C22:6 ω -3)	0.58	0.46
Total Omega-6	16.25	16.39
Total Omega-3	19.46	15.95
Total Polyunsaturated Fatty Acid (PUFA)	35.71	32.34

Results of gonad fatty acid analysis for sea urchin *Tripneustes gratilla* overall show that there are differences in the levels of saturated fatty acids and unsaturated fatty acids (SFA) on the Lapango coast and Manalu coast. The sample from the Manalu coast (53.37%) was higher than the Lapango coast (51.19%). The monounsaturated fatty acid (MUFA) content was higher on the Manalu coast (13.94 %) if we compared it to the Lapango coast (12.94%), while the polyunsaturated fatty acid (PUFA) content on the Lapango coast was 35.71 % higher than the Manalu coast by 32.34%. This discrepancy is likely due to several factors, including environmental influences such as seawater temperature, availability of feed, and maturity gonads. In marine ecosystems, two main factors can affect the fat in the tissue: water temperature and food type. Martínez-Pita [46] reported that animal invertebrates, including sea urchins, tend to increase in fatty acid levels and polyunsaturated fatty acids when the water temperature decreases. Overall, the total saturated fatty acid SFA was higher in gonads for sea urchin *Tripneustes gratilla* than monounsaturated fatty acids (MUFA) and polyunsaturated PUFA. Saturated fatty acids can increase LDL cholesterol levels as well as HDL so that they automatically increase total cholesterol, which is a combination of LDL and HDL [47]. HDL cholesterol plays a role in reversing cholesterol transport, which enables the liver to remove excess cholesterol in the poriferous tissue, thereby preventing the formation of atherosclerosis which is the cause of cardiovascular disease [48].

HDL cholesterol also plays a role in preventing excess fat tissue, which can reduce insulin sensitivity so that diabetes mellitus can be prevented and provides protection against endotoxin toxicity. Other research also reported the results of a study on the gonads of sea urchins *Diadema setosum*, *Echinothrix calamari*, and *Echinothrix diadema* contain fatty acid profile SFA (30.46-34.99), MUFA (8.75 - 9.74), PUFA (14.05 - 20.17), Arachidonic (6.76 - 10.24) EPA (2, 3 - 2.89) DHA (0.38 - 0.73), Oleate (3.06 - 3.84) Omega-3 (3.16 - 4.84) Omega-6 (9.21 - 13.88), and Omega-9 (3.95 - 5.01) [33]. The gonadal analysis of five types of sea urchins in the South Coast of Gunung Kidul Regency showed that the levels of saturated fatty acids were higher than unsaturated fatty acids, especially myristic acid 27.20% and 24.44% of palmitic [49]. The results showed that the gonads of sahoaki (*Tripneustes gratilla*) on the Manalu coast have a total content of omega-6 fatty acids of 16.39% and omega-9 of 13.94% which is higher than on the coast of Lapango, namely 16.25% and 12.94%. Meanwhile, the total omega-3 fatty acids on the Lapango coast were 19.46% higher than the Manalu coast, which was 15.95%. Previous research reported the results of the analysis of the gonadal fatty acid profile of *Tripneustes gratilla*, showing that saturated fatty acids were higher than unsaturated fatty acids, namely SFA of 58.78%, MUFA 8.53%, PUFA 11.95%, Omega-3 11.95% and Omega-9 5.5% [50]. The highest Omega-3 content in the gonads of the sea urchin *Tripneustes gratilla* is Eicosapentaenoic Acid (EPA), which is 10.72%, then Linolenic Acid, which is 7.61%, on the coast of Lapango, while on the coast of Manalu, EPA is 10.63% and Linolenic Acid is 7.52%.

The highest Omega-6 content on the Lapango coast was Arachidonic Acid (AA), which was 9.77% and 7.62% on the Manalu coast, while the DHA content was very low in both locations (0.58 and 0.46%). The low content of DHA in the gonads of sahoaki is not a problem because the gonads of sea urchins contain relatively high MUFA and PUFA. Humans have an enzyme system for chain elongation and desaturation to make DHA and arachidonic acid from omega-6 fatty acids [51]. Omega-6 fatty acids are necessary for normal growth and development as well as the functioning of the liver and brain [52]. Another benefit of omega-3 fatty acids is preventing asthma, diabetes, and kidney disease [53]. Endogenous bioregulators in regulating ion homeostasis, gene transcription, hormone signal transduction, fat synthesis, and influencing protein formation are all complex functions of unsaturated fatty acids [54]. Good quality fatty acids have a ratio of omega-3 to omega-6 fatty acids that do not exceed 1:5. If the ratio of omega-6 fatty acids is higher than omega-3, it will harm cognitive, mood, and behavior [55]. People are recommended to consume omega-6 and omega-3 in a 4:1 ratio to maintain a healthy body, mainly to prevent heart disease. Cardiovascular sufferers are advised to consume omega-6 and omega-3 in a 1:1 ratio. ARA and DHA work together to grow the baby's brain and eyes and prevent cardiovascular disease. Linolenic acid functions as an ARA precursor in the presence of the omega-6 desaturase enzyme. Oleic acid, omega-9, is vital in reducing LDL blood cholesterol or bad cholesterol and increasing HDL.

The Amino Acid Profile

The results of sahoaki (*Tripneustes gratilla*) gonad analysis contain seven types of amino acids consisting of 6 essential amino acids: L-arginine, L-histidine, L-lysine, L-phenylalanine, L-isoleucine, L-leucine and one non-essential amino acid L-tyrosine. The complete gonadal amino acid profile of Sahoaki is presented in Table 3.

Table 3. The Amino Acid Profile of Sahoaki (*Tripneustes gratilla*) Gonads

Amino Acid Composition	µg/g	
	Lapango Coast	Manalu Coast
L-Arginine	1879,0	1772,9
L-Histidine*	494,1	476,7
L-Lysine*	689,1	667,0
L-Phenylalanine*	78,7	75,9
L-Isoleucine*	262,5	232,4
L-Leucine*	165,0	139,7
L-Tyrosine	28,3	25,1
L-Methionine*	0	0
L-Valin*	0	0
L-Proline	0	0
L-Glutamic Acid	0	0
L-Aspartic Acid	0	0
L-Cytosine	0	0
L-Threonine*	0	0
L-Serine	0	0
L-Alanine	0	0
L-Glycine	0	0
L-Cysteine	0	0

*Essential Amino Acids

Amino acids have traditionally been categorized as nutritionally essential (indispensable) or non-essential (dispensable) for animals and humans based on growth or nitrogen balance [56]. The term "essential amino acids" (EAA) refers to either those whose carbon skeletons cannot be synthesized de novo in animal cells or those whose syntheses are sufficient for the needs of their metabolism [57]. Non-essential amino acids (NEAA), on the other hand, can be synthesized by animals de novo in sufficient quantities [58]. Although in this study, the number of amino acids detected was only 7, five are essential amino acids that greatly determine the quality of the protein content in the gonads of sahoaki. The highest detected non-essential amino acid was arginine (1879.0 and 1772.9) µg/g, while the lowest was tyrosine (28.3 and 25.1) µg/g. The gonads of sea urchins as reproductive organs are high-quality protein stockpiles rich in amino acids that the human body needs. The amino acids are glycine, valine, alanine, methionine, and glutamic acid. Apart from that, nucleotides from the types of Inosine Mono Phosphate (IMP) and Guanosine Mono Phosphate (GMP) also influence the characterization of the gonadal taste of sea urchins, especially in the formation of "umami" taste, which is a distinctive taste like that of the meat group [59]. Protein quality depends on the completeness of its essential amino acid levels [60]. Histidine is important for brain homeostasis and the inflammatory response because it is an essential amino acid involved in aspartate and glutamate synthesis and histamine production [61].

Histidine is the most active and adaptable of the 20 natural amino acids. It plays multiple roles in protein interactions and is frequently the key residue in enzyme catalytic reactions [62]. The most adaptable player in protein architectures and bioactivities might be histidine. Because of its unique structure, histidine has multiple functions in molecular interactions. During development, the body requires the amino acid histidine to repair tissues and transform excess glucose into glycogen processed in the liver. The body converts histidine to histamine, which stimulates gastric acid release. But it is also often necessary to supplement histidine itself in old age because there is a disturbance of its synthesis and absorption by the body. Most higher organisms cannot produce lysine; As a result, to maintain protein synthesis, it is an essential amino acid that must be consumed in sufficient quantities [63]. Lysine is a necessary nutrient for animal growth and an essential amino acid for human diets [63]. Most post-translational modifications are performed on lysine, the most modified amino acid [64]. Lysine contains a long flexible side-chain

containing three methyl groups and a terminal ϵ -amino group (a primary amine) [64]. Lysine is an essential amino acid directly related to growth because of its role in arginine, glycine, and ornithine, which can activate the human growth hormone [65].

Phenylalanine is an amino acid and an essential nutrient in many foods [66]. Because it is converted into tyrosine and, consequently, into catecholamine neurotransmitters, this amino acid is essential to the human body [66]. Phenylalanine is an aromatic α -amino acid of the chemical formula C9H11NO2 [66]. Phenylalanine is a necessary amino acid for human development because it is used as a precursor in the synthesis of tyrosine and other compounds with a six-membered aromatic ring, like dopamine and catecholamines: epinephrine, also known as adrenaline, norepinephrine, and melanin, also known as skin pigment [66]. Additionally, it is used to improve memory and alleviate hunger pains [67]. In the brain's nervous system, compounds such as phenylalanine, taurine, and tryptophan function as messengers or neurotransmitters [68]. Due to its ability to regulate thyroid gland secretion and suppress appetite, this amino acid is responsible for weight management. Red eyes (bloodshot eyes), cataracts, and behavioral changes (psychotic and schizophrenic) are all symptoms of phenylalanine deficiency. One of the three essential branched-chain amino acids for the human body is isoleucine, which contains one carboxyl and one amine [69]. It performs various physiological functions and is necessary to life metabolism; Consequently, it is utilized extensively in the food, pharmaceutical, and feed industries [69]. Isoleucine plays a crucial role in growth, immunity, protein metabolism, fatty acid metabolism, and glucose transport throughout the body [70]. Isoleucine can potentially enhance the immune system, including cells, immune organs, and reactive substances [70].

According to recent research, host defense peptides (also known as β -defensins), which can control both innate and adaptive immunity, may be induced by isoleucine [70]. Leucine is a branched-chain amino acid (BCAA) necessary for human and animal nutrition [71]. In foods high in protein, it is typically one of the most abundant amino acids [71]. In skeletal muscle, adipose tissue, and placental cells, Leu activates the mammalian target of the rapamycin (mTOR) signaling pathway, which leads to an increase in protein synthesis [71]. Leu inhibits protein degradation and promotes energy metabolism (glucose uptake, mitochondrial biogenesis, and fatty acid oxidation) to support protein synthesis [71]. Therefore, leucine-enriched amino acid supplements in older sarcopenic adults who consume less food maintain muscle mass and physical function [72]. Leucine in the diet is regarded as an additional treatment for obesity-related insulin resistance [73]. Our bodies can produce arginine, a conditionally non-essential amino acid [74]. Arginine is the precursor for the synthesis of numerous biologically active molecules, including nitric oxide (NO), ornithine, polyamines (putrescine, spermidine, and spermine), creatine, and agmatine [75]. It is also a building block for proteins. Human embryos require the amino acid arginine for growth and survival [75]. In women who had a poor response to in vitro fertilization, oral administration of L-arginine (16 g/day for eight days) can increase uterine blood flow, ovarian response to gonadotrophin, endometrial receptivity, and pregnancy rate [75].

In living things, tyrosine is very important and plays a crucial role. Using L-phenylalanine, the human body produces tyrosine, a non-essential amino acid found in sweat, breast milk, urine, saliva, plasma, feces, and cerebrospinal fluid [76]. L-tyrosine, one of the essential amino acids, is used by cells to make proteins in living systems [77]. It plays a crucial role in signal transduction through phosphorylation and is a building block for proteins and a precursor for melanin, several neurotransmitters, and hormones [78]. Varying levels of L-tyrosine in living systems have also been linked to dementia, depression, Parkinson's disease, tyrosinemia, and kidney and liver dysfunction [77]. Tyrosine and its analogs play a role in the production of important chemicals in the brain as well as melanin, the regulation of mood, the reduction of stress, the regulation of thyroid function, and other important metabolic activities [76]. Studies on the connection between tyrosine and epilepsy, Parkinson's disease, Alzheimer's disease, myocardial infarction, viral infections, schizophrenia, colon cancer, thyroid cancer, lung cancer, leukemia, obesity, hypothyroidism, autism, alcoholism, depression, and other conditions [76].

IV. CONCLUSION

Sahoaki (*Tripneustes gratilla*) gonads in Sangihe Islands Regency have good nutritional value because they contain high omega-3, Omega-6, and Omega-9 fatty acids and are equipped with essential amino acids which have the potential as functional food ingredients. Based on the research we have done, the gonads of sahoaki (*Tripneustes gratilla*) on the Lapango coast and Manalu coast, Sangihe Islands Regency, contain: (1) Chemical composition: water content (84.24 and 85.99)% , fat (3.77 and 2.67)% , protein (10.12 and 9.34)% , carbohydrates (0.08 and 0.05)% , and ash (3.19 and 2.95)% ; (2) There were 14 saturated fatty acids (SFA) profiles, with the highest content being palmitic acid (27.52 and 28.97%), then myristic acid and stearic acid; 7 profiles of monounsaturated fatty acids (MUFA).

With the highest content of eicosanoic acid (4.15 and 3.69%), then palmitoleic acid, oleic acid; 9 polyunsaturated fatty acids (PUFA) profiles with the highest content were eicosapentaenoic acid (EPA) (10.72 and 10.63%), then arachidonic acid (9.77 and 7.62 %), Linolenic Acid (7.61 and 7.52%), Docosahexaenoic Acid (DHA) (0.58 and 0.46 %). The total content of Omega-3 (19.46 and 15.95%), Omega-6 (16.25 and 16.39 %), and Omega-9 (12.94 and 13.94%). In addition, the content of saturated fatty acids is higher than monounsaturated and polyunsaturated fatty acids; and (3) Amino acid profiles were detected consisting of 5 essential amino acids with the highest content, namely lysine (689.1 and 667.0) $\mu\text{g/g}$; histidine (494.1 and 476.7) $\mu\text{g/g}$; isoleucine (262.5 and 232.4) $\mu\text{g/g}$; leucine (165.0 and 139.7) $\mu\text{g/g}$; phenylalanine (78.7 and 75.9) $\mu\text{g/g}$; and two non-essential amino acids namely arginine (1,879.0 and 1,772.9) $\mu\text{g/g}$; tyrosine (28.3 and 25.1) $\mu\text{g/g}$.

V. ACKNOWLEDGMENTS

We are grateful to the Indonesian Ministry of Education, Culture, Research, and Technology for funding this study through the Higher Education Excellence Basic Research scheme in 2022 with contract number: 228/E5/PG.02.00.PT/2022.

REFERENCES

- [1] Handiani, D. N., Heriati, A., & Gunawan, W. A. (2022). Comparison of coastal vulnerability assessment for Subang Regency in North Coast West Java-Indonesia. *Geomatics, Natural Hazards and Risk*, 13(1), 1178-1206. <https://doi.org/10.1080/19475705.2022.2066573>
- [2] Afriansyah, A., Darmawan, A. R., & Pramudianto, A. (2022). Enforcing law in undelimited maritime areas: Indonesian border experience. *The International Journal of Marine and Coastal Law*, 37(2), 1-18. <https://doi.org/10.1163/15718085-bja10092>
- [3] Hermansyah, H., Setia, T. M., Utomo, C., Ramadhani, A. R., Sabihis, S., & Sahril, N. (2020). Preliminary study of coral reef conditions in marine park and coral reef education centre plan area, Seribu Islands National Park, Indonesia. *Indonesian Journal of Biotechnology and Biodiversity*, 4(1), 1-9. <https://doi.org/10.47007/ijobb.v4i1.50>
- [4] Mamede, R., Duarte, I. A., Caçador, I., Tanner, S. E., Silva, M., Jacinto, D., Fonseca, V. F., & Duarte, B. (2022). Elemental fingerprinting of sea urchin (*Paracentrotus lividus*) gonads to assess food safety and trace its geographic origin. *Journal of Food Composition and Analysis*, 114, 104764. <https://doi.org/10.1016/j.jfca.2022.104764>
- [5] Uneputty, P. A., Pattikawa, J. A., & Rijoly, F. (2017). Status populasi bulu babi *Tripneustes gratilla* di perairan Desa Liang, Pulau Ambon. *Omni-Akuatika*, 12(3), 98-105. <http://dx.doi.org/10.20884/1.oa.2016.12.3.131>
- [6] Huda, M. A. I., Sudarmadji, S., & Fajariyah, S. (2017). keanekaragaman jenis echinoidea di zona intertidal Pantai Jeding Taman Nasional Baluran. *Berkala Sainstek*, 5(2), 61-65. <https://doi.org/10.19184/bst.v5i2.5531>
- [7] Fraulaine, F. F., & Akobiarek, M. N. (2016). Penentuan kualitas protein berdasarkan kandungan asam amino pada bulu babi (*Tripneustes gratilla* Linnaeus). *Novae Guinea Jurnal Biologi*, 7(1). Retrieved from: <https://ejournal.uncen.ac.id/index.php/NG/article/view/745>
- [8] ITPC Osaka. (2014). **Market brief: bulu babi (Uni)**. Jakarta. Retrieved from: <https://itpc.or.jp/2014/08/19/bulu-babi-2014/>
- [9] Wessel, G. M., Kiyomoto, M., Reitzel, A. M., & Carrier, T. J. (2022). Pigmentation biosynthesis influences the microbiome in sea urchins. *Proceedings of the Royal Society B*, 289(1981), 20221088. <https://doi.org/10.1098/rspb.2022.1088>

[10] Sabilu, Y., Jafriati, & Madjid, R. (2022). Test of bioactivity and antioxidant activity of sea urchin (*Diadema setosum*) gonads as medicinal ingredients based on marine biodiversity. *Journal of Southwest Jiaotong University*, 57(1). <https://doi.org/10.35741/issn.0258-2724.57.1.13>

[11] Fitriansyah, M. (2018, October). Identifikasi echinodermata di pesisir Pulau Denawan, Kecamatan Pulau Sembilan. In *Prosiding Seminar Nasional Lingkungan Lahan Basah* (Vol. 3, No. 1). Retrieved from: <https://snllb.ulm.ac.id/prosiding/index.php/snllb-lit/article/view/36>

[12] Ibrahim, I., Devira, C. N., & Purnawan, S. (2018, April). Struktur komunitas Echinoidea (bulu babi) di perairan pesisir pantai Teluk Nibung Kecamatan Pulau Banyak Kabupaten Aceh Singkil. In *Prosiding Seminar Nasional Biotik* (Vol. 5, No. 1). <http://dx.doi.org/10.22373/pbio.v5i1.2145>

[13] Pringgenies, D. (2019). **Bioprospeksi bahan hayati laut untuk pengembangan industri farmasi di Indonesia**. Semarang: UNDIP Press.

[14] Nane, L., & Paramata, A. R. (2020). Impact of overfishing on density and test-diameter size of the sea urchin *Tripneustes gratilla* at Wakatobi Archipelago, South-Eastern Sulawesi, Indonesia. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 25(2), 53-56. <https://doi.org/10.14710/ik.ijms.25.2.53-56>

[15] Deli, T., Mohamed, A. B., Attia, M. H. B., Zitari-Chatti, R., Said, K., & Chatti, N. (2019). High genetic connectivity among morphologically differentiated populations of the black sea urchin *Arbacia lixula* (Echinoidea: Arbacioida) across the central African Mediterranean coast. *Marine Biodiversity*, 49(2), 603-620. <https://doi.org/10.1007/s12526-017-0832-y>

[16] Kroh, A.; Mooi, R. (2022). **World Echinoidea Database. *Tripneustes gratilla* (Linnaeus, 1758)**. Accessed through: World Register of Marine Species at: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=212453> on 2022-11-04

[17] Silahoy, V. B., & Toha, H. (2013). Spatial distribution and genetic diversity of *Tripneustes gratilla* in Ambon Island. *Journal of Tropical Life Science*, 3(3), 177-181. Retrieved from: <https://jtrolis.ub.ac.id/index.php/jtrolis/article/view/122>

[18] Firmandana, T. C. (2014). Kelimpahan bulu babi (sea urchin) pada ekosistem karang dan lamun di Perairan Pantai Sundak, Yogyakarta. *Management of Aquatic Resources Journal (MAQUARES)*, 3(4), 41-50. <https://doi.org/10.14710/marj.v3i4.7030>

[19] Toha, A. H. A., Sumitro, S. B., & Hakim, L. (2015). Color diversity and distribution of sea urchin *Tripneustes gratilla* in Cenderawasih Bay ecoregion of Papua, Indonesia. *The Egyptian Journal of Aquatic Research*, 41(3), 273-278. <https://doi.org/10.1016/j.ejar.2015.05.001>

[20] Lubis, S. A., Yolanda, R., Purnama, A. A., & Karno, R. (2016). The sea urchin (Echinoidea) from Panjang Island Water, Bangka Belitung Province. *Omni-Akuatika*, 12(2), 125-129. <http://dx.doi.org/10.20884/1.oa.2016.12.2.111>

[21] Buwono, N. R., & Fitri, N. L. (2017). Diversity of Echinoderms in Balekambang Beach, Malang District, East Java. *El-Hayah: Jurnal Biologi*, 6(2), 35-40. <https://doi.org/10.18860/elha.v6i2.4532>

[22] Wainwright, B. J., Arlyza, I. S., & Karl, S. A. (2018). Population genetics of the collector urchin, *Tripneustes gratilla*, in the Indonesian archipelago. *Marine Ecology*, 39(6), 1-12. <https://doi.org/10.1111/maec.12530>

[23] Noviana, N. P. E., Julyantoro, P. G. S., & Pebriani, D. A. A. (2019). Distribusi dan kelimpahan bulu babi (Echinoidea) di perairan pulau Pasir Putih, desa Sumberkima, Buleleng, Bali. *Current Trends in Aquatic Science*, 2(1), 21-28. Retrieved from: <https://ojs.unud.ac.id/index.php/CTAS/article/view/42514>

[24] Nomleni, A., Widodo, M. S., Kilawati, Y., & Valen, F. S. (2020). Contemporary records of sea urchin *Tripneustes gratilla* (Echinodermata: Echinoidea) in Timor Island, Indonesia. *Aquaculture, Aquarium, Conservation & Legislation*, 13(4), 1899-1905. Retrieved from: <http://www.bioflux.com.ro/home/volume-13-4-2020/>

[25] Tamti, H., Rappe, R. A., & Omar, S. B. A. (2021, May). Preliminary assessment of *Tripneustes gratilla* populations in Seagrass Beds of the Spermonde Archipelago, South Sulawesi, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 763, No. 1, p. 012008). IOP Publishing. doi: 10.1088/1755-1315/763/1/012008

[26] Toha, A. H. A., Ambariyanto, A., Widodo, W., Hakim, L., Sumitro, S. B., & Aminin, A. L. (2022). Genetic diversity and connectivity of sea urchin *Tripneustes gratilla* in region surrounding Cenderawasih Bay, Papua-Indonesia and Indo-Pacific. *HAYATI Journal of Biosciences*, 29(2), 155-163. <https://doi.org/10.4308/hjb.29.2.155-163>

[27] Umagap, W. A. (2013). Keragaman spesies landak laut (Echinoidea) filum Echinodermata berdasar morfologi di Perairan Dofa Kabupaten Kepulauan Sula. *Jurnal Bioedukasi*, 1(2), 94-100. <http://dx.doi.org/10.33387/bioedu.v1i2.4345>

[28] Radjab, A. W., & Purbiantoro, W. (2019, April). Effect of long-term maintenance on gonad characteristics and egg quality of collector sea urchin *Tripneustes gratilla* (LINNAEUS 1758). In *IOP Conference Series: Earth and Environmental Science* (Vol. 253, No. 1, p. 012009). IOP Publishing. doi: 10.1088/1755-1315/253/1/012009

[29] Dako, F. X., Purwanto, R. H., Faida, L. R. W., & Sumardi, S. S. (2019). Tipologi pola konsumsi pangan untuk menjaga ketahanan pangan masyarakat sekitar kawasan hutan lindung Mutis Timau KPH Kabupaten Timor Tengah Selatan. *Jurnal Ketahanan Nasional*, 25(1), 92-106. <https://doi.org/10.22146/jkn.39544>

[30] Machovina, B., Feeley, K. J., & Ripple, W. J. (2015). Biodiversity conservation: The key is reducing meat consumption. *Science of the Total Environment*, 536, 419-431. <https://doi.org/10.1016/j.scitotenv.2015.07.022>

[31] Tupan, J., & Silaban, B. (2017). Karakteristik fisik-kimia bulu babi *Diadema setosum* dari beberapa perairan Pulau Ambon. *TRITON: Jurnal Manajemen Sumberdaya Perairan*, 13(2), 71-78. Retrieved from: <https://ojs3.unpatti.ac.id/index.php/triton/article/view/786>

[32] Hadinoto, S., Sukaryono, I. D., & Siahay, Y. (2017). Kandungan gizi gonad dan aktivitas antibakteri ekstrak cangkang bulu babi (Diadema setosum). *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan*, 12(1), 71-78. <http://dx.doi.org/10.15578/jpbkp.v12i1.281>

[33] Afifudin, I. K., Suseno, S. H., & Jacoeb, A. M. (2014). Profil asam lemak dan asam amino gonad bulu babi. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 17(1), 60-70. <https://doi.org/10.17844/jphpi.v17i1.8138>

[34] Henry-Unaeze, H. N. (2017). Update on food safety of monosodium l-glutamate (MSG). *Pathophysiology*, 24(4), 243-249. <https://doi.org/10.1016/j.pathophys.2017.08.001>

[35] Prihatno, M. R., Harahap, M. S., Akbar, I. B., & Bisri, T. (2014). Penurunan kadar glutamat pada cedera otak traumatis pasca pemberian agonis adrenoseptor alpha-2 dexmedetomidin sebagai indikator proteksi otak. *Jurnal Neuroanestesi Indonesia*, 3(2), 69-79. <https://doi.org/10.24244/jni.vol3i2.138>

[36] Putra, M. D. H., Putri, R. M. S., Oktavia, Y., & Ilhamdy, A. F. (2020). Karakteristik asam amino dan asam lemak bekasam kerang bulu (Anadara antiquata) di desa benan kabupaten lingga. *Marinade*, 3(02), 159-167. <https://doi.org/10.31629/marinade.v3i02.3404>

[37] Suriani, N. W., Wola, B. R., & Komansilan, A. (2022). Development of biological macromolecules three-tier test (BM-3T) to identify misconceptions of prospective science teachers. *Jurnal Penelitian Pendidikan IPA*, 8(4), 1798-1805. <https://doi.org/10.29303/jppipa.v8i4.1297>

[38] [AOAC] Association of Official Analytical Chemist. 2005. **Official Method of Analysis of The Association of Official Analytical of Chemist**. Arlington, Virginia (US): The Association of Official Analytical Chemist Inc.

[39] Hasan, F. (2002). Pengaruh konsentrasi garam terhadap mutu produk fermentasi gonad bulu babi jenis *Tripneustes gratilla* (L). B.Sc. Thesis, Bogor Agricultural University, Bogor, Indonesia.

[40] Chasanah, E., & Andamari, R. (1997). Komposisi kimia, profil asam lemak, dan asam amino gonad bulu babi *Tripneustes gratilla* dan *Salmacis* sp. dan potensi pengembangannya. In *Prosiding Seminar Kelautan LIPI-UNHAS ke-1. Balitbang Sumberdaya Laut, Puslitbang Oseanologi-LIPI* (pp. 269-274).

[41] Purwaningsih, S. (2012). Aktivitas antioksidan dan komposisi kimia keong matah merah (*Cerithidea obtusa*). *ILMU KELAUTAN: Indonesian Journal of Marine Sciences*, 17(1), 39-48. <https://doi.org/10.14710/ik.ijms.17.1.39-48>

[42] Pais, A., Saba, S., Rubattu, R., Meloni, G., & Montisci, S. (2011). Proximate composition of edible sea urchin *Paracentrotus lividus* roe commercialised in sardinia/composizione centesimale di gonadi del riccio di mare edule *Paracentrotus lividus* commercializzate in sardegna. *Biologia marina mediterranea*, 18(1), 390-391.

[43] McAlister, J. S., & Moran, A. L. (2012). Relationships among egg size, composition, and energy: a comparative study of geminate sea urchins. *PLoS One*, 7(7), e41599. <https://doi.org/10.1371%2Fjournal.pone.0041599>

[44] Byrne, M., Sewell, M. A., & Prowse, T. A. A. (2008). Nutritional ecology of sea urchin larvae: influence of endogenous and exogenous nutrition on echinopluteal growth and phenotypic plasticity in *Tripneustes gratilla*. *Functional Ecology*, 22(4), 643-648. <https://doi.org/10.1111/j.1365-2435.2008.01427.x>

[45] Akerina, F. O., Nurhayati, T., & Suwandi, R. (2015). Isolation and characterization of antibacterial compounds from sea urchin. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 18(1), 61-73. <https://doi.org/10.17844/jphpi.v18i1.9564>

[46] Martínez-Pita, I., García, F. J., & Pita, M. L. (2010). Males and females gonad fatty acids of the sea urchins *Paracentrotus lividus* and *Arbacia lixula* (Echinodermata). *Helgoland Marine Research*, 64(2), 135-142. <https://doi.org/10.1007/s10152-009-0174-7>

[47] Tuminah, S. (2009). Efek asam lemak jenuh dan asam lemak tak jenuh "trans" terhadap kesehatan. *Media Penelitian dan Pengembangan Kesehatan*, 9(3), 13-20.

[48] Tuminah, S. (2009). Peran kolesterol HDL terhadap penyakit kardiovaskuler dan diabetes mellitus. *Gizi Indonesia*, 32(1), 69-76. <http://dx.doi.org/10.36457/gizindo.v32i1.70>

[49] Purnami, S. E., & Pratiwi, R. T. (2014). Profil asam lemak lima spesies landak laut (Echinoidea) dari Pantai Selatan Kabupaten Gunung Kidul Daerah Istimewa Yogyakarta. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, 19(1), 9-14. <https://doi.org/10.24002/biota.v19i1.449>

[50] Siahaya, D. M. (2009). Analisis kandungan asam lemak pada gonad bulu babi (*Tripneustes gratilla* L.). *Ichtyos*, 8(2), 75-79.

[51] Tangkilisan H. A., & Lestari H. (2001). Peran penambahan DHA pada susu formula. *Sari Pediatri*, 3(3), 147-151. <https://dx.doi.org/10.14238/sp3.3.2001.147-51>

[52] Castle, F. L. D., & Gooder, R. P. (2010). **Omega-3 and omega-6 fatty acid**. NebGuide, University of Nebraska-Lincoln Extensions, Lincoln, NE, USA. Retrieved from: <https://extensionpublications.unl.edu/assets/html/g2032/build/g2032.htm>

[53] Muhamad, N. A., & Mohamad, J. (2012). Fatty acids composition of selected Malaysian fishes. *Sains Malaysiana*, 41(1), 81-94. Retrieved from: https://www.ukm.my/jsm/pdf_files/SM-PDF-41-1-2012/10%20Nur%20Airina.pdf

[54] Basmal, J. (2010). Lepidocybium flavobrunneum as a resource of essential fatty acids. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, 5(3), 109-117. <https://doi.org/10.15578/squalen.v5i3.54>

[55] Ruxton, C. H. S., Reed, S. C., Simpson, M. J. A., & Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Journal of Human Nutrition and Dietetics*, 17(5), 449-459. <https://doi.org/10.1111/j.1365-277X.2004.00552.x>

[56] Hou, Y., Yin, Y., & Wu, G. (2015). Dietary essentiality of “nutritionally non-essential amino acids” for animals and humans. *Experimental Biology and Medicine*, 240(8), 997-1007. <https://doi.org/10.1177/1535370215587913>

[57] Hu, X., & Guo, F. (2021). Amino acid sensing in metabolic homeostasis and health. *Endocrine Reviews*, 42(1), 56-76. <https://doi.org/10.1210/endrev/bnaa026>

[58] Eugenio, F.A., van Milgen, J., Duperray, J. Sergheraert, R., & Floc'h, L. (2022). Feeding intact proteins, peptides, or free amino acids to monogastric farm animals. *Amino Acids*, 54, 157-168 (2022). <https://doi.org/10.1007/s00726-021-03118-0>

[59] Hariani, Wiralis, Fathurrahman, T., Suwarni, Askrenning, Imanuddin, Devianti, R., Rahayu, D. Y. S., Yunus, R., Arsulfa, & Sahmad. (2021). Aplikasi “TETOY” dan optimalisasi pemanfaatan gonad bulu babi dalam mengatasi stunting untuk mewujudkan desa sehat di Bajo Indah Kecamatan Soropia Kabupaten Konawe. *Krida Cendekia*, 1(4), 11-18. Retrieved from: <http://kridacendekia.com/index.php/jkc/article/view/42>

[60] Silaban, B. B., & Srimariana, E. S. (2013). Kandungan nutrisi dan pemanfaatan gonad bulu babi (Echinothrixs calamaris) dalam pembuatan kue bluder. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 16(2), 108-118. <https://doi.org/10.17844/jphpi.v16i2.8045>

[61] Scalise, M., Galluccio, M., Console, L., Pochini, L., & Indiveri, C. (2018). The human SLC7A5 (LAT1): the intriguing histidine/large neutral amino acid transporter and its relevance to human health. *Frontiers in chemistry*, 6(243), 1-12. <https://doi.org/10.3389/fchem.2018.00243>

[62] Liao, S. M., Du, Q. S., Meng, J. Z., Pang, Z. W., & Huang, R. B. (2013). The multiple roles of histidine in protein interactions. *Chemistry Central Journal*, 7(1), 1-12. <https://doi.org/10.1186/1752-153X-7-44>

[63] Matthews, D. E. (2020). Review of lysine metabolism with a focus on humans. *The Journal of Nutrition*, 150(Supplement_1), 2548S-2555S. <https://doi.org/10.1093/jn/nxaa224>

[64] Azevedo, C., & Saiardi, A. (2016). Why always lysine? The ongoing tale of one of the most modified amino acids. *Advances in Biological Regulation*, 60, 144-150. <https://doi.org/10.1016/j.jbior.2015.09.008>

[65] Nurina, M. E., Maryanto, S., & Pontang, G. S. (2020). The effect of giving modified modisco with soybeans against growth on protein energy malnutrition rats. *Jurnal Gizi dan Kesehatan*, 12(27), 59-64. <https://doi.org/10.35473/jgk.v12i27.61>

[66] Dinu, A., & Apetrei, C. (2020). A review on electrochemical sensors and biosensors used in Phenylalanine Electroanalysis. *Sensors*, 20(9), 2496. <https://doi.org/10.3390/s20092496>

[67] Niu, X., Yang, X., Mo, Z., Guo, R., Liu, N., Zhao, P., & Liu, Z. (2019). Perylene-functionalized graphene sheets modified with β -cyclodextrin for the voltammetric discrimination of phenylalanine enantiomers. *Bioelectrochemistry*, 129, 189-198. <https://doi.org/10.1016/j.bioelechem.2019.05.016>

[68] Suprayitno, E., & Sulistiyati, T. D. (2017). **Metabolisme Protein**. Malang: Universitas Brawijaya Press.

[69] Wang, X. (2019). Strategy for improving L-isoleucine production efficiency in *Corynebacterium glutamicum*. *Applied Microbiology and Biotechnology*, 103(5), 2101-2111. <https://doi.org/10.1007/s00253-019-09632-2>

[70] Gu, C., Mao, X., Chen, D., Yu, B., & Yang, Q. (2019). Isoleucine plays an important role for maintaining immune function. *Current Protein and Peptide Science*, 20(7), 644-651. <https://doi.org/10.2174/1389203720666190305163135>

[71] Duan, Y., Li, F., Li, Y., Tang, Y., Kong, X., Feng, Z., ... & Yin, Y. (2016). The role of leucine and its metabolites in protein and energy metabolism. *Amino Acids*, 48(1), 41-51. <https://doi.org/10.1007/s00726-015-2067-1>

[72] Yoshimura, Y., Bise, T., Shimazu, S., Tanoue, M., Tomioka, Y., Araki, M., ... & Takatsuki, F. (2019). Effects of a leucine-enriched amino acid supplement on muscle mass, muscle strength, and physical function in post-stroke patients with sarcopenia: A randomized controlled trial. *Nutrition*, 58, 1-6. <https://doi.org/10.1016/j.nut.2018.05.028>

[73] Taylor, P. M. (2014). Role of amino acid transporters in amino acid sensing. *The American Journal of Clinical Nutrition*, 99(1), 223S-230S. <https://doi.org/10.3945/ajcn.113.070086>

[74] Kuo, M. T., Chen, H. H., Feun, L. G., & Savaraj, N. (2021). Targeting the proline–glutamine–asparagine–arginine metabolic axis in amino acid starvation cancer therapy. *Pharmaceuticals*, 14(1), 72. <https://doi.org/10.3390/ph14010072>

[75] Wu, G., Bazer, F. W., Satterfield, M. C., Li, X., Wang, X., Johnson, G. A., ... & Wu, Z. (2013). Impacts of arginine nutrition on embryonic and fetal development in mammals. *Amino Acids*, 45(2), 241-256. <https://doi.org/10.1007/s00726-013-1515-z>

[76] Öztürk, G., Saylan, Y., & Denizli, A. (2021). Designing composite cryogel carriers for tyrosine adsorption. *Separation and Purification Technology*, 254, 117622. <https://doi.org/10.1016/j.seppur.2020.117622>

[77] Chauhan, P., Mundekkad, D., Mukherjee, A., Chaudhary, S., Umar, A., & Baskoutas, S. (2022). Coconut carbon dots: Progressive large-scale synthesis, detailed biological activities and smart sensing aptitudes towards tyrosine. *Nanomaterials*, 12(1), 162. <https://doi.org/10.3390/nano12010162>

[78] Ménard-Moyon, C., Venkatesh, V., Krishna, K. V., Bonachera, F., Verma, S., & Bianco, A. (2015). Self-assembly of tyrosine into controlled supramolecular nanostructures. *Chemistry—A European Journal*, 21(33), 11681-11686. <https://doi.org/10.1002/chem.201502076>