Physico-chemical characteristics and amino acid profile of fermented sauce made from tuna loin by-product

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Abstract. The processing of tuna loin produces by-products which can be used to increase the total economic value of tuna. One way to use these by-products is to make fish sauce through a fermentation process. Fermentation can increase the nutritional value and extend the shelf life of the product. This study aimed to determine the physico-chemical characteristics and amino acid profiles of fermented fish sauce made from tuna loin by-products. All experimental treatments were conducted with three replicates. The data are reported as mean values ± standard deviation. Physical characteristics measured included colour (L: 4, a: 0.6, b: 2.3) and viscosity (9.58 cP). Chemical characteristics measured included salt content (26.57%); acidity (0.11%), pH (5.00), TVBN (26.00 mg% N), Protein (11.30%), and moisture content (59.00%). The amino acid with the highest concentration was glutamate (1.01%).

1. Introduction
Maluku is one of the regions in Indonesia which has been producing tuna loin for the past two decades. Tuna loin is defined as a cut taken from the backbone and cut lengthwise into quarters, without spines, bones, skin, or red meat. The processing of tuna loin from whole tuna produces on average 60.3% of waste products which comprise 23.1% discarded meat (offcuts); 17.8% head; 8.5% bones and fins; 3.7% skin; 3.2% stomach/gut content and other internal organs; 0.9% blood, and 0.6% heart [1,2].

Waste is a by-product of production which has a low economic value. The discarded tuna meat is one waste product (by-product) of tuna loin production that is under-utilised. Usually referred to locally as tetelan, this discarded meat (tuna offcuts) can be processed in a number of ways. The utilization of this waste through further processing will increase the value of the discarded meat, and thus of the whole tuna. For example, tetelan can be included in the mixes used to make meatballs, nuggets, rissoles, and the regional specialty called otak-otak. Another example is the utilization of tuna loin by-product to make fermented fish sauce.

Fish sauce is a product with high water, protein, and mineral content. Fish sauce can be made from crushed meat to which salt, spices and some kind of hydrolytic enzyme are then added. The fermentation process that occurs in fish meat when making fish sauce is a biological or semi-biological decomposition process in which complex compounds, especially proteins, are broken down into smaller compounds under controlled conditions. During the fermentation process, fish protein will be hydrolysed into peptides and amino acids which are then further decomposed into other compounds, including compounds which can enhance the taste (flavour) of the product. Peptides and
amino acids in the fish sauce are known to be produced by proteolytic enzymes together with indigenous protease from fish meat and various other microorganisms [3–5].

Several fish sauce products are traditionally produced around the world, for instance, Yu-lu in China [6], Jeotkal in Korea [7], Bakasang in Indonesia [5,8], Lanhouin in Benin, Togo, and Ghana [9], Momoni in Ghana [10], Plaa-som in Thailand [11] and Pekasam in Malaysia [12]. This study aimed to determine the physico-chemical characteristics and amino acid profiles of fish sauce made from tuna loin by-product.

2. Materials and Methods

2.1. Raw material

The raw materials used in this study were leftover pieces of white meat and red tuna meat (tuna offcuts), obtained from the waste produced by the tuna processing facility in Passo village in Ambon, Indonesia. The collected samples were placed in a cooler filled with ice for transport to the laboratory where they were prepared and fermented to produce fish sauce.

2.2. Fish sauce preparation

The fish sauce was prepared following the method of Simanjorang et al. [13] with modification. Briefly, the tuna offcuts were washed, then cut into smaller pieces measuring ± 1 cm³. The meat was then coarsely ground. Portions of ground tuna weighing 200 g were mixed with 5% of papain enzyme (Paya brand) and 20% of salt. This mixture was transferred into sterilized glass bottles which were then tightly closed and placed in an incubator at 50°C for 6 days of fermentation. The fermented product from each bottle was diluted with 200 ml of distilled water and filtered (filtering I). The filtrate was heated and kept at 80°C for 10 minutes. After this first heating, 17% of seasoning and cellulose gum powder (CMC brand) were added and the mixture was further heated at 70°C-80°C for 20 minutes, then filtered (filtering II). The filtrate produced was the fish sauce analysed.

2.3. Determination of physical characteristics (colour and viscosity)

The colour of the fish sauce was measured using a colour reader (Minolta CR-10) to determine the value of the parameters L *, a *, and b * of the sample, where L* varies from 0 = black to 100 = white, a* = redness, b* = yellowness. Viscosity was measured in cp, using a rotational viscometer (Elcometer 2300).

2.4. Determination of chemical characteristics

The chemical characteristics of the samples were determined following methods approved and described by the Association of Official Analytical Chemists (AOAC) [14].

2.4.1. pH and acidity. The pH of the fish sauce was measured by using a Senz pH digital tester (Trans instruments), while the total acid analysis was conducted using the titration method. Three drops of phenolphthalein indicator were added to 10 ml of suspension, which was then titrated using 0.1 N. NaOH solution. Colour change was used as the end of titration indicator. The quantity of 0.1 N. NaOH solution used for titration was recorded to provide the formula to calculate the acidity of the fish sauce.

2.4.2. Salt content. One gram of each sample was weighed and placed in a 250 mL Erlenmeyer glass to which 25-50 mL AgNO3 0.1 N and 20 mL of concentrated HNO3 were added. The mixture was then placed in an acid chamber and heated on a hot plate until all the solids except AgCl were dissolved. The temperature of the mixture was then reduced to room temperature before adding 50 mL of halogen-free water. A ferric indicator was added and the solution was titrated with NH4CNS 0.1 N until the solution changed colour to a light brown. The volume of 0.1 N NH4CNS used for titration was recorded in order to provide the formula to calculate the salt content of the fish sauce.
2.4.3. Total volatile base nitrogen (TVBN). Five grams of each sample were mixed with 15 ml of 5% trichloroacetic acid (TCA) and homogenized. The solution was filtered with filter paper to obtain a clear filtrate. Then, 1 ml of boric acid solution was poured into the inner chamber of a Conway cup, while 1 ml of the sample was placed into the outer chamber. A K₂CO₃ solution was added to the other side of the outer chamber which was then tightly closed. A solution of 3% TCA was used as blank. The cup was closed, then shaken for a minute and incubated for 2 hours. After incubation, the boric acid solution in the inner chamber was titrated with 0.01 NHCl until the colour turned pink. The TVBN was calculated from the volume of NHCl needed.

2.4.4. Analysis of Protein and Moisture Content. Analysis of protein and moisture content used methods approved and described by the Association of Official Analytical Chemists (AOAC) [14].

2.5. Amino-acid Analysis. Amino acids were analysed following [14] by weighing 0.5 g of each sample into a 25 mL beaker to which 10 mL of 6 N HCl was then added. The samples were heated at 100°C for 24 hours and then filtered to obtain a filtrate to which 5 mL of a drying solution (methanol, picolotiocianate, triethylamine) was added, and then dried. The derivative solutions (methanol, Na-acetate, and triethylamine) were added and the samples were left for 20 minutes. Approximately 200 mL of 1 M acetic acid solution was added and an aliquot of each sample was injected into a High Performance Liquid Chromatography (HPLC) instrument. The following HPLC settings were used: at room temperature, using the pico column tag 3.9 × 150 mM, a flow rate of 1.5 mL • min⁻¹, a pressure limit of 3,000 psi (1 psi = 108,219 kPa), gradient program, mobile phase 60% acetonitrile, 1 M sodium acetate buffer, and UV detector with wavelength 254 nM.

2.6. Statistical Analysis
All experiments were conducted with three replicates. The data were reported as mean values ± standard deviation.

3. Results

3.1. Physical Characteristics of Tuna Fish Sauce
The physical characteristics (colour and viscosity) of tuna fish sauce from tuna loin waste (off-cuts) are presented in Table 1.

| Colour* | Viscosity (cp) |
|---------|---------------|
| L⁺      | a⁺           | b⁺           |
| 36.05 ± 0.57 | 18.76 ± 1.44 | 15.65 ± 0.53 |
| 9.58 ± 0.02 |

*L⁺ (0 = black; 100 = white), a⁺ = red, b⁺ = yellow

3.2. Chemical Characteristics
The chemical characteristics of the tuna fish sauce produced from tuna loin waste (off-cuts) are presented in Table 2.
### Table 2. Chemical Characteristics of tuna Fish Sauce

| Parameter          | Content (%) |
|--------------------|-------------|
| pH                 | 5.00 ± 0.02 |
| Salt (%)           | 26.57 ± 0.05|
| Acidity (%)        | 0.11 ± 0.03 |
| TVBN (mgN/gr)      | 26.00 ± 0.11|
| Protein (%)        | 11.30±0.01  |
| Moisture (%)       | 59.00±0.02  |

### 3.3. Amino Acid Compositions

The amino acid composition of the tuna fish sauce produced from tuna loin waste (off-cuts) contained a number of essential amino acids and non-essential amino acids which are needed by the human body (Table 4). During the fermentation process, the hydrolysis of proteins produces a number of peptides and amino acids. The composition is influenced by several factors such as fermentation conditions, fermentation time, and total lactic acid bacteria (LAB).

#### Table 3. Amino-acid composition of the tuna fish sauce

| Amino Acid        | Contents (% w/w) |
|-------------------|------------------|
| Aspartic          | 0.54             |
| Glutamic          | 1.01             |
| Serine            | 0.28             |
| Histidine         | 0.12             |
| Glycine           | 0.32             |
| Threonine         | 0.26             |
| Arginine          | 0.20             |
| Alanine           | 0.35             |
| Tyrosine          | 0.19             |
| Methionine        | 0.13             |
| Valine            | 0.38             |
| Phenylalanine     | 0.29             |
| L-leucine         | 0.32             |
| Leucine           | 0.48             |
| Lysine            | 0.08             |
| Total             | 4.97             |

### 4. Discussion

Based on the measured colour of the fish sauce (L * (4), a * (0.6), b * (2.3)), the calculated degree(O) of Hue value was 14.69. Based on the colour table, the colour of the fish sauce was purplish red. This is similar to the results of research by Wenno et al. [5]. The colour of the fish sauce is influenced by factors influenced by the type of fish meat used, additional ingredients used, and fermentation conditions [15]. Fish muscle tissues contain myoglobin which is responsible for the red coloration of the meat. In addition, the addition ingredients such as brown sugar can also affect the colour.

The mean viscosity of the fish sauce was 9.58 cP. As compared to the results reported by Citra [16], sauces made from inferior raw fish material showed a comparable viscosity (9.79 cp) when using 1% CMC as a thickener. The ingredients used such as a thickening agents, sugar, and salt can greatly influence fish sauce viscosity. Salt can attract water contained in food and can thus reduce the water content and water activity (aw) of food products, thus increasing the viscosity [5].

A high salt concentration can promote denaturation of the protein. This protein denaturation is a process of change or modification of the secondary, tertiary, and quaternary structure of protein molecules without breaking the covalent bonds, including peptide bonds. The development of denatured protein molecules will open reactive groups in the polypeptide chain, then the same or
molecules by microorganisms to produce the organic acid flavour. The growth of fermentation can attract a certain ss are proteolytic lactic acid bacteria molecular weight amino acids, fatty acids, peptides, and others) through th reported TVBN levels of about 76.41 mgN/g for dayok, actic acid bacteria that grow during the fermentation process. AT are strongly related to the number of lactic acid bacteria within the fermentation dominant amino acids in fish sauce (yu protein showed that proteolytic activity is proteins and peptides which can be further degraded into amino acids. A higher amount of total protein produced consists of low molecular weight proteins and water activities of indigenous la proteolytic activity can occur due to enzymatic processes of endogenous enzymes or proteolytic which will degrade the fish protein into peptides and amino acids contributing the taste. The presence of lactic acid bacteria is supported by the availability of food or nutrients in the media during the fermentation process. The total acid produced from this research was 0.11%. Hwanhlem et al. also reported that plasom (fish sauce in Thailand) fermented with the addition of 100 g of salt for 8 days at room temperature resulted in a total acidity of 0.3-0.9%.

The Total Volatile Base Nitrogen (TVBN) level in fishery products can generally be used as an indicator of damage. TVBN values indicate the process of protein hydrolysis through the activity of enzymes and bacteria that produce amine compounds and reduce the product quality and value. During the fermentation, there is a process that causes changes in total titrated acid (TAT). Increases in TAT are strongly related to the number of lactic acid bacteria within the fermentation medium. The role of lactic acid bacteria is to produce organic acids such as lactic acid, acetic acid, and other secondary metabolites. The lactic acid produced can create an organic acid flavour. The growth of lactic acid bacteria is supported by the availability of food or nutrients in the media during the fermentation process. The total acid produced from this research was 0.11%. Hwanhlem et al. also reported that plasom (fish sauce in Thailand) fermented with the addition of 100 g of salt for 8 days at room temperature resulted in a total acidity of 0.3-0.9%.

The salt content of the fish sauce in this study was 26.57%. This value is comparable with fish sauce in Taiwan with 22.2% - 37.0% salt produced through 6-12 months of fermentation, but higher than the salt content of Mahyaveh (traditional fish sauce in Iran) which ranges from 8.19%-17.1% [4], and Bakasang with a salt content around 13.72% and a fermentation time of 7-14 days [5].

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The water content of the tuna fish sauce produced in this study was high. Fish sauce is a semi-solid product or paste that has high water content. The water content of fish sauce comes from the fish meat and a number of dissolved solids resulting from hydrolysis [22]. The added salt can attract a certain amount of water so that it can reduce the free water content.

During the fermentation process, the population of lactic acid bacteria will increase. Some strains of lactic acid bacteria that grow during the fermentation process are proteolytic lactic acid bacteria which will degrade the fish protein into peptides and amino acids contributing the taste. The proteolytic activity can occur due to enzymatic processes of endogenous enzymes or proteolytic activities of indigenous lactic acid bacteria that grow during the fermentation process. [23–28]. The total protein produced consists of low molecular weight proteins and water-soluble proteins. The presence of proteolytic activity causes the degradation of proteins into smaller molecular weight proteins or water-soluble peptides. Hydrolysis of muscle proteins will be digested into water-soluble proteins and peptides which can be further degraded into amino acids. A higher amount of total protein showed that proteolytic activity is well conducted during the fermentation process.

Fermented fish products often contain glutamate which has a flavouring function. For example, the dominant amino acids in fish sauce (yu-lu) produced through traditional fermentation processes in
China are glutamic acid, lysine, leucine, valine, and alanine, while proline is present in small amounts [6]. Analysis of the amino acid composition of the fish sauce produced from tuna loin waste (offcuts) in this study showed that amino acids with charged side chains were more dominant than amino acids with polar or hydrophobic side chains, in particular glutamate, the amino-acid which had the highest concentration (1.01%).

5. Conclusion
The present study demonstrated that the addition of 5% papain enzyme and 20% salt to tuna loin waste (offcuts) produced tuna fish sauce with good physical and chemical characteristics. The fish sauce had a purplish red colour, with a viscosity of 9.58 cp, salt level 26.57%, TVBN level of 26 mgN%, a total acid concentration of 0.11%, a protein content of 11.3%, and moisture content of 59%. Glutamic acid was the dominant amino acid contained in the tuna fish sauce produced.

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