Antioxidant activity of protein hydrolysate from snakehead fish (*Channa striata*) viscera obtained by enzymatic process

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Abstract. Snakehead fish (*Channa striata*) in South Sumatra, Indonesia has been widely used as a raw material in the processing of typical Palembang food industry, namely pempek, kemplang, and kerupuk. During its processing, not all parts of the fish can be utilized. In general, only 40% of the fish is used for consumption and 60% is wasted as by-products. One of the by-product produced during processing is viscera which can still be used as value-added products. One alternative is to use viscera as raw material in the production of fish protein hydrolysate (FPH) which is well known to have functional properties such as antioxidant, antibacterial, antihypertensive, and anticancer. This research was aim to determine the antioxidant activity of FPH made from snakehead fish viscera as a source of natural antioxidants. The research was conducted by papain treatments with the concentration of enzymes used were 1%, 2% and 3%. Evaluation of the hydrolysis process was done by measure the soluble protein and DH (degree of hydrolysis) while antioxidant activity was carried out by DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-Azinobis 3-ethyl benzothiazoline6-sulfonic acid) assay. DH of fish protein hydrolysate was found to be 68.79%, 77.06% and 72.63%, respectively while protein solubility gave values of 5.69%, 6.15% and 6.56% respectively. The highest antioxidant activity was attained at enzyme concentration of 3% with DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-Azinobis 3-ethyl benzothiazoline 6-sulphonic acid) value were 37.26% and 58.58% respectively.

1. Introduction
Snakehead fish (*Channa striata*) in South Sumatra, Indonesia has been widely used as a raw material in the processing of typical Palembang food industry, namely pempek, kemplang and kerupuk. During its processing, not all parts of the fish can be utilized. In general, only 40% of the fish is used for consumption and 60% is wasted as by-products [5]. One of the by-product produced during processing is viscera which can still be used as value-added products. One alternative is to use viscera as raw material in the production of fish protein hydrolysate (FPH) which is well known to have functional properties such as antioxidant, antibacterial, antihypertensive and anticancer [2]. Antioxidant compounds are important in neutralizing the potential damage caused by free radicals. Among the functional properties of protein hydrolysate, antioxidant properties are interesting to be explored. In
food industry, antioxidants are used to prevent oxidative damage that can cause rancidity, color, aroma changes and other physical damage [17].

Several studies have shown that FPH have potential as a good source of antioxidant peptides, as has been done by [21] hydrolysates from mackerel, Grass carp (*Ctenopharyngodon idella*) skin hydrolysate [19] and hydrolysate from salmon head (*Salmon salar*) [6]. Parameters such as enzyme, incubation time, temperature and controlled pH can affect FPH with the desired functional and nutritional value. Papain has been selected and reported as one of the most widely used protease enzymes to produce protein hydrolysates. This research was aimed to determine the antioxidant activity of FPH made from snakehead fish viscera by enzymatic process as a source of natural antioxidants.

2. Materials and methods

2.1 Materials

Materials and tools used in this research are blender, water bath, Erlenmeyer, beaker glass, spectrophotometer, centrifuge, shaker and hotplate. Snakehead fish by-product (viscera), Papain (Sigma-Aldrich 1,5-10 units/mg solid), NaOH, HCL, aquaedest, 1,1-diphenyl-2-picrylhydrazyl (DPPH) Sigma-Aldrich, 2,2-Acinobis 3-ethyl benzothiazoline 6-sulphonic acid (ABTS) Sigma-Aldrich, ethanol, Buffer acetate, TCA 20%, follin-ciocalteu, NaK-tartrate, NaCO3, CuSO4, Bovine serum albumin (BSA) (Sigma-Aldrich) and Trolox (Sigma-Aldrich).

2.2 Methods

Snakehead fish viscera obtained from typical food industry in Palembang. Frozen viscera was transported to the laboratory using Styrofoam and ice gel. This research was making hydrolysates with enzyme concentrations (1%, 2% and 3%). Evaluation of the hydrolysis process and antioxidant activity was carried out by DPPH assay, ABTS assay, Degree of hydrolysis (DH) and soluble protein.

2.3 Preparation of Fish Protein Hydrolysate

The protein hydrolysates of snakehead fish were prepared according to the method reported by [16] with modification. Viscera (50 g) were first cooked to inactivate endogenous enzymes. The cooked viscera sample was mixed with distilled water (1:1) and homogenized. The hydrolysis was performed under the following condition: pH=7, t=55°C. After the required digestion time the reaction was stopped by heating the solution at 80 °C for 20 min to inactivate the enzyme. The fish hydrolysate was centrifuged at 1900 x g for 20 min to separate insoluble and soluble fractions. Finally the soluble phase was freeze dried, samples were stored as hydrolyzed snakehead protein powder.

2.4. Determination of degree of hydrolysis

The determination of the degree of hydrolysis refers to [1]. 20 mL of protein hydrolysate was added with 20 mL of TCA 20% (w/v). The mixture was allowed to stand for 30 minutes for precipitation occur, then centrifuged (7,800 x g for 15 minutes). The supernatant was analyzed for nitrogen content using the Kjeldahl method.

2.5. Determination of protein solubility

The soluble protein content of snakehead fish protein hydrolysate was performed according to [11]. Sample (0,5 mL) was added 0,7 mL D solutions then vortex. The mixture was incubated for 20 minutes. Then added 0,1 mL E solutions, vortex and kept for 30 minutes. The resulting solutions was measured at 600 nm.

2.6. DPPH (1,1-diphenyl-2-picrylhydrazyl)

DPPH radical scavenging activity was determined according to the method of [8]. Sample (1,5 mL) was added with 1,5 mL of 0,1 mM DPPH in 95% ethanol. The mixture was incubated at room
temperature for 30 minutes in the dark. The resulting solution was measured at 517 nm. The control was prepared in the same manner except that distilled water was used instead of the sample.

2.7. ABTS (2,2-Azinobis 3-ethyl benzothiazoline 6-sulphonic acid)
ABTS radical scavenging activity of both enzymatic hydrolysates were determined by ABTS assay, as described by [4]. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. Sample (150 μl) was mixed with 2850 μl of ABTS solution and the mixture was left at room temperature for 2 hours in the dark. The absorbance was then measured at 734 nm using a spectrophotometer.

2.8. Statistical analysis
All experiments were performed in complete random design, the data were analyzed one-way ANOVA using statistical analysis software (IBM SPSS Ver 24).

3. Result and discussion

3.1. Proximate analysis
The proximate composition of snakehead fish are depicted in Table 1. Snakehead fish viscera in present study had lower protein but higher moisture and lipid than values reported for [15] 17.49%, 76.06% and 0.67% respectively. However, [22] documented higher protein (23%), lipids (5.7%) and ash (1.8%) in whole snakehead fish, respectively. These variations in proximate analysis might be due to the differences in part of fish, age, sex and variation in the level nutrition.

| Sample  | Moisture | Protein | Lipid | Ash  |
|---------|----------|---------|-------|------|
| Viscera | 80.05    | 5.60    | 3.20  | 0.75 |
| Fish meat | 70.41   | 20.38   | 1.66  | 1.15 |
| Head    | 72.68    | 14.29   | 0.62  | 1.58 |

3.2. Yield
Yield is one of the important values in product manufacture. Yield is the ratio of the dry weight product which produced by the weight of raw material. The yield results are illustrated in Figure 1. The yield was related to the DH, such as the highest yield (9.02±1.73%) was achieved at DH 77% with no significant difference. These results express that the maximum cleavage of peptides occurred during the first hour of hydrolysis. Our findings were in agreement with [7] who reported that hydrolysates prepared from the viscera of skipjack tuna has no significant differences to amount of yield from DH 16.71% to DH 19.71%.

![Figure 1. Yield of Snakehead fish hydrolysates prepared with different concentration of papain. Same letters indicate not significant differences (P>0.05).](image-url)
3.3. Degree of hydrolysis (DH)
Degree of hydrolysis (DH) is defined as the proportion of cleaved peptide bonds in a protein hydrolysate. Enzymatic hydrolysis of snakehead fish viscera was carried out under optimized pH and temperature conditions. The percent degrees of hydrolysis of snakehead fish viscera were 68.79%, 77.06% and 72.63% respectively (Figure 2). DH values were non-significant (P>0.05) at enzyme concentration 1%, 2% and 3%. DH increased when concentration increased, there was slight decrease in DH might due to some of the peptides released were highly so that increase amino acids and smaller peptides that existed in hydrolysate product when the concentration of papain increased [15]. According to [10] it was found that it has no significant effect on DH when increasing over the optimal enzyme concentration. It might be due to saturation of the reaction rate caused by enzyme aggregation.

![Figure 2. Effect degree of hydrolysis in different concentration. Same letters indicate not significant differences (P>0.05).](image)

3.4. Protein solubility
The protein content of FPH snakehead fish viscera was performed by calculating the absorbance of hydrolysate on standard absorbance using Bovine Serum Albumin (BSA). The concentration of protein solubility using snakehead fish viscera illustrated in Figure 3. The highest soluble protein concentration 6.56±0.815% was obtained from hydrolysis process for 3 hour with 3% papain was not significantly (P>0.05). Protein solubility increased when concentration increased, increase of protease concentration was associated with a quadratic increase insoluble nitrogen at fish protein hydrolysates [13]. These results were in agreement with the findings of [20] who reported that protein solubility level of WPH using biduri protease at various concentration and hydrolysis time ranged between 44.78 mg/ml to 65.90 mg/ml. The solubility protein values obtained from all treatments increased with increasing the concentration enzymes and the hydrolysis time.

![Figure 3. Effect protein solubility in different concentration. Same letters indicate not significant differences (P>0.05).](image)
3.5. **DPPH radical scavenging activity**

DPPH radical scavenging activity of snakehead fish protein hydrolysates at different concentrations. The protein hydrolysates resulting from the enzymatic hydrolysis process showed that the radical scavenging activity increased significantly (P<0.05). Percent inhibition of DPPH from 27.41%-37.26% for 3 hours reaction time was shown in Figure 4. As the DH increased, DPPH radical scavenging activity increased when the enzyme concentration increased from 1% to 3% (P<0.05). Our findings were in agreement with previous works reported by [9] and [3], who reported that the DPPH scavenging activity increased with increasing concentrations. Compared to FPH from the viscera of skipjack tuna [7], the antioxidant activity of FPH from snakehead fish viscera was still higher. The size of peptides and the composition of free amino acid affected the antioxidant activity of FPH. During hydrolysis, small peptides and free amino acids were formed, depending on enzyme specificity. Antioxidative activity might be affected by size, level, composition of free amino acids and small peptides.

![Figure 4. Antioxidant activities of protein hydrolysates at different concentration. Different letters indicate significant differences (P<0.05).](image)

3.6. **ABTS radical scavenging activity**

In our study, the peptides from snakehead fish viscera presented free radical scavenging activity and the results were shown in Figure 5. ABTS activity increased significantly (P<0.05) when concentration increased. Percent inhibition of ABTS radical cation were 46%, 52% and 58% respectively. The highest activity of FPH from snakehead fish viscera with 3% enzyme concentration is supported by higher DH and the generated peptides might be varying in terms of amino acid composition, amino acid sequence etc. Antioxidant activity were 46%, 52% and 58% was substantially lower than in previous reports by [18] showed that protein hydrolysate from porcine liver using papain with percent inhibition of ABTS ranged 37.67%-70.63%. The low percent inhibition might be attributed to various factors such as the enzyme, DH, solubility of hydrolysates and existence of free amino acids [12].

![Figure 5. Antioxidant activities of protein hydrolysates at different concentration. Different letters indicate significant differences (P<0.05)](image)
4. Conclusion
FPH was successfully prepared from Snakehead fish viscera using papain. Good antioxidant activities could be achieved by papain hydrolysis at enzyme concentration of 3%. The snakehead fish protein hydrolysate could offer antioxidant properties, thereby expanding its application in industry. However, for better result the next research will increase enzyme percentage and pharmaceuticals application should be further studied.

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