

## Functional Properties of Fish Protein Hydrolysate from Dark Flesh of Skipjack Tuna (*Katsuwonus pelamis*)

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### Abstract

The dark flesh of skipjack tuna (*Katsuwonus pelamis*) hydrolysate was produced by using two different enzymes; Alcalase 2.4L® (SDFPH-A) and Protamex® (SDFPH-P). Samples treated with SDFPH-A and SDFPH-P were analyzed for their functional properties and compared to commercial cod fish hydrolysate as a control sample (FPH-C). The result showed that the oil holding capacity of the FPH-C was significantly higher ( $P < 0.05$ ) than that of SDFPH-A and SDFPH-P. There was no significant difference ( $P > 0.05$ ) in water holding capacity between SDFPH-A and SDFPH-P. Solubility of both SDFPH-A and SDFPH-P were higher (85.31-99.62%) than FPH-C (57.61-63.02%) over a wide pH range. The foaming capacity and stability of samples were affected by pH range. The hydrolysates showed same trend of emulsifying activity index which was also affected by pH. The emulsifying activity index of all samples were low at pH 2-6 (25.26-40.15 m<sup>2</sup>/g) and tended to be higher starting from pH 8 (29.18-52.43 m<sup>2</sup>/g) until pH 10 (190.08-220.33m<sup>2</sup>/g). The emulsion stability of SDFPH-A, SDFPH-P and FPH-C were quite similar at pH 8-10. This study suggests that SDFPH-P had better functional properties compared to SDFPH-A in terms emulsifying activity index.

**Keywords:** skipjack tuna hydrolysate, dark flesh functional properties

### Introduction

Tuna (*Thunus* sp.) and tuna-like species are economically very important and significant source of food. Their global production tends to increase continuously from less than 0.6 million ton in 1950 to almost 6 million ton today [1]. Tuna is generally processed as raw meat and marketed as loins/steaks or as canned foods. Due to global competition, the profit margin on tuna loins/steaks is limited. In the canning process, only about one-third of the whole fish is available for value addition. Canned fish processing generates solid wastes from the processing industry composed of muscles (after the loins are taken), fish viscera, gills, dark flesh /dark muscle, head, bone, and skin; these can be as high as 70% of the original material [2]. Processing discards from tuna canning industries are estimated to be 450000 tons annually [3]. Although the tuna industry in Malaysia is relatively smaller compared to other countries like Thailand and Indonesia the government's pioneering efforts to make the country a major player in international tuna trade is commendable. The Malaysian government has identified one of the ports in Penang Island as an international tuna port as a catalyst for tuna industry in Malaysia [4].

Hydrolysates can be defined as protein that are chemically or biologically broken down into peptides of varying size. Chemical hydrolysis are more commonly used in industrial practice, but biochemical hydrolysis holds the most promising future because it results in food grade products of high functional and nutritive value. Biochemical hydrolysis is performed by utilizing enzymes to hydrolyze peptide bonds that can be done via proteolytic enzymes already present in fish viscera or by adding enzymes from other sources [5].

Protein hydrolysates are produced for a wide variety of uses in the food industry, such as milk replacers, protein supplements to cereal foods, soups, bread and crackers, stabilizer in beverages and flavour enhancers in confectionary products [6]. Fish protein hydrolysate has a potential use for functional food ingredients as emulsifier and binder agents [7]. However, different raw materials and enzymes used in hydrolysis process could produce different functional properties of Fish protein hydrolysates. This study aimed to characterize the functionality of Skipjack dark flesh protein hydrolysate produced by using Protamex® and Alcalase 2.4L® enzymes.

## Materials and Methods

### Raw Material and Chemicals

Frozen blocks of Skipjack tuna (*Katsuwonus pelamis*) by-product (dark flesh parts) were obtained from PT. Medan Tropical Canning & Frozen Industries (Medan, Indonesia). The frozen materials were transported in a cold storage truck at -20°C to the laboratory of Food Technology Division, in the School of Industrial Technology, Universiti Sains Malaysia and were stored under -20°C until further used. Prior to the hydrolysis, one packet from each sample block was thawed overnight in a refrigerator at 4°C. The following enzymes were obtained from Novo Nordisk (Denmark): Industrial endo and exopeptidase mixtures, Protamex®(1.5MG) and Alcalase® 2.4LFG. These enzymes comply with the recommended purity specifications for food-grade enzymes recommended by Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC). These enzymes were stored at 4°C until further used. All chemical reagents used for this experiment were of analytical grade.

### Production of Protein Hydrolysate

The thawed dark flesh tuna were minced in a blixer (Robot Couple, France) followed by heating at 85°C for 20 minute in water bath (WiseBath<sup>(R)</sup> Daihan Scientific, Korea) to inactivate the endogenous enzymes [8] and to facilitate the removal of fat presented in the material. The heat treated raw material was then allowed to cool and preceded with centrifugation at 3500 rpm for 20 min at 4°C (Union 5KR centrifuge, Hanil Science Industry, Korea) for oil separation. The separated oil was then removed and the protein rich solid was used for subsequent experiments. The protein rich solid samples were mixed with sodium phosphate buffer 1:2 (w/v) and homogenized (IKA T25 digital Ultra Turrax, Germany) for about 2 min at ambient temperature. The condition of FPH production was based on RSM optimized condition from previous research [9, 10]. The condition for Alcalase 2.4L was 65.41°C, pH 8.87, and 2.04% Alcalase 2.4L® for 5.73 h whereas for protamex® it was 58°C, pH 6.57, and 3% Protamex® for 4 h. After each treatment, the reaction was terminated by heating the solution in water bath (JP Selecta, Spain) at (Alcalase® 2.4L FG optimum at 85°C for 10 min, and Protamex® at 85°C for 10 min), ensuring the inactivation of the enzymes. The hydrolysates were then cooled on ice to room temperature and centrifuged at 10000 rpm at 4°C for 20 min in a Kubota 6500 centrifuge (Japan), to collect the supernatant. The supernatant was then freeze-dried for hydrolysate functional properties analyses.

## Functional Properties

### Foaming Capacity

Foam capacity (FC) was determined according to the method of Klompong [12] with slight modifications. Freeze dried protein hydrolysate was diluted with distilled water to make 1.0% protein solution. Twenty ml of 1.0% sample solutions were adjusted to pH 2, 4, 6, 8 and 10 and then homogenized in 50 ml cylinder at a speed of 16,000 rpm to incorporate air for 2 min (IKA T25 digital Ultra Turrax, Germany). The difference in volume was expressed as the volume of the foam. FC was then calculated using the following Eq. 1:

$$FC(\%) = \left( \frac{V_T}{V_0} \right) \times 100 \quad (1)$$

where  $V_T$  is total volume after whipping,  $V_0$  is the original volume before whipping.

## Emulsifying properties

The emulsifying activity index (EAI) was determined according to the method of Nalinanon [11] with slight modification. Freeze dried protein hydrolysate was diluted in distilled water to make 0.5% protein solution. Six ml of 0.5% protein solution was mixed with 2 ml soybean oil and then homogenized at 20,000 rpm for 1 min (IKA T25 digital Ultra Turrax, Germany). An aliquot of the emulsion (50µl) was pipetted from the middle portion of the container at 0 and 10 min after homogenization and subsequently diluted 100-fold using 0.1% sodium dodecyl sulphate (SDS) solution. The mixture was mixed thoroughly for 10 s using a vortex mixer (Stuart® SA7, Stone, Staffordshire ST15 0SA, UK).  $A_{500}$  of the resulting dispersion was measured using a spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan). EAI was calculated by the following Eq.2:

$$EAI (m^2/g) = (2 \times 2.303 \times A \times DF) / l \phi C \quad (2)$$

where  $A = A_{500}$ ,  $DF$  = dilution factor (100),  $l$  = path length of cuvette (m),  $\phi$  = oil volume fraction and  $C$  = protein concentration in aqueous phase ( $g/m^3$ );

## Solubility

Solubility was determined according to the procedure of Klompong [12] with slight modifications. To determine protein solubility, 200 mg of skipjack dark flesh hydrolysate samples were dispersed into 20 ml of deionized water and pH of the mixtures were adjusted to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 with 1 or 6 N HCl and 1 or 6 N NaOH. The mixtures were stirred at room temperature for 30 min and centrifuged (Thermo Scientific Heraeus Multifuge X1R Centrifuge, Germany) at 7500 g for 15 min. Protein contents in the supernatant were determined using Biuret method [13]. Total protein content in the sample was determined after solubilization in 0.5 N NaOH. Protein solubility was calculated as follows (Eq. 3):

$$Solubility(\%) = \frac{\text{protein content in supernatant}}{\text{total protein content in sample}} \times 100 \quad (3)$$

## Results and Discussions

### Foaming properties

Functionalities such as foaming properties are often affected by their solubility [14]. The foaming capacity result in this study is shown in Fig. 1. From the graph, it could be seen that in the range of acidic condition (pH 2-4), all treated samples had quite similar range of foaming capacity. However, FPH-C showed a slightly higher foaming capacity in acidic condition. Significant differences were clearly shown starting from higher pH of 6. At pH 6, the SDFPH-P had foaming capacity (118.33%) which was significantly lower ( $P < 0.05$ ) than that of SDFPH-A (140.00%) and FPH-C (149.17%). In the alkali condition, FPH-C had foaming capacity around 139.17- 148.33%. Meanwhile the foaming capacity of FPH-C was still stable with increasing pH (8-10), the foaming capacity of SDFPH-A had decreased significantly, even lower than the foaming capacity of SDFPH-P. In contrast, the foaming capacity of SDFPH-P was slightly increased when the pH was raised (pH 8-10). The results mostly suggested that an increase in surface activity, probably due to the initial greater number of polypeptide chains that arose from partial proteolysis, allowed more air to be incorporated [15]. This result also suggested that SDFPH-A had higher foaming capacity when it is used at a pH of 6 than SDFPH-P, however the SDFPH-P had more stable foaming capacity in pH changing than SDFPH-A. In a previous study we also found lower foaming capacity of FPC of Tilapia (*Oreochromis niloticus*) hydrolyzed with by Alcalase 2.4 L compared to FPC hydrolyzed with Flavourzyme and Neutrase [15]. The pH had a major effect on the foaming stability of hydrolysates [16]. The emulsion stability was also affected by pH range (unpublished data).

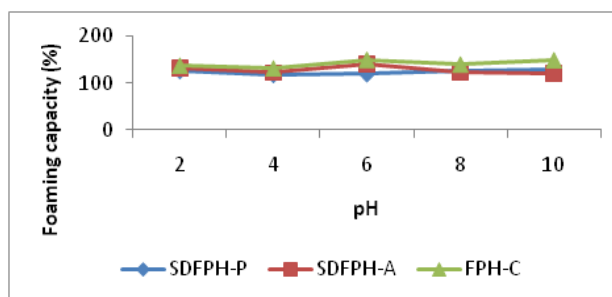


Figure 1. Foaming capacity (%) in different pH

### Emulsification properties

Emulsion activity index (EAI) is presented in Fig. 2. The graph (a) shows that EAI of all samples were slightly similar at pH 2 and 4. FPH-C showed slightly higher EAI ( $52.43 \text{ m}^2/\text{g}$ ) at pH 6 than that of SDFPH-P ( $36.19 \text{ m}^2/\text{g}$ ) and SDFPH-A ( $29.18 \text{ m}^2/\text{g}$ ). EAI increased with increasing pH. EAI of SDFPH-P was higher than that of SDFPH-A at pH 8 and 10. The trend for FPH-C was also similar to that of EAI with increasing of pH, but at pH 8 it had lower EAI than that of SDFPH-P and SDFPH-A. At pH 10, the EAI of FPH-C was quite similar with SDFPH-P. A relationship was found between solubility and emulsion properties, as hydrolysates with high solubility can rapidly diffuse and adsorb at the interface [12]. An increase of emulsification properties was possibly contributed by the increase in degree of hydrolysis and thus led to increase solubility [17]. The peptide profile of the final product is affected not only by the specificity of the enzymes, but also by the process conditions (such as temperature and pH), which greatly affect the enzymes' reaction kinetics [18]. Lower EAI at pH 4 of protein hydrolysates from yellow stripe trevally (*Selaroides leptolepis*) meat hydrolyzed by Alcalase 2.4L was also found by Klompong [12]. The emulsion stability of SDFPH-A, SDFPH-P and FPH-C were quite similar at pH 8-10 (unpublished data).

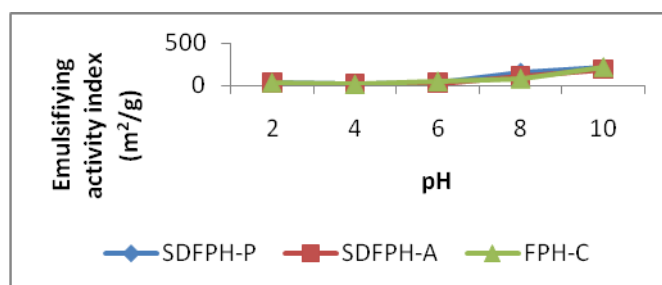


Figure 2. Emulsifying activity index/EAI of SDFPH-P, SDFPH-A and FPH-C

### Nitrogen solubility

The nitrogen solubility result is presented in Fig. 4. From this graph, it could be seen that the nitrogen solubility of SDFPH-P and SDFPH-A had the same trend and with similar range values at all pH observed. The nitrogen solubility of SDFPH-P and SDFPH-A were significantly higher ( $P < 0.05$ ) than that of FPH-C. Nitrogen solubility of SDFPH-P were 88.29-99.37% and SDFPH-A were 89.20-99.62% which were higher than that of FPH-C (57.61-63.02%). The lowest solubility of SDFPH-P and SDFPH-A were found at pH 4. It probably caused the lowest EAI of SDFPH-A and SDFPH-P at pH 4. At this pH the peptides could not move rapidly to the interface. Higher EAI of hydrolysates resulted in higher solubility, while lower EAI of hydrolysates resulted in lower solubility [12].

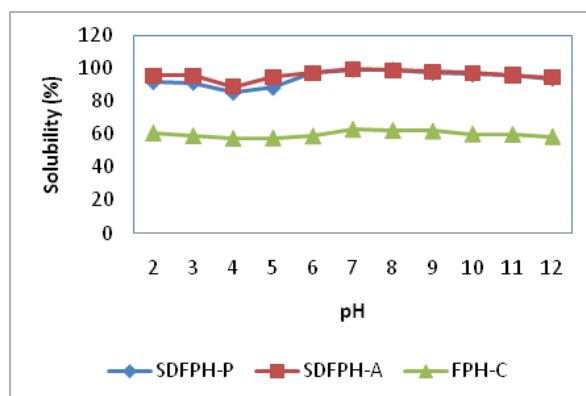


Figure 4. Nitrogen solubility (%) of SDFPH-P, SDFPH-A and FPH-C

## Conclusion

Protein hydrolysates have been obtained from skipjack tuna (*Katsuwonus pelamis*) hydrolysed by using Alcalase 2.4L®(SDFPH-A) and Protamex®(SDFPH-P). The foaming and emulsifying properties changed and varied depending on pH changes. Both SDFPH-P and SDFPH-A had an excellent solubility over a wide pH range. SDFPH-P and SDFPH-A had comparable WHC, but were still lower than that of FPH-C. Results of these protein functionality assessments indicated that skipjack tuna protein hydrolysed under the process conditions used in this work has potential as an ingredient in food systems.

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