Amino acid profile of tuna (Thunnus albacores) fish sauce fermented by salt and papain enzyme

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Abstract. Fish sauce has been long known as a flavoring by many communities in the world, especially in Asia including Indonesia. Fish sauce's function is to enhance the food taste and the people's appetite to eat. The aim of this research was to identify the amino acid profile of tuna (Thunnus albacores) fish sauce obtained through salt and enzyme fermented. The research was carried out by fermenting the fish flesh using 8% of salt and 15% of papain enzyme followed by 6 weeks fermentation process. The result showed that 15 amino acids of fish sauce were identified consists of 9 amino acids essential and 6 amino acids non-essential. The total amount of amino acid found on fish sauce was 10.57%, where the largest part was in the form of glutamate acid 1.82%. It was believed that the source of flavoring in the fish sauce was glutamate acid.

1. Introduction

Fish sauce is a processed food produced through fermentation of used fish or fish waste as a raw material. Fish sauce has a unique flavor and long shelf life [1]. Traditionally, fish sauce is produced by mixing the minced fish with salt in a specified concentration followed by incubation at room temperature (±30oC) during 6-12 months or more [2]. Sometimes fermentation process is carried out through a combination of the salt, enzyme, and bacterial [3]. Fish sauce is the clear brown liquid manufactured by fermenting fish or fish waste using salt [4].

Fish sauce is famous in South Asia countries used as seasoning also as a source of protein. Nitrogen content in fish sauce could reach 20 g/L where 80% of this number consisted of amino acids [5]. During fermentation processed, fish meat will be hydrolyzed into a short-chain amino acid form, which was considered contributing to the fish sauce taste, aroma, texture, and unique flavor [6]. The fermentation process increased the soluble nitrogen total and the food quality because it produces not only essential amino acids but also minerals such as sodium (Na), calcium (Ca), and magnesium (Mg) [2].

Protein degradation is influenced by the presence of the enzyme in fish meat. Bacterial activity is limited by the salt content as the main component in fish sauce preparation [7]. The addition of proteolytic enzymes, such as papain, pronase, trypsin, chymotrypsin, to fish meat increases the rate of protein solubilization during the fermentation. Endogen fish enzymes such as chymotrypsin have a high catalytic activity consequently, it could hydrolyze more peptide bonds in protein substrate and shorten the fermentation period. The most changes in the fermentation process were the conversion of protein into small peptides and amino acids. The total nitrogen value would be a principle indicator that indicates the fish sauce quality.

The present research was aimed to study the amino acid profile of fish sauce processed by using salt and papain enzyme in order to shorten the fermentation time.
2. Materials and Method

2.1. Materials
The main raw material was tuna loin waste obtained from PT. Harta Samudera, located in Tantui Village, Sirimau District, Ambon City. Other auxiliary materials were papain enzyme (“Paya” Branded), salt, distilled water, labeling paper, aluminum foil, and plastic wrapping. Devices used were blender, glass bottle, plastic container, spoon, knife, incubator, balancing, Erlenmeyer, vortex, and measuring cup.

2.2. Fish sauce preparation
First, tuna loin waste milled using a blender, about 100 grams of minced fish were then mixture with 8% of salt and 15% of papain enzyme. The mixture was then placed in a glass bottle sealed tightly and incubated at 50°C for six weeks. After fermentation, the fermented product from each bottle was diluted with 200 ml of distilled water and filtered. Next, the filtrate was heated and kept at 80°C for 10 minutes. After this first heating, 17% of seasoning was added and the mixture, further heated at 70°C-80°C for 20 minutes, then filtered. The fish sauce produced was then analyzed.

2.3. Amino acid analysis
Amino acids were analyzed following [8] 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, picoletiocianate, 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 acetate 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 = 108,2197kPa), gradient program, mobile phase 60% acetonitrile, 1 M sodium acetate buffer, and UV detector with wavelength 254 nM.

3. Result and Discussion
Table 1 showed the result of the amino acid profile of fish sauce originated from tuna loin waste. There were 15 types of amino acids found consisted of 9 essential amino acids and six non-essential amino acids. The total amino acids were 10.57% consisted of 5.88% essential amino acid and 4.69% non-essential amino acid.

The result of this research is displayed in Table 1. This table shows that glutamate acid of non-essential was the amino acid component with the highest content number followed by phenylalanine and histidine with the percentage of 1.82 %, 1.31 %, and 1.27 % respectively. Amino acid profile with the highest component signed by glutamate acid was also found in some research on both soy sauce and fish sauce. Fish sauce was found to have the highest glutamate acid number followed by leucine and valine with the number of 128.50 mg/100mL, 92.70 mg/100 mL, and 78.20 mg/100 mL correspondingly [9]. It was also found that glutamate acid was the highest component of amino acid found in green-been sauce followed by leucine and lysine with the percent concentration of 0.93%, 0.62%, and 0.54% correspondingly [10]. Other research on soy sauce also reports that glutamate acid showed the highest number of concentration followed by aspartate acid and isoleucine with the value of 2.2 – 2.9 %, 1.05 - 1.46%, and 1.04 – 1.28% in that order [11].

Domination of alanine and glutamate acid on even soy sauce and fish sauce was vital in enhancing the flavor of either fish sauce or soy sauce [12]. Some peptide had been recognized from fish sauce and was concluded that this sort of oligopeptide has a high contribution to the strong and complete taste of the fish sauce. Tripeptide glutathione can strengthen the fish sauce characteristics but was not influence
the intensity of the original flavor [13]. It was found that oligopeptide acidic fraction in fish hydrolysate provides a considerable broth taste and favorable after taste [14].

Table 1. Amino acid profile of fish sauce sample

| Amino acids | Content % (w/w) |
|-------------|-----------------|
| **Essential** |                |
| Valine      | 0.38            |
| Methionine  | 0.20            |
| Isoleusine  | 0.28            |
| Leusine     | 0.58            |
| Phenylalanine | 1.31        |
| Histidine   | 1.27            |
| Lysine      | 0.79            |
| Threonine   | 0.36            |
| Arginine    | 0.71            |
| **Non-essential** |         |
| Aspartic acid | 0.84          |
| Serine      | 0.33            |
| Glutamate   | 1.82            |
| Glycine     | 0.87            |
| Alanine     | 0.72            |
| Tyrosine    | 0.11            |
| **Amino Acid Total** | **10.57** |

Compared to the total amino acid of tuna loin that amounts to 18.51% - 21.25% [15], the total amino acid of fish sauce of tuna loin waste was smaller with an amount of 10.57%. Therefore, the present fish sauce obtained was not recommended to be the main source of protein or amino acid, but more likely as a seasoning in enhancing the flavor of the food. In comparison with the fish sauce obtained by Wenno and Loppies [16] studied with the total amino acid of 4.97%, the current study produced the higher total amino acid with the value of 10.57%. The same as the previous study [9, 10, 11] described, glutamate acid also has the highest component in fish sauce.

The brown color of fish sauce resulted in a Maillard reaction between protein and sugar reduction indicates lysine blocking. The decreasing of lysine in fish sauce caused by $\varepsilon$-amino lysine deamination is due to the Maillard reaction formed melanoidin a brown color indication. Thus foodstuff contains lysine, its color tends to change to be brown color [11].

4. Conclusion
Fish sauce was not proposed to be the vital source of protein or amino acid, but more likely as a seasoning in enhancing the flavor of the main food. Domination of glutamate acid in the fish sauce was very important in enhancing the flavor of either fish sauce or soy sauce. There were 15 types of amino acids found consists of nine essential amino acids and six non-essential amino acids. The total amino acid was 10.57% consist of 5.88% essential amino acid and 4.69% non-essential amino acid. The presence of lysine in fish sauce caused $\varepsilon$-amino lysine deamination due to the Maillard reaction formed melanoidin a brown color indication.
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