Chemical properties of dried anchovy (*Stolephorus sp*) from Buru Island

I K E Savitri¹*, V D Tohata², R B D Sormin¹, V M N Lalopua¹, M N Mailoa¹ and F Rieuwpassa¹

¹Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Pattimura University
²Master Programe in Marine Science Pattimura University

*E-mail: endahsavitri@gmail.com

Abstract. The objective of this study was determined proximate, amino acid and some minerals of dried anchovy from Kayeli bay Buru Island. Dried anchovy from Buru island has a good quality indexes and a good feasibility business while the nutritional composition unexplored, so determine the proximate, amino acid and ash content are appropriate to complete its nutritional data. Protein in dried anchovy is relatively high, almost 60% and the amino acids profile were Phenylalanine, Valine, Threonine, Isoleucine, Methionine, Histidin, Lysine, Leucine, Arginine, Aspartic, Tyrosine, Serine, Glutamate and Glysine. There are 8 essential amino acids, 1 semi essential amino acid and 6 non essential amino acids and the highest non essential amino acid is Glutamate and followed by Aspartic. Dried anchovy has a good amino acid profile especially essential group with a good taste and also highly Phosphorous, Calcium, Iron and Iodine.

1. Introduction

Buru island is one of the small islands in Maluku with some fisheries products like fresh tuna, dried cucumber, fresh demersal fish and dried anchovy. Dried anchovy produced by the artisanal community at Kayeli bay is a prominent fisheries product from Buru island. It has a good quality indexes and good feasibility business for the community’ income [1]. Dried anchovy from Kayeli bay is popular locally and other places, it can be found easily in the market with affordable prices. As a durable fish product reaches a wide distribution, preferred by the community as an alternative daily menu and fulfill the availability proteinous foodstock through the year especially during high wave season when fishermen can not catch fish.

Fish is highly nutritional value, its cheap and highly quality protein with a high biological value contain the good essential amino acid especially lysine, also rich mineral sources. Food proteins provide amino acids which serve as building block of all vital organs, muscles (including heart muscle), hormones and biological fluids such as blood. A constant supply of good quality protein is needed to maintain growth and other physiological function. Insufficient intake of protein especially during periods of growth and development can affect all organs in the body including brain, heart, immune system and other vital organs [2]. Some minerals are essential for cellular metabolism, among them calcium, phosphorous, iron and iodine. Minerals comprise only a fraction of total body weight, they are crucial for many functions including transporting oxygen, normalizing the nervous system and stimulating growth, maintenance and repair of tissues. Adding fish to traditional bland staple diets can stimulate appetite and increase food consumption of the young child and the aged [3, 4].
Dried anchovy is a durable proteinous foodstock for the community in small islands through the year especially during the wave season where the fisherman can not go to sea. Its sustain the good quality protein intake containing essential amino acid moreover the small size of the anchovy enable to consumed whole with the bones which is a good mineral sources. Investigation of proximate, Amino acid profile and mineral constitut are important for nutritional data. Evaluation parameters of dried anchovy were done to determine nutritional value related to the health benefits.

2. Material and Method

2.1. Sampling Dried anchovy
Dried anchovy were collected in June and July 2019 from Siahoni village at the coastal of Kayeli bay Namlea Buru island. The dried anchovy brought to Integrated Chemistry Laboratory Bogor Agriculture University for Amino Acid and Mineral. Proximate determination carried out at Fisheries Product Technology Laboratory, Faculty of Fishery and Marine Science, Pattimura University.

2.2. Proximate Analysis
Moisture was determined according to AOAC (2005) method \[5\] by drying sample in a hot air at 135°C for 1 hour, cooled down in desicator for 15 minutes and weighed. Protein analysis was done according to AOAC (2005) procedure. Total protein in the homogenized sample determined by using Kjeldhal method, total N was multiplied by 6.25 to get the crude protein of dried anchovy. The crude fat estimation was carried out by continuous extraction of fat in petroleum ether according to AOAC (2012) method \[6\]. Ash content determination was done according to AOAC (2012), determination by incinerating the sample in a furnace at 550°C for 6 hours.

2.3. Determination of Calcium (Ca) and Iron (Fe) (AOAC 2005)
Ca and Fe determination were performed according to AOAC (2005). 2.5 grams sample in the Erlenmeyer/digestion flask added 25 ml glacial HNO\(_3\) and boiled slowly for 30-45 minutes to remove all of oxidized compound, cooled down and added 10 ml 70-72% HClO\(_4\) then boiled slowly till till colorless. Cooled and added 50 ml H\(_2\)O then boiled again till all NO\(_2\) gas comes out then cooled and filtration into 100 ml volumetric flask and added distilled water up to the tera mark, homogenized and ready for the test. Ca and Fe determinated by using AAS on 624 nm and 510 nm respectively for Ca and Fe.

2.4. Determination of Iodine
Iodine dried fish determination was done according to Monitoring Universal Salt Iodization Programmes, Publish by PAMM/MI/ICCIDD \[6\]. 5-10 grams in the 250 ml Erlenmeyer with stopper flask added 100 ml aquades, swirled and let it for 30 minutes then filtered by using Whatman no. 41. Added 2 ml 2 N sulfide acid and 5 ml 10% KI solution then incubated in dark for 10 minutes. Titrated with 0.025 N Natrium Thiosulphate till the color turns light yellow, added 2 ml starch indicator and stirred till the color turns blue. Titrated with Natrium Tiosulfate solution till colorless.

Iodine (mg I\(_2\)/kg) = \(\frac{(A-B) \times N \times 127000}{g \text{ sample}}\)
A : volume blanco titrane  
B :  volume sample titrane 
N : normalitas titrane 
127000 : MW I₂ x 1000

2.5. Determination of Phosphorus

Determination was carried out according to AOAC (2005). 2 grams sample was ashing in the furnace at 600°C for 4 hours and forms white ash. Cooled and added 40 ml 6 N HCL and few drops of HNO₃ then heated to boiling. Cooled and transferred into volumetric flask, diluted up to 200 ml by using distilled water. Filtered the fluid which is has a 5-15 ppm P in 100 ml and added 20 ml molibdovanadate, diluted with aquades and homogenized. Left for 10 minutes then read %P at 400 nm and compare it with 0.5 mg standard at 100%.

\[
\text{mg P/g sample} = \frac{(A_{\text{sample}} - i_{\text{reg}}) \times 10}{S_{\text{reg}} \times 2 \times 100}
\]

\(A_{\text{sample}}\) : absorbance of Sample
\(i_{\text{reg}}\) : intercept of calibration curve
\(S_{\text{reg}}\) : slope of calibration curve

2.6. Amino Acid Analysis (ICI Instrument In House Method IPB, 1998) [7]

Firstly, hydrolyzed sample containing 6 mg protein in 2 ml 6 N HCl and heated in oven at 110 °C for 24 hours and dried. Cooled in room temperature and dried by using evaporator. Diluted dried hydrolyzate sample in 10 ml 0.01 N HCl and filtered with Millipore paper. Added Kalium Borate pH 10.4 on the filtrate by comparison 1:1. Pipetted 5 µl filtrate into an empty vial and added 25 µl ophthaldehyde (OPA) reagent, left for 1 minute so that derivatization process perfectly performs. After that, injected 5 µl into HPLC column, the separation process of amino acid will be finish in 25 minutes (this step can be done by using autosampler).

2.7. Evaluation of Protein Quality

The ASS and EAAI were used to describe protein quality of dried anchovy, calculated by using formula below [8, 9]

\[
\text{AAS} (\%) = \left[ \frac{\text{mg AA in 1 g of protein tested}}{\text{mg AA in 1 g reference protein}} \right] \times 100
\]

\[
\text{EAAI} (\%) = n \cdot \log \text{EAA}
\]

Where \(\log \text{EAA} = \left[ \frac{1}{n} \right] \times \left[ \log(100a_i/a_iR) + \ldots + \log(100a_n/a_nR) \right] ; a_i\) in mg of amino acid in g of tested protein; \(a_iR\) in mg of amino acid in 1 g of reference protein; \(n\) is the number of amino acid and essential amino acids considered for the calculation (the pair Methionine-Cysteine count as 1). The amino acid pattern defined by the World Health Organization and the Good and Agriculture Organization (FAO/WHO/ONU) were used as the reference protein [10]. The lowest ASS value within essential amino acid defined as the AAS of protein.

3. Result and Discussion

The average value of proximate, amino acid and mineral composition in dried anchovy from Kayeli bay Buru island shown on Table 1.
Table 1. Proximate, Amino Acid and Mineral of Dried Anchovy from Kayeli Bay Buru Island

| Proximate (%) | Amino Acid (%w/w) | Mineral (mg/kg) |
|---------------|-------------------|-----------------|
| Protein       | 59.32             | Phenylalanine    | 2.53           | Phosphorous | 160 |
| Fat           | 2.63              | Valine           | 2.89           | Calcium     | 219 |
| Moisture      | 11.23             | Threonine        | 2.28           | Iodine      | 5.08 |
| Ash           | 11.92             | Isoleucine       | 2.55           | Iron        | 60.83 |
|               |                   | Methionine       | 1.72           |            |     |
|               |                   | Histidine        | 1.71           |            |     |
|               |                   | Lysine           | 3.29           |            |     |
|               |                   | Leucine          | 4.51           |            |     |
|               |                   | Arginine         | 4.91           |            |     |
|               |                   | Aspartic         | 5.14           |            |     |
|               |                   | Tyrosine         | 2.29           |            |     |
|               |                   | Serine           | 1.95           |            |     |
|               |                   | Glutamate        | 8.65           |            |     |
|               |                   | Glysine          | 3.67           |            |     |
|               |                   | Alanine          | 3.98           |            |     |

Dried anchovy from Kayeli bay Buru island contain protein almost 60%, a highly ash and a relatively low moisture and fat. Those constitute support the durability of the product as proteinous food reserves. The low moisture is a good microbial growth inhibition and the low fat keep the product get rancid uneasily. The amino acid profile comprise of 8 essential amino acid, 1 semy essential amino and 6 non essential amino acid. The highest essential amino acid is Leucine and followed by the Lysine, while the lowest is Histidine and followed by the Methionine. The highest non essential amino acid is Glutamate and followed by Aspartic, while the lowest is Serine and followed by Tyrosine.

Refer to the Amino Acid Scoring Pattern, the Amino Acid Score (AAS) and Essential Amino Acid Index (EAAI) of the dried anchovy were 73.1% and 53.8% respectively for AAS and EAAI. Those value above 50% determine that dried anchovy is good enough as the protein sources for the community daily menu, eventhough Lysine is a limited amino acid (Table 2). Moreover, dried anchovies is not good enough for children for some amino acid intakes (Table 3). The dried anchovy weakness as protein sources and amino acid intakes can be complimented by the addition of other types of protein sources such as peanuts and tempeh which have been done by the community in preparing and processing dried anchovies for daily menu based on local wisdom. Dried anchovy from Buru island might contribute to fulfill the suggested pattern Essential Amino Acid requirements for adults as shown on Table 3.

Table 2. Amino Acid Score (ASS) and Essential Amino Acid Index (EAAI) for Dried Anchovy From Kayeli Bay Buru Island

| Essential Amino Acid | Amino Acid Scoring Pattern (mg AA/g) [10] | Essential Amino Acid dried Anchovy (mgAA/g) |
|----------------------|-------------------------------------------|-------------------------------------------|
| Phenylalanine+Tyrosine| 38                                        | 48.2                                      |
| Valine               | 39                                        | 28.9                                      |
| Threonine            | 23                                        | 22.8                                      |
| Isoleucine           | 30                                        | 25.5                                      |
| Methionine           | 22                                        | 17.2                                      |
| Histidine            | 15                                        | 17.1                                      |
| Lysine               | 45                                        | 32.9                                      |
Leucine 59 45.1
Limited Amino Acid (LAA) Lysine
AAS (%) 73.1
EAAI (%) 53.8

Table 3. Suggested Pattern Estimated Amino Acid Requirement of Children and Adult

| Essential Amino Acid | EAA dried anchovy (mg/g protein) | Children (mg/g protein) | Adult (mg/g protein) |
|----------------------|----------------------------------|-------------------------|---------------------|
| Histidine            | 17.2                             | 0                       | 0                   |
| Isoleucine           | 25.5                             | 37                      | 18                  |
| Leucine              | 45.1                             | 56                      | 25                  |
| Lysine               | 32.9                             | 75                      | 22                  |
| Phenylalanine+Tyrosine | 48.2                         | 34                      | 25                  |
| Threonine            | 22.8                             | 44                      | 13                  |
| Tryptophan           | -                                | 46                      | 6.5                 |
| Valine               | 28.9                             | 41                      | 18                  |

Sources : [10]

The Minerals constitute as shown on Table 1 determined that dried anchovy from Buru Island can contribute as a good sources of phosphorous (160 mg/kg), Calcium (219 mg/kg), Iron (60.83 mg/kg) and Iodine (5.08 mg/kg) for community daily menu. Especially for Iodine and Iron, Dried anchovy from Buru island can fulfill daily intake recommendation by WHO and FAO [11]. Daily intake recommendation of Iron based on the bio-availabilities for children 3.9-17.8 mg/day, for males 9.1-37.6 mg/day and for females 9.3-65.4 mg/day, while the recommendation of daily intake Iodine for children 90-120 μg/day and for adults 150 μg/day.

4. Conclusion
Dried Anchovy from Kayeli Bay Buru Island is good enough as essential amino acid sources, Phosphorous, Calcium, Iodine and Iron, but need to combine with other kinds of proteinous and mineralous foods for an excelent nutrients intakes in community daily menu.

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