Analysis of Amino Acids, Protein Profile, Calcium and Phosphorus Levels of *Upeneus moluccensis Waste (Thorns and Scales)*

I Dewa Ayu Ratna Dewanti[1](✉), I Dewa Ayu Susilawati[1,2], Pujiana Endah Lestari[1], Erawati Wulandari[2], Ristya Widi Endah Yani[3], and Sunlip Wibisono[4]

[1] Department of Biomedical Science, Faculty of Dentistry, University of Jember, Jember, Indonesia
idewadewanti@yahoo.com

[2] Department of Dental Conservative, Faculty of Dentistry, University of Jember, Jember, Indonesia

[3] Department of Public Dental Health Faculty of Dentistry, University of Jember, Jember, Indonesia

[4] Department of Statistics, Faculty of Economics and Business, University of Jember, Jember, Indonesia

Abstract. *Upeneus molucensis* is seawater fishes that are consumed by many people so it leaves a lot of waste (including scales and thornss). On the other hand, the utilization of fish waste is still not much. Whereas, waste contains a lot of amino acids, protein, Calcium and Phosphorus. This study analyzed amino acids, protein profiles, levels of Calcium and Phosphorus in the thornss and scales of *U. moluccensis.* Analyze of amino acids used HPLC: The samples were injected into the HPLC column, waiting until the separation of all amino acids were complete. The amino acids standard used a mixed standard consisting of 15 types of amino acids. Protein analyzes used SDS-PAGE: 15% acrylamide, 50-95 V for 5 h in electoda buffer (glycine 192mM, Trisbase 25mM, SDS0, 1%). Staining used Coomassie Brilliant Blue. While the analysis of Calcium and Phosphorus using Atomic Absorption Spectrophotometer (AAS) and Spectrophotometer UV-Vis. The sample was added with nitric acid, HClO4:HNO3, distilled water and HCl, heated and filtered. Statistical analysis was carried out descriptively (amino acids and protein profiles), T test (Calcium and Phosphorus level). *U. moluccensis* amino acids found 12 amino acids there are 5 types of essential amino acids and 7 non-essential. Protein profiles of scales were 11 bands, thorns 13 bands. Calcium level (ppm) of *U. molucensis* (scales 3227, thorns 2782), Phosphorus level (scales 16514, thorns 23082). The scales and thorns of *U. moluccensis* fish were found in various types of amino acids, proteins profiles with various molecular weights and higher Calcium content were found in scales, but higher phosphorus was found in thornss.

Keywords: Amino acid · protein · Calsium · Phosphorus · Upeneus moluccensis

A. Lelono et al. (Eds.): ICOLIB 2021, ABSR 27, pp. 98–109, 2023.
https://doi.org/10.2991/978-94-6463-062-6_11

1 Introduction

Fish waste (fish spines and scales) is usually not utilized or disposed of. Some literature states that fish spines and fish scales contain organic and inorganic materials which are also found in bone and dentin. So that it can be used for various materials in the health sector. The outer layer of fish scales is significantly more mineralized and is often referred to as the 'bone layer', 'while the inner layer ('basal' or 'collagenous' layer) [1-3]. Chemical compounds contained in fish spines and scales such as water, fat and ash, organic proteins (collagen and ichtylepidine) and the rest are minerals and inorganic residues such as magnesium carbonate and calcium carbonate (4% Calcium, 3% Phosphorus, and 32% protein). [1, 2-6]. These proteins can be separated from other types of proteins based on their size, solubility, charge and binding affinity. Protein separation must be carried out in order to study the character and function of the protein. One way to analyze proteins is by electrophoresis. In addition, fish waste in addition to containing amino acids, protein, also contains a lot of Calcium and Phosphorus [3-6]. The content of Calcium and Phosphorus can be analyzed using AAS (atomic absorption spectrophotometer).

Based on the above description we are interested in analyzing of the amino acids, protein profile, Calcium and Phosphorus levels being further utilized. One of the demersal fish that has significant economic value is the yellow fish (*Upeneus moluccensis*). Thorns and fish scales are derived from the yellow fish that many found in the district of Jember, Indonesia. *U. moluccensis* is one type of fish with low economic value which has a high protein nutritional component, but the utilization is not yet optimal. *U. moluccensis* has the following physical characteristics: the average length of 20–22 cm, has a tail and a horizontal yellow line along its body, and has a chin eagle for feeding in the sand, living in tropical/subtropical climates and inhabiting a slightly muddy beach with a depth of 100 m. Fish protein consists of myofibril protein, sarcoplasmic protein, and stroma protein [7-9]. The purpose of this study was Analyze of the amino acids, protein profile, Calcium and Phosphorus levels of *U. moluccensis* waste (thorns and scales).

2 Material and Methods

2.1 Analysis of Calcium and Phosphorus Levels

Measurement of Calcium levels using atomic absorption spectrophotometer (AAS) (Perkin-Elmer) and use UV-Vis spectrophotometer (Perkin-Elmer Lambda 25) to determine phosphorus levels. Previously, fish bone meal was made by boiling, drying, and mashing fish bones. The next step is to dissolve the fish bone meal using chemicals in the form of HNO_3, H_2SO_4, HCl, $HClO_4$, and aquades.

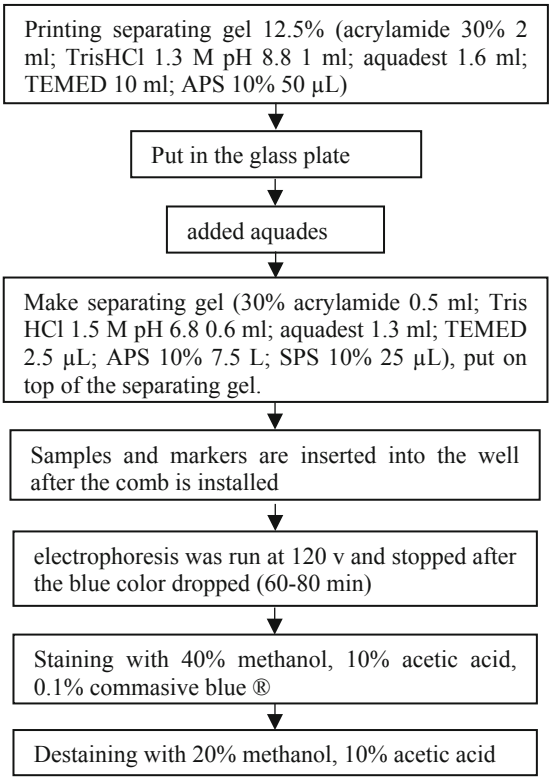
The dry sample was weighed as much as ±1 g and put in a 125 mL Erlenmeyer. 5 mL of HNO_3 was added and allowed to stand for 1 h at room temperature in a fume hood. The sample was heated on a hot plate at low temperature for 4–6 h in a fume hood and then left overnight in a closed condition. 0.4 mL of H_2SO_4 was added and then heated on a hot plate until the solution was reduced (more concentrated) usually 1 h. Add 2–3 drops of a mixed solution of $HClO_4$:HNO_3 (2:1). The sample was allowed to remain on the hot plate because heating was continued until there was a color change from brown to dark yellow to light yellow (usually ± 1 h). After there is a color change, heating is still continued for 10–15 min. Remove the sample from the hot plate, cool, and add 2 mL of distilled water and 0.6 mL of HCl. The sample was reheated on a hot plate for ± 15 min and then put into a 100 mL volumetric flask. If there is a precipitate filtered with glass wool or filter paper. Furthermore, measurements were made with an atomic absorption spectrophotometer at a maximum wavelength of 422.7 nm according to [10].

Phosphorus levels were measured using a UV-Vis spectrophotometer as follows. The sample to be analyzed is placed in a cuvette/cell made of translucent glass or plastic. The photocell captures light which will be converted into electrical energy which will be delivered to the detector. A detector is a material that can absorb energy from photons and convert it into electrical energy. The display converts the electric light from the detector into a reading in the form of numbers according to the results of the analysis [11].

2.2 Analysis of Amino Acids

The thornss and fish scales extract was added with 6N HCL, vortexed, nitrogen gas was added, hydrolyzed at 110 °C for 22 h. The sample is injected until the sample shows the maximum peak height of the compound. Different compounds have different retention times. For some compounds, the retention time will vary greatly and depend on the on the pressure applied, the condition of the stationary phase, the solvent composition and the temperature of the column. Polar compounds in the mixture through the column will adhere longer to polar silica than non-polar compounds. Therefore, non-polar compounds will pass through the column faster [11].

2.3 Protein Profile Analysis



The data obtained from the atomic absorption spectrophotometer were tested for normality and homogeneity using the Kolmogorov-Smirnov test and Levene's test. The data obtained were then analyzed using T test. Meanwhile, the protein profile and amino acid content were analyzed descriptively.

3 Results

3.1 Calcium and Phosphorus Levels

Calcium content of fish scales of *U. moluccensis* 3227, fish thorns 2782, while Phosphorus fish scales 16514 and fish thorns 23082. It can be seen that the Calcium content of fish scales is higher than fish thorns, on the other hand the Phosphorus content of fish thorns is higher.

Shapiro Wilk's normality test and homogeneity test used Levene's test. The result is $p > 0.05$, which means that the data is normally distributed and homogeneous. Statistical analysis was continued with the results of the Independent T Test on the data on calcium and phosphorus levels, the value of $p = 0.000$ ($p < 0.05$) showed that there was a significant difference.

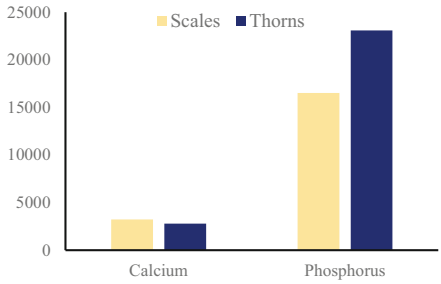


Fig. 1. Calcium and Phosphorus level of *U. moluccensis.*

Table 1. Farmer's Acceptance of Each Crop Per Year in The Research Area in 2021

No	Amino acids	Sampel	
		Scales	Thorns
1	Alanine (%)	0.059	0.076
2	Leucine-Isoleucine (%)	0.085	0.051
3	Valine (%)	0.174	0.214
4	Arginine (%)	0.035	0.081
5	Methionine (%)	0.097	0.144
6	Proline (%)	0.030	0.037
7	Glutamic Acid (%)	0.064	0.050
8	Histidine (%)	0.021	0.033
9	Lysine (%)	0.068	0.099
10	Glycine (%)	0.059	0.053
11	Serine (%)	0.275	0.029
12	Tyrosine (%)	0.177	0.254

3.2 Amino Acids

The results of the analysis of the amino acid content of the thorns and scales of *U. moluccensis* fish, found 12 types of amino acids. Of the 12 types, there are 5 types of essential amino acids (methionine, histidine, leucine-isoleucine, lysine, valine) and 7 non-essential amino acids, namely: arginine, alanine, glutamic acid, glycine, tyrosine, proline (Table 1., Figs. 2 and 3).

Fig. 2. Essential amino acids of Scales and thorns) of *U. moluccencis.*

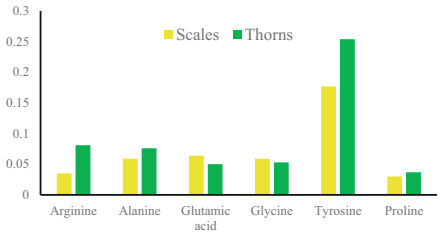


Fig. 3. Non-essential amino acids of Scales and thorns of *U. moluccencis.*

Table 2. Protein content of scales and thorns *U. moluccensis*

Regresion = y = 0.0187x + 0.0231.					
Sample	abs	y = 0.0187x + 0.0231	10 ug	25 ug	30 ug
Scales	0.382	21.66	2.31 ul	5.77 ul	6.92 ul
Thorns	1.778	96.32	0.52 ul	1.30 ul	1.56 ul

3.3 Profil Protein

The result of determination of protein content was done by using Bradford method is as shown in Table 2 below. Fish scales contain the more protein than thorns.

The results of SDS-PAGE (Figs. 4 and Table) show that the thick bands found. The more protein volume. The thinner the band. The less volume of protein. On the scale of fish volume of 3352 in the protein with molecular weight 27. While the smallest 1386 on protein with molecular weight 46. The most curved fish bunch 5365 with molecular weight 27. The smallest 1387 with molecular weight 275. The smallest 1825 with the molecular weight of 23. The protein fraction 23. 27. 31. 275 kDa is a protein fraction equally found in fish thorns and scales. The protein fractions 23. 27. 31. 275 kDa are the same protein fractions found in fish spikes and scales. While the 27 kDa protein fraction is the protein with the greatest intensity that there are scales and fish thorns *U. moluccensis.* The largest intensity of yellow fish scales occurred on Line 5. Fish thorns on lene to line 5 (Fig. 5).

Table 3. Cost of Using Agroforestry Agricultural Fertilizer in Research Area in 2021

No	Volume	Molecule weight	Volume	Molecule weight
	Scales		Thorns	
1	1469	275	1387	275
2	1386	46	1800	88
3	1502	39	3070	37
4	2120	31	4755	31
5	3352	27	5365	27
6	2607	23	4256	23

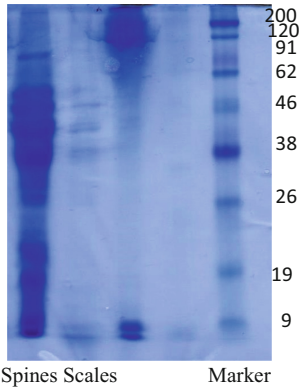


Fig. 4. Protein profile of scales. Thornss of *Upeneus moluccensis.*

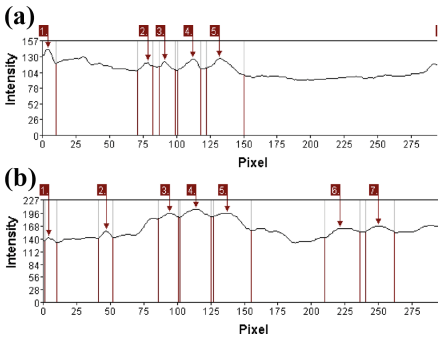


Fig. 5. The intensity of protein fraction of *U. moluccensis.* a) thorns b) scales.

4 Discussion

4.1 Calcium and Phosphorus Level

Phosphorus found in hard tissue have lower levels than calcium. But if in soft tissues. Phosphorus levels are much higher than calcium [12]. This can be understood why in this study obtained higher levels of calcium in fish scales. While higher levels of Phosphorus in thornss.

In general. Phosphorus bioavailability is positively correlated with the solubility of minerals in water. The bioavailability of phosphorus is largely influenced by its form and source. In nature. Phosphorus is available in the form of phosphates. Which can be tribasic calcium phosphate or monobasic calcium phosphate. Some nutrients affect the availability of certain minerals. One of them is the intake of iron. Aluminum. And magnesium can reduce the absorption of phosphorus by forming insoluble phosphate [13].

Calcium solubility is affected by species fish because different fish species have different bone structures. Compounds in bone structures such as fluoride and magnesium can affect calcium solubility. A lot of research which states that fluoride compounds can inhibit the solubility of calcium. While the compounds Magnesium can increase the solubility of calcium [14].

Calcium is an essential nutrient required for many functions in human health. Calcium functions in various important roles in the human body. Including: 1) providing structure and hardness to bones and teeth. 2) allowing muscles to contract and nerves to send signals. 3) dilating and constricting blood vessels. 4) helping the formation of blood clots. And 5) supports protein function and hormone regulation. Currently. There is a lot of interest in finding natural food substances or products that can increase calcium absorption [14, 15].

Phosphorus has several important roles in our body. Phosphorus is required for bone mineralization and is also a constituent of hydroxyapatite crystals. In addition. Several phosphorylated proteins such as osteopontin and Dentin Matrix Protein 1 (DMP1) play a role in regulating bone mineralization. Phosphorus is also a constituent of biomembranes and nucleic acids. Many phosphorylated metabolites such as adenosine triphosphate. 2.3-diphosphoglycerate. Glucose-6-phosphate and phosphorylated proteins are required for various activities of all cells such as energy metabolism. Proliferation. And in particular cell differentiation. Besides being very important for bone mineralization. Phosphorus also plays a role in signaling cells and storing energy in the form of ATP [16-19].

4.2 Amino Acids

Amino acids found in the scales and thorns of *U. moleccensis* consist of essential amino acids (can be produced by the body) and non-essential (cannot be produced by the body).

U. moluccensis essential amino acids: methionine. Histidine. Leucine-isoleucine. Lysine. Valine. Methionine contains sulfur which is not nucleophilic and will react with several electrophilic centers. Its function is to eliminate toxins. Improve cardiovascular health. Assist the liver in processing fat. Other functions: make creatine (a natural nutrient in muscles. Heart and blood vessel function). Formation of nails. Skin and

connective tissue. Reduce inflammation. Allergies. Histidine to maintain the health of body tissues. Especially in the myelin sheath of nerve cells to send nerve impulses from the brain throughout the body. Helps mental disorders. Sexual dysfunction. Leucine and isoleucine for muscle recovery. Proteinogenesis. Forming antibodies. Activating hormones. Providing energy. Making ketones and glucose. Lysine is involved in the synthesis of enzymes and growth hormones. Lysine is important in the production of various tissue-building proteins. Hormones. Enzymes. And antibodies. Valine functions to regulate blood sugar. Prevent muscle breakdown. Remove excess nitrogen. Maintain body energy and maintain mental function [20, 21].

U. moluccenis non-essential amino acids: arginine. Alanine. Glutamic acid. Glycine. Tyrosine. Proline. Arginine protects the liver. Skin. Joints and muscles. Strengthens the immune system. Regulates hormones and blood sugar. Increases male fertility. Improves blood circulation. Treats impotence and heart disease. Alanine or 2-aminopropanoic acid (L-alanine) helps the body convert glucose into energy. Removes excess toxins in the liver. Protects cells from damage. Alanine is important for processing B vitamins. Especially B5 and B6. Glutamate amino acid helps sugar and fat metabolism. Potassium transport. Brain energy. Improve personality disorders. Overcome epilepsy. Ulcers. Increase salivary secretion. Suppress obesity. Nourish the brain. Glycine acts as a neurotransmitter and accelerates wound healing. Aiding muscle growth. Tyrosine plays a role in the production of thyroid hormones T3 and T4. Serine (mostly in scales) helps muscle growth. Tyrosine (mostly in the thorns) plays a role in the production of thyroid hormones T3 and T4. Proline for the production of collagen and cartilage. Keeping muscles and joints flexible. Cell production. High content of glycine and proline may indicate the presence of collagen. Since glycine and proline appear to be important in collagen formation [2, 20, 21].

4.3 Protein Profile

The result of total protein content analysis showed that fish scales have greater content than fish thorns. However. Thorns with heating have a total protein content greater than unheated thorns. This indicates that heating will increase the protein content. Results SDS_PAGE scales. Thorns have a protein with the same molekul weight that is 23kDa. The protein fractions 23. 27. 31. 275 kDa are the same protein fractions found in fish thorns and scales. While the 27 kDa protein fraction is the protein with the largest volume of fish scales and thorns.

Proteins that have a thickness and a greater color intensity than other proteins and always present in each processing are called major protein fractions. The thick bands exhibit a large concentration. While thin bands exhibit fewer levels of protein. The thick and thin band difference is due to the difference in the number of migrated molecules. The thick band is the fixation of some bands. Bands that have greater ionic strength will be migrated further than small ionic-strength bands [22].

The greater the distance and the intensity of each fraction. The greater the intensity of the protein fraction. The greater the intensity of the fraction the higher the protein content [22]. The largest distance of *U. moluccensis* scales occurs in the 5th Line (125–150 pixels). The fish thorns on the line to the 5th (125–150 pixels) line. This shows that fish thorns have higher levels of protein than fish scales.

The scales and thorns of *U. molucensis* fish are found in various types of amino acids. Proteins with various molecular weights and high content of calcium and phosphorus.

Acknowledgments. Thanks to DRPM RISTEKDIKTI (7/E/KPT/2019) for supporting this research through fundamental grants.

Authors' Contributions. IARD carried out the research design. Coordination. Writing of manuscript. IAS contributed to an analysis of Calcium and Phosphorus levels. PEL contributed to the analysis of Amino acids. EW made fish scale powder. RWEY contributed to the collection and checking of data. Assisted with the writing of the manuscript. SW contributed to the data analysis. All authors read and approved the final manuscript.

References

1. Begum Mohajira. Md. Zia Uddin Al Ma Mun and Dr. Md. Abdus Satter Miah. Nutritional profiling of selected fish's scales: An approach to determine its prospective use as a biomaterial. International Journal of Fisheries and Aquatic Studies. 2021: 9(3): 26–31. https://www.fisheriesjournal.com.
2. Masood Zubia. Rehana Yasmeen. Muhammad Samee Haider. Omer Mukhtar Tarar. Lakht-e-Zehra. Md. Yeamin Hossain. Evaluations of crude protein and amino acid contents from the scales of four mullet species (Mugilidae) collected from Karachi fish harbour. Pakistan. Indian Journal of Geo-Marine Science. May 2015; 44(5):724–731. http://nopr.niscair.res.in
3. [3] Kalfas. I. H. Principle of Bone Healing. *Neurosurg Focus.* 2001; 10(4): 7-10.
4. Boskey. A. L. Bone Composition: Relationship to Bone Fragility and Antiosteoporotic Drug Effects. *BoneKey Reports.* 2015; 4: 710. Published online 2015 Jun 3. doi: https://doi.org/10.1038/bonekey.2015.79
5. Dewanti. I.D.A.R.. Lestari. P.E.. Purwanto. Wulandari. E.. Yani. R.W.E.. Wibisono. S.. Burlakovs. J.. Bones. scales of Upeneus sulphureus and Osphronemus gouramy increase adhesion and decrease IL-1β expression on monocytes against Streptococcus mutans. Ann. Trop. Med. Public Heal. 2020; 23: 1253–1258. https://doi.org/10.36295/asro.2020.23810.
6. Dewanti. I.D.A.R.. Susilawati. I.D.A.. Purwanto. P.. Lestari. P.E.. Budirahardjo. R.. Setyorini. D.. Widi. R.. 2019. thornss and scales in increasing salivary leukocyte and monocyte cells viability against Streptococcus mutans Dental Journal. 2018;52(1): *p.45–50.* https://doi.org/10.20473/j.djmkg.v52.i1.p4
7. Deju Zhu. Cesar Fuentes Ortega. Ramak Motamedi. Lawrence Szewciw. Franck Vernerey and Francois Barthelat. Structure and Mechanical Performance of a "Modern" Fish Scale. Advanced Engineering Materials. 2011; 13. No. XX: B1-B10. https://barthelat-lab.mcgill.ca/files/papers/ABM2011.pdf.
8. Fadlian. Rikza. Boer. Mennofatria. Kurnia. Rahmat. Study of Yellow Fish Stock (Upeneus moluccensis. Bleeker 1855) in Sunda Strait Waters Landed at PPI Labuan. Banten. Scientific Repository. 2012. http://repository.ipb.ac.id/handle/123456789/56765. Copyright © Central Library of Bogor Agricultural University.
9. Uci Novian. dry miofibril characteristics of jelly fish (upeneus sp) were extracted using papain enzyme by hot press method. 2014. http://repository.unej.ac.id/handle/123456789/25650.
10. Garcia. R. dan A. P. Baez. 2012. Atomic Absorption Spectrometry (AAS). http://www.intechopen.com/books/atomic-absorptionspectroscopy/atomic-absorption-spectrometry-aas-. [Diakses pada tanggal 20 April 2018].

11. Wijayanti Imas Eva. Vol.2. No.1. Januari 2017. Jurnal Kimia dan Pendidikan. 2(1): 40–51. e-ISSN 2502–4787
12. [12] Warono. D. dan Syamsudin. 2013. Unjuk Kerja Spektrofotometer untuk Analisa Zat Aktif Ketoprofen. KONVERSI. 2(2): 57-65.
13. Loppies Cindy Regina Magdalena. Daniel A.N. Apituley. & D. Soukotta. Scales mineral composition of red snapper fish (Lutjanus sp.) and parrotfish (Scarus sp.) with acid immersion. Proceedings of the VII National Symposium on Marine and Fisheries 2020. Faculty of Marine and Fisheries Sciences. Hasanuddin University. Makassar. 5 June 2020. ISBN 978–602–71759–7–6.
14. Terech-majewska. E.. J. Pajdak.. dan A. K. Siwicki. 2016. Water as A source of Macronutrients and Micronutrients for Fish. with Special Emphasis on the Nutritional Requirements of Two Fish Species: The Common Carp (Cyprinus carpio) and The Rainbow Trout (Oncorhynchus mykiss). Journal of Elementology. 21(3): 947–961.
15. [15] Yin T. Du H. Zhang J. Xiong S. Preparation and characterization of ultrafine fish bone powder. Journal of Aquatic Food Product Technology. 2016; 25(7) : 1 – 27.
16. [16]Westermeier. 2004. Electrophoresis in Practice: A Guide to Theory and Practice. New Jersey: John Wiley & Sons inc.
17. Marezs. K. Proper Calcium Use: Vitamin K as a Promoter of Bone and Cardiovascular Health. *Integrative Medicine: A Clinician's Journal*. 2015;14(1): 34–39. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4566462/.
18. Nicoll. R.. J. M. Howard. dan M. Y. Henein. Cardiovascular Calcification and Bone: A Comparison of The Effects of Dietary and Serum Calcium. Phosporus. Magnesium. and Vitamin D. *International Cardiovascular Forum Journal*. 2014; 1(5): 209–218. https://pdfs.semanticscholar.org.
19. Lin Yanlan. Xixi Cai. Xiaoping W. Shengnan Lin. haoyun Wan. Fabrication of snapperfish scales protein hydrolysate-calcium complex andthe promotion in calcium cellular uptake. Journal of Functional Foods 65 (2020): 1037172. https://doi.org/10.1016/j.jff.2019.103717.
20. Lopez Michael J.; Shamim S. Mohiuddin.. Biochemistry. Essential Amino Acids. March 26. 2021. Copyright © 2021. StatPearls Publishing LLC. https://www.ncbi.nlm.nih.gov/books/NBK557845/.
21. TakahashToru i. Eri Toda. Ram B. Singh. Fabien De Meester. Agnieszka Wilczynska. Douglas Wilson. Lekh R. Juneja. Essential and Non-Essential Amino Acids in Relation to Glutamate. The Open Nutraceuticals Journal. 2011; 4:205–212. https://benthamopen.com/ABSTRACT/TONUTRAJ-4-205
22. Hou Yongqing . Yulong Yin. and Guoyao Wu. Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. Exp Biol Med (Maywood). 2015 Aug; 240(8):997–1007. Published online 2015 Jun 2. doi: https://doi.org/10.1177/1535370215587913.