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Antioxidant activity of skin and bone collagen hydrolyzed from striped catfish (*Pangasius pangasius*) with papain enzyme

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ABSTRACT

To produce bioactive peptides from collagen was hydrolyzed from Striped Catfish (Pangasius pangasius) using protease (papain enzyme) and the peptides were evaluated for antioxidant activity. The degree of hydrolysis (DH), DPPH radical-scavenging activity, and reducing power of the peptides were investigated. Papain enzyme was further used to produce collagen peptides with different time of hydrolysis. Within 160 min of hydrolysis, the maximum cleavage of peptide bonds from fish skin and fish bone occurred were found with DH 4.57% and 1.75%, respectively. Collagen peptide from fish skin and fish bone exhibited the highest antioxidant activity after 160 min incubation. DPPH radical scavenging activity of collagen hydrolysate from fish bone was higher (71.55%) than that of these hydrolysed collagen from fish skin (63.06%). However reducing power activity of the collagen peptide hydrolysed from fish skin (0.817) was higher than that of the collagen peptide hydrolysed from fish bone (0.788). Therefore, papain enzyme could be used to produce the collagen peptides possessing antioxidative activities.

Keywords: Antioxidant, collagen, DPPH, Reducing power

INTRODUCTION

Enzymatic hydrolysis is widely applied to improve and upgrade the functional and nutritional properties of food proteins [1]. Enzyme from different sources are commonly used to obtain a more selective hydrolysis since they are specific for peptide bonds adjacent to certain amino acid residues [2].

Numerous peptides derived from hydrolyzed food proteins have been shown to have antioxidative activities. Fish protein hydrolysate such as skin gelatin hydrolysate from Alaska Pollack [3], yellow fin sole [4], and Alaska Pollack [5], have been reported to exhibit antioxidative activity. Moreover, preliminary data suggest that hydrolysated fish protein could represent an interesting source of anticancer peptides [6], angiotensin I-converting enzyme (ACE) inhibitors [7], anti anemia agent [8], and component of microbial growth media [9]. However, there is a little information regarding collagen peptide from Striped Catfish (*Pangasius pangasius*) and their antioxidative activity.

EXPERIMENTAL SECTION

Materials

DPPH (2,2-diphenyl-2-picrylhydrazyl) and all solvents used were of analytical grade and purchased from Sigma chemical (St. Louis, MO, USA). Striped Catfish (*Pangasius pangasius*) purchased from local market (Palembang).

Preparation of skin and bone collagen hydrolysate

Fish collagen was prepared from skin and bone from Striped Catfish (*Pangasius pangasius*). To remove non-collagenous proteins, the skin and bone fish was mixed with 0.1 mol/L NaOH at a solid to alkali solution (NaOH) ratio of 1:10 (w/v), followed by continuous stirring for 8 h using an overhead stirrer. The alkali solution was

changed every 2 h. Pretreated skin fish was soaked in 1.5% acetic acid with a solid to solvent ratio 1:2 (w/v) for 24 h. Skin was washed with cold water until neutral pH, followed by extraction with aquades with a solid to solvent ratio of 2:1 (w/v) for 3 h at 50^0 C. Collagen solutions were incubated at optimal temperature for proteolytic activity of each species for 10 min. Papain enzyme was added into the mixtures. At hydrolysis time designated (0, 15, 30, 60, and 90 min).

Degree of hydrolysis

The degree of hydrolysis was estimated according to the method established by Hoyle and Merritt [10]. To the supernatant, one volume of 20% trichloroacetic acid (TCA) was added, followed by centrifugation at 10000 rpm at 4°C for 10 min to collect the 10% TCA-soluble materials. Total nitrogen in the 10% TCA soluble material and the substrate was estimated by Kjeldahl method using Kjeltec protein analyzer. The degree of hydrolysis (DH) was calculated as follows:

$$\%DH = 100 \times [(10\%\ TCA - Soluble\ nitrogen\ in\ sample) / (Total\ nitrogen\ in\ sample)]$$

DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured based on methods described in Hanani *et al*. [11]. DPPH solution concentration used was 1 mM. The solution used in fresh condition and protected from light. A total of 4.5 ml of test solution included in test tube is then reacted with 0.5 ml of DPPH solution. Test tube is covered with aluminum foil and incubated at 37°C for 30 minutes then the absorbance was measured using a UV-Vis spectrophotometer at length wave 517 nm.

The antioxidant activity of each sample was expressed in percentage inhibition of free radicals which is calculated by the formula:

$$\%\ Inhibition = \frac{blank\ absorbance - sample\ absorbance}{blanko\ absorbance} \times 100\%$$

Reducing power

Reducing powerwas determined by the method of Oyaiza [12]. The sample solution (0.5 ml, 40 mg protein/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6)and 2.5 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 ^{0}C for 20 min. An aliquot (2.5 ml) of 10%trichoroacetic acid was added to the mixture, followed by centrifugation at 700 g for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 2.5 ml of 0.1% (w/v) ferric chloride and the absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicates increasing reducing power.

RESULTS AND DISCUSSION

Degree of hydrolysis

Research efforts have been focused on the generation of bioactive peptides from a myriad of food sources, including collagen, envisaging potential utilization by the food industry. In particular, investigations have been carried out to obtain bioactive peptides through the hydrolysis of meat and fish [3-4, 13]. In the current study, the biological activities of collagen hydrolysates were investigated, on which there are relatively few studies in the literature. The progression in DH during the hydrolysis of by papain enzyme shown in Fig. 1.

Degree of hydrolysis (DH), which indicates the percentage of peptide bonds cleaved [14]. The degree of hydrolysis (DH) measures the content of peptide bonds cleaved in the substrate by a proteolytic agent (papain, in the current case): the higher the DH, the higher the content of released amino groups. The DH value increased during hydrolysis time, reaching 4.57% for collagen hydrolysate from fish skin and 1.75% for collagen hydrolysate from fish bone in 160 min, which are similar to DH of tuna backbone protein by α-chymotripsin, neutrase and papain [5].

DPPH radical scavenging activity

Peptides obtained from the proteolysis of various food proteins, are reported to possess antioxidant activities. Antioxidant mechanisms include radical-scavenging(both hydrogen-donating capability and free radical quenching)activity, inhibition of lipid peroxidation, metal ion chelation, or acombination of these properties [15]. Antioxidant activities might protect biological systems against damage related to oxidative stress in human disease conditions. These antioxidant peptides might also be employed in preventing oxidation reactions (such as lipid peroxidation) that leads to deterioration of foods and foodstuffs [16]. Fish protein hydrolysates with antioxidant activity obtained by enzymatic hydrolysis have been reported. Functional foods with such natural antioxidants are

interesting since they can be potentially employed without toxic side effects associated with the use of synthetic equivalents. Also, antioxidants from protein hydrolysates might confer nutritional value besides functional/physiological properties, which are additional advantages over the synthetic counter parts [17-18].

DPPH radical scavenging activities of fish collagen with different time of hydrolysis and source of papain depicted in Fig. 1. The collagen peptide from skin exhibited the highest activity (63.06%) after 160 min incubation and collagen peptide from fish bone exhibited the highest activity (71.55%) after 160 min incubation.DPPH radical scavenging activitiy of collagen hydrolysate from fish bone was higher (71.55%) than that of these hydrolysed collagen from fish skin (63.06%) (Fig. 2).

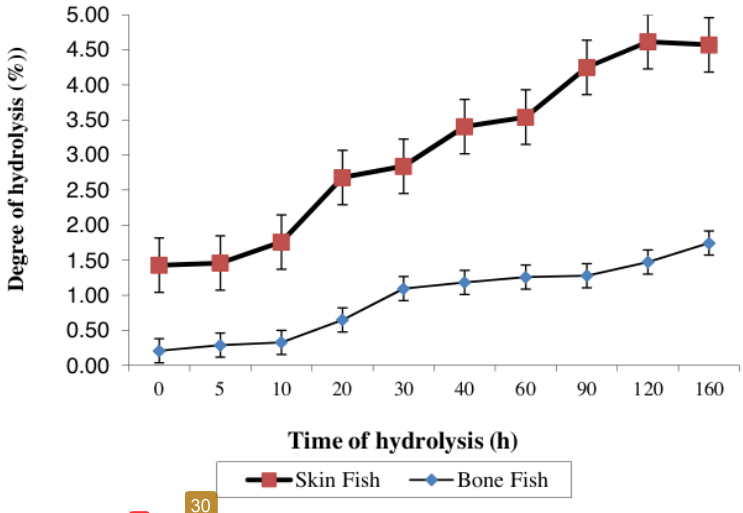

Figure 1. Degree of hydrolysis of collagen peptide

Bars represent the standard deviation from triplicate determinations

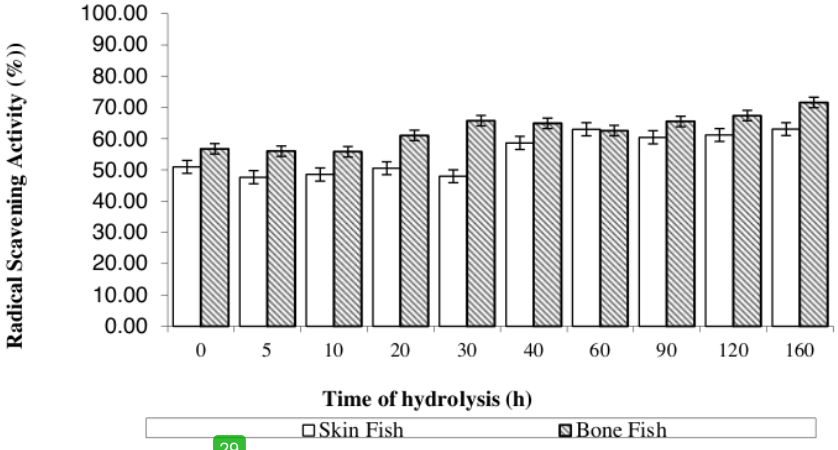

Figure 2. DPPH radical scavenging of collagen peptide

Bars represent the standard deviation from triplicate determinations

DPPH is a stable free radical that shows maximal absorbance at 517 nm in ethanol. When DPPH encounters a proton-donating substance, such as an antioxidant, the radical is scavenged. The color is changed from purple to yellow and the absorbance is reduced [19]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability [20]. DPPH radical scavenging activities were found in protein hydrolysates

derived from round scad (*Decapterusmaruadsi*) and yellow stripe trevally (*Selaroidesleptolepis*) by Alcalase and Flavourzyme [21-22].

Proteolysis of food proteins is usually reported to enhance the DPPH-scavenging activity of hydrolysates [23]. (Phelan *et al*., 2009).The DPPH-scavenging activity of yak milk protein hydrolysates obtained with Alcalase was observed to increase during the hydrolysis process for up to7 h [24]. Nevertheless, this is not always observed [25]. Specifically, bovine casein hydrolysates obtained with diverse proteolytic enzymes were shown to possess lower DPPH activity than the whole protein [26].

Collagen, both hydrolyzed and non-hydrolyzed contain some molecular part which act as electron donors that could react with free radicals, converting them into more stable molecules and terminating the radical chain reaction. His, Phe, Tyr, Trp, among other aromatic and hydrophobic amino acids, seem to be involved in the antioxidant activity of protein hydrolysates [15, 23].

Reducing power

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron or hydrogen [27]. In this assay, the ability of a compound to reduce the Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). Fig 3 shows the reducing power activities (as indicated by the absorbance at 700 nm) of the collagen peptide hydrolysed from skin and bone from Stiped Catfish (*Pangasius pangasius*).

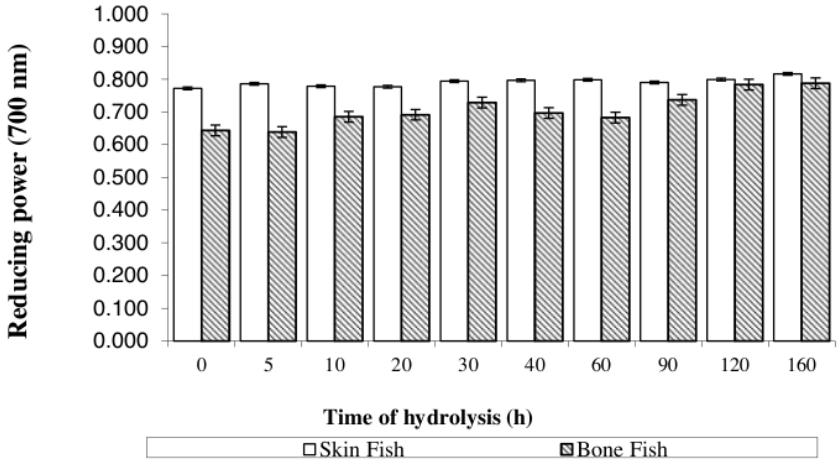

Figure 3. Reducing power of collagen peptide
Bars represent the standard deviation from triplicate determinations

The collagen peptide hydrolysed from skin and bone fish exhibited the highest activity at time of hydrolysis of 160 min. The reducing power activities of collagen hydrolysate from fish skin was higher (0.817) than that of these hydrolysed collagen from fish bone (0.788). Consequently, the reducing ability of collagen peptide indicates that they could act as electron donors, reducing the oxidized intermediates of lipid peroxidation processes, and suggesting that the reducing power likely contributes to the antioxidant activity [1]. At a similar concentration, wheat germ protein isolates treated with Alcalase showed a reducing power comparable to that of 60 min peptide of ovine collagen hydrolyste [1]. On the other hand, the proteolysis of porcine hemoglobin resulted in decreased reducing power compared to the intact protein [25].

CONCLUSION

Bioactive peptide from fish skin and fishbone collagen were produced using papain enzyme (protease). Peptides collagen exhibited DPPH scavenging, and reducing power activity.

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