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Amino acid profile of biologically processed fish protein hydrolysate (FPH) using local enzyme to combat stunting

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Abstract. Fish protein hydrolysates (FPH) which contains mixture of small protein or peptide and free amino acids may be good to be applied in children's diet, preventing and combating malnutrition problem through readily absorbed essential amino acid. Malnutrition is still a big issue in East Asian countries including Indonesia. According to FAO, in 2005—2015, the percentage of children under five who experience stunting increased from 28.6% to 36.4%, while the percentage of stunting for Indonesian children was 37.2% (2013), 35.6% (2010), 36.8% (2007). Malnutrition in children can lead to serious problems such as abnormal brain development and susceptibility to various infectious diseases. The last report in 2016 revealed that stunted children have lower content of essential amino acid in their serum compared to the normal children. The aims of this study were to produce and analyze the amino acid profile of two fish hydrolysate protein (FPH) products from *Sardinella* using local microbial protease isolated from hot marine water. The FPH-1 and FPH-2, both from soluble and solid part of hydrolyzed fish were dominated by amino acid lysine and leucine as well as glutamic acid. Both FPH products can potentially be used as ingredients for developing food for malnourished children.

Keywords: malnutrition, peptide, *Sardinella*, stunting

1. Introduction

Stunting has been a serious problem faced worldwide including in Indonesia. It is a form of chronic malnutrition in children under five. It is reported that one-quarter children have been affected globally [1]. In Indonesia, there is an increasing percentage of children suffering from stunting, as can be seen from the 2007, 2010 and 2013 data, which amounts to 35.6%, 36.8%, and 37.2% respectively [2]. Stunted children show indications of being physically short accompanied by a deteriorating or underdeveloped brain development, and later, these children will have problems in education such as failing to graduate high school. Consequently, these children cannot have a good carrier like normal children when they are grown up. This causes serious problems for the Indonesian economy [3].

International organizations have been developing programs focusing on protein sufficiency for malnourished children in developing countries to overcome malnutrition problems worldwide since the 1950s and 1960s, followed by micronutrient sufficiency programs in the 1970s [1]. Tryptophan and lysine were reported to be the key amino acids that should be consumed in enough quantity to avoid stunting in children, several researches reported on the importance of protein and essential amino acid in malnourished children [4-6]. Stunting children had lower serum concentration in all nine essential amino acids [1]. Consequently, the synthesis of both proteins and lipids is limited due to the mTORC1 gene being repressed. The availability of the nine essential amino acids in the children's blood serum



has to be enough in quantity to avoid stunting children. Protein hydrolysate, which is rich in amino acid, peptide, and shorter protein, should be a good and ideal diet for stunting children.

Fish is well known as an aquatic animal that can serve as an excellent source of nutrition; they contain high quality proteins. Fish protein provides human's need of essential amino acids (EAA) in the quantity needed. Fish protein hydrolysate (FPH) is a hydrolyzed fish protein, either biologically or chemically, so that the protein will be changed into its simple form. In the form of fish hydrolysate, the fish protein is more readily absorbed and increases the availability of amino acid in plasma blood compared to their native protein. FPH from various fish, either fresh water fish such as tilapia carp and marine fish such as *Sardinella* (local name *tembang*), yellowstripe scad (*selar*) and splendid ponyfish (*petek*) have been made using papain enzyme [7]. FPH from fish waste was reported to be produced using alcalase and flavorzyme; from *bibisan* fish using enzyme of *biduri* and papain [8], from catfish waste [9]. The FPH prepared using plant enzyme was reported to have a bitter and salty taste [7]; therefore, the bitter taste was used as a screening protease that will be used in this FPH process. In this study, we used local microbial enzyme screened previously and lab-scaled produced [10-12]. The enzyme work best at 55°C, thermostable, produced from heat-loving *Bacillus* isolated from hot spring water in the near coast of Banyuwedang, Bali [13, 14].

In Indonesia, underutilized fish that have white flesh is available in abundance. In the fish-landing sites (TPI) along north coast of Java, by catch which is mixed of white flesh fish is usually onboard as frozen fish. The mixed fish contains locally named *kuniran* (sulphur goat fish; *Upeneus moluccensis*), *swangi/mata besar*, *swangi batu* (purple-spotted bigeye; *Priacanthus tayenus*), *ikan pari* (rays), *kurisi* (omate-treadfin bream; *Nemipterus nematoporus*), *kapasan* (false trevally; *Geres punctatus*), *beloso* (lizardfish; *Saurida micropectoralis*), *manyung* (sea catfish, *Arius thalassinus*), and *ikan sebelah* (Indian halibut; *Isettodes irumei*). The aims of this study was to produce FPH using local bacterial protease and underutilized fish as raw material. In this case, we used *Sardinella*. FPH-1 is an FPH powder product derived from the soluble part of hydrolysate which have been added with binder material (malto dextrin) by 20% and spray dried, while FPH-2 product is the meat fish residue which is dried in 50°C vacuum oven.

2. Material and Methods

The fish (*Sardinella*) used for the study was from TPI Cirebon, brought frozen and kept frozen by putting the frozen fish in the styrofoam on the way to the laboratory in Jakarta. The fish was eviscerated and filleted before hydrolyzed.

2.1. Preparation of enzyme

Local protease enzyme was produced according to the best result of Fawzya's trial [13]. *Bacillus* sp. BII.1 was used to produce microbial protease. The isolate was grown in Minimal Synthetic Medium (MSM) containing 0.1% K₂HPO₄, 0.1% NaCl, 0.7% (NH₄)₂SO₄, 0.05% yeast extract, 0.01% MgSO₄, 1% skim milk or 0.6% technical grade skim milk, incubated at 37°C 125 rp for eighteen hours. Crude enzyme was obtained by centrifuging the culture using 8,000 rpm, 4°C for twenty minutes. The supernatant was the crude enzyme. The activity of the enzyme was calculated [15].

2.2. Preparation of protein hydrolysate

Fish hydrolysate was prepared based on the result of a previous study on the optimization process [13]. Meat of the *Sardinella* fish was chopped and blended with water (ratio 1:2 w/v). Local protease enzyme was added (500 U/25g fish meat), then hydrolyzed at 55°C. Hydrolysis process was optimized by sampling the slurry and analyzing the α -amino and total protein content. After the optimum time of the hydrolysis was achieved, the enzyme was inactivated at 80-90°C for 20 minutes. The supernatant (FPH-1) was obtained by separation using centrifuge (8,000 g) for ten minutes, added 20% filler (maltodextrin) and spray dried. The residue left was calculated, and dried by vacuum oven (50°C) (FPH-2).

2.3. Determination of hydrolysis time

Hydrolysis degree was analyzed every hour by calculating the α -amino nitrogen formed divided by total nitrogen of the fish hydrolysate or the number of the peptide bond broken divided by total peptide bond which was calculated [16].

2.4. Analysis

Moisture and protein content analysis were determined according to AOAC 985.35/50.1.14.2005; while the amino acid profile was analyzed following EZ: Faast amino acid testing kit - Phenomenex. The sample was hydrolyzed [17], i.e 20 mg of sample in a bottle, added with 1 mL of 6N HCl, heated 180°C for 60 minutes. An amount of 100 μ L hydrolyzed sample was then prepared with EZ: Faast amino acid testing kit - Phenomenex, and read using GC. The standard amino acid used was Phenomenex (AG0-7184).

3. Result and Discussion

The *Sardinella* used in this study had moisture content of 74.89% and protein content of 19.6%. Hydrolysis was optimally obtained at 7 hours, achieving about 57% degree of hydrolysis. The soluble part (1 L liquid) was obtained from 1 kg of *Sardinella* fish or 548.2 *Sardinella* meat), then added with maltodextrin 20% as filler, homogenized and spray-dried to produce 366 gram of white powder FPH-1 product. The process produced some residue which was dried in vacuum oven 50°C, producing FPH-2 powder.

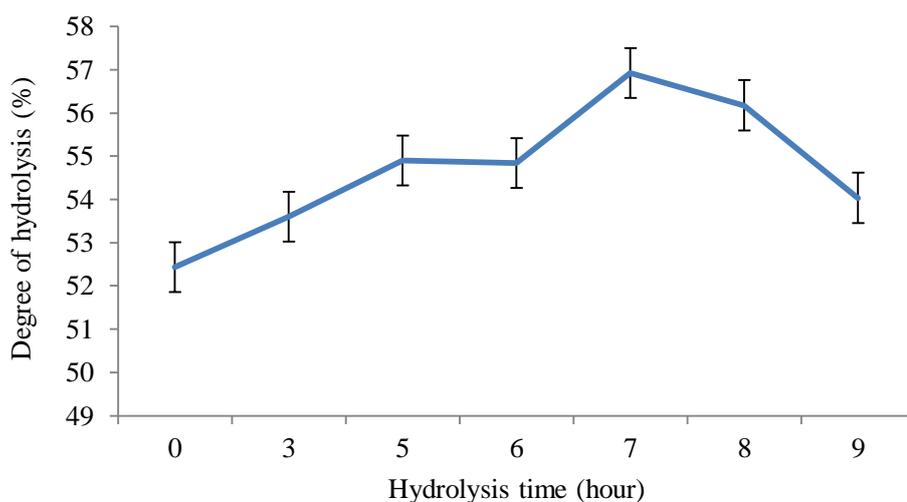


Figure 1. Optimization process of fish hydrolysis at 55°C using local bacterial protease.

The EAA profile of FPH (FPH-1 product) and residue (FPH-2 product) was dominated by lysine, followed by leucine, while non-essential amino acid (NEAA) was dominated by glutamic acid and aspartic acid. Except for lysine and glutamic acid, the amino acid of FPH-1 was lower than that of the raw material (fish) and residue/FPH-2.

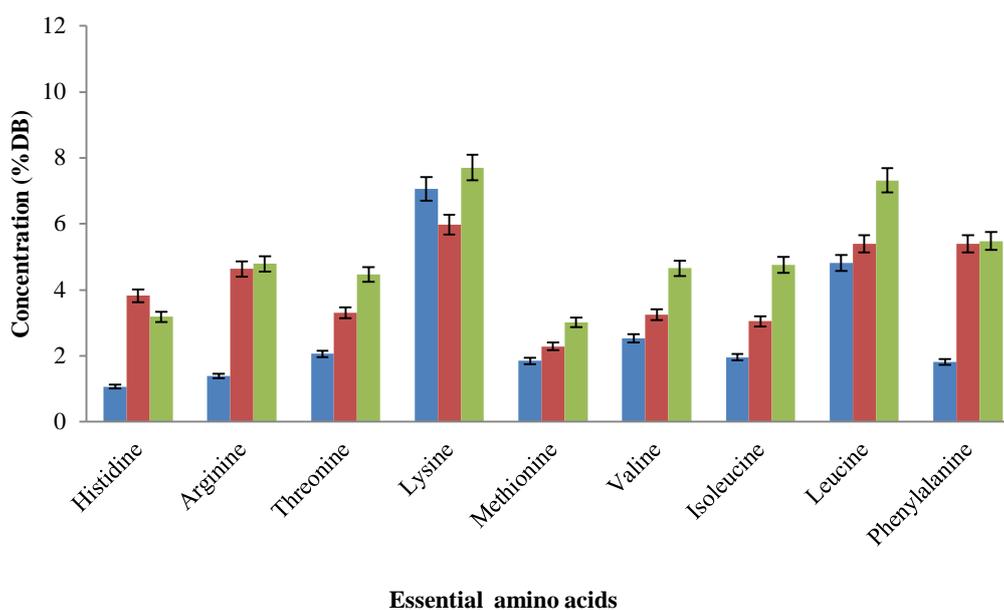


Figure 2. Essential amino acids profile of fish as raw material, and the FPH-1, FPH-2 (residue) products, ■ = FPH, ■ = raw fish, ■ = residue.

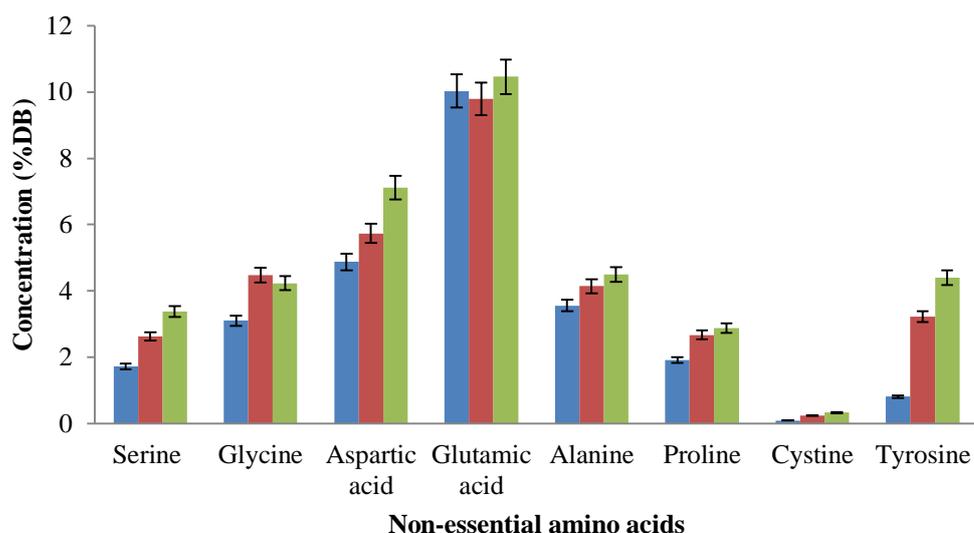


Figure 3. Non-essential amino acids profile of fish as raw material, and the FPH-1, FPH-2 (residue) products, ■ = FPH, ■ = raw fish, ■ = residue.

Using 500 U protease /25 gram fish meat, *Sardinella* meat was hydrolyzed optimally after seven hours at 55°C incubation, which is longer compared to those using *Selaroides* as raw material (six hours). Study on the hydrolysate protein from *Sardinella longiceps* showed that the optimum time of hydrolysis was 25.12 ± 0.87 % using 2% trypsin [18]. While other research used five hours of hydrolysis as hydrolysis time for *Sardinella* [19]. Depending on the fish species and enzyme used, the time of hydrolysis varies. Our previous study showed that lean fish like *Selaroidae* need six hours, while smaller fish which was processed as whole fish achieved its optimum hydrolysis time after eight hours [12]. *Sardinella* is categorized as fatty fish, and although the washing of minced fish was conducted in the processing of *Sardinella* FPH to remove the fat, it seems that *Sardinella* meat needs longer time to achieve optimum hydrolysis compared to the lean, white meat fish.

The amino acid profile of *Sardinella* as the raw material in this study was dominated by lysine followed by isoleucine and phenylalanine (figure 1). *Sardinella* harvested from coast of Kerala, India was dominated by lysine [19]. Lysine and leucine have been reported as the key amino acids in marine fish.

Meanwhile, lysine was reported as the key amino acid of fresh water fish harvested from cold water [20]. Previous research on fresh water fish found that key essential amino acid of *Channa striata* [11], *Hemibagrus nemurus* [21], *O.gouramy* [22], *Chanos chanos* [23] was lysine, which is in accordance with Mohanty's report .

Figure 1 and 2 shows the essential and non-essential amino acids of fish (*Sardinella*) as well as the FPH-1 and FPH-2 products. The FPH-1 product was superior in lysine and glutamic acid, for essential and non-essential amino acid. However, the FPH-1 product contained lower amino acids for both EEA and NEAA compared to the raw material and the FPH-2 products. This is understandable because the FPH-1 product used 20% maltodextrin as filler in the spray-dried process producing the powder. Maltodextrin is a complex carbohydrate derived from the starch that is usually used as food ingredients. High lysine content and other essential amino acid compared to the fish as raw material proved that hydrolysis can have a positive effect in improving the amino acid content in the FPH product. It seems that heat treatment applied in spray drying was not destructive to lysine. Heat processing was reported to reduce protein value as well as amino acid by destroying or making them unavailable. Amino acids are lost in the processing of catfish; the worst process is frying with palm oil since it decreases amino acid content in the catfish processed. The loss of amino acids in processed catfish is more pronounced than that of tilapia; however, in total the essential amino acid is increased [24]. In this study, the spray drying applied was not detrimental to lysine, an important EAA in the FPH. Lysine is an essential amino; its presence is very important for obtaining optimal growth. Therefore, lysine is absolutely needed to prevent stunting.

Other amino acids that are prevalent in raw fish, FPH-1 and FPH-2, are glutamic acid. Glutamic acid is also reported as an important material of umami taste. Therefore, the highest content of glutamic acid in FPH-2 indicates that this product can be used as fish enhancer, meaning that the residue/FPH-2 has a potential to be used as food ingredient, especially as umami taste enhancer. Glutamic acid is important in transamination reactions, which is essential reaction in metabolism. Glutamic acid is reported as one of the functional amino acids (FAA), along with other amino acids like arginine, cysteine, leucine, methionine, tryptophan, tyrosine, aspartate, glutamic acid, glycine, proline, and taurine. The amino acids that have an important role in the prevention and treatment of diseases related to metabolism is grouped as FAA. The FAA is also reported essential in metabolic pathways to obtain primary health status, survival, growing, lactation, and reproduction [25]. As depicted in figure 1 and 2, the residue or HFP-2 product contained higher EAA and NEAA than that of the FPH-1 except for lysine and glutamic acids. The residue/FPH-2 also contained leucine and phenylalanine as major EAA after lysine. Therefore, FPH-1 and FPH-2 have a good profile of amino acid, having similar profile as the raw material.

Food from animal sources such as milk, eggs, and meat have been suggested to be included in children's diet to prevent and combat stunting [2]. Fish offer good quality and quantity of amino acid, low fat and sources of ω -3 fatty acid naturally. Fish is abundantly available, both fresh water fish and marine fish, and Indonesians should take advantage of this resource to fulfill their high quality diet. However, due to the perishable property of the fish meat, fish processing should be conducted while maintaining the nutrition. FPH is one of alternative processing to obtain ingredient products such as FPH-1 and FPH-2 for malnourished children who need high quality and quickly absorbed protein in their diet for normal growth.

4. Conclusion

FPH from *Sardinella* could be obtained thorough hydrolysis time of seven hours, achieving 57% degree of hydrolysis. The products which were FPH-1 and FPH-2 had excellent lysine and leucine and other essential amino acids, indicating that the products could potentially be used as ingredients for children's food to prevent and combat stunting. The FPH-2 product was rich in glutamic acid that could potentially be used as fish flavoring as well.

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