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Collagen Hydrolysis from skin and bone of Pangasius catfish Prepared by Bromelain Enzyme and Antioxidant Activity of Hydrolysate

Ace Baehaki*, Shanti Dwita Lestari and Ida Desliani

Study Program of Fisheries Product Technology, Faculty of Agriculture, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatera, Indonesia

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ABSTRACT

The purpose of the research was to produce collagen hydrolysate from pangasius catfish skin and bone using bromelain enzyme. The resulted collagen peptides were expected to have antioxidative activity. Five different enzyme concentration ranged from 1-5% and different hydrolysis time (0, 5, 10, 20, 30, 40, 60, 90, 120 and 160 min) were used in the experiment. The parameters observed were yields of both skin and bone collagen, degree of hydrolysis and antioxidative activity (DPPH and reducing power). The result showed that collagen hydrolysate prepared by bromelain enzyme has antioxidative activity. The yield for both skin and bone collagen hydrolysate was 45.84% and 12.86% respectively. The highest degree of hydrolysis skin collagen hydrolysate was 2.58% at 160 minutes whereas the bone collagen hydrolysate had the highest degree of hydrolysis was 2.97% at 160 minutes. Based on the DPPH method skin and bone collagen hydrolysate had the antioxidative activity of 61.67% and 71.83%. In the other hand the reducing power method skin and bone collagen hydrolysate had the antioxidative activity of 0.35 and 0.38.

Keywords: *Antioxidant, collagen, bromelain, DPPH, reducing power*

INTRODUCTION

Bromelain is proteolytic enzyme found in pineapple plant [1]. The name bromelain was first applied to the fruit enzyme. Later, the term 'bromelain' was introduced and originally applied to any protease from any plant member of the plant family Bromeliaceae [2].

Several fish skin collagens were hydrolyzed with proteolitic enzyme and hydrolysates have potent antioxidant such as striped pangasius catfish skin by papain enzyme [3] and tuna skin by alcalase [4]. However, there is a little information regarding collagen peptide from Pangasius catfish (*Pangasius pangasius*) with bromelain enzyme and their antioxidative activity.

MATERIALS AND METHODS

Preparation of skin collagen

Fish collagen was prepared from skin and bone from Pangasius 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 of 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.

Yield of extraction
The extraction yield is ratio the mass extraction in mg with mass of sample in mg,

Y (%) = 100 Mext / Msamp.

Where in: Y is the% yield; Mext is the mass of the extract in mg and the Msamp is dry mass of the organ sample in mg.

Degree of hydrolysis
Collagen solutions were incubated at optimal temperature for proteolytic activity of each species for 10 min. Bromelain enzyme was added into the mixtures. At hydrolysis time designated (0, 5, 10, 20, 30, 40, 60, 90, 120 and 160 min).

The degree of hydrolysis was estimated according to the method established by Hoyle and Merritt [5]. The degree of hydrolysis (DH) was calculated as follows:

%DH= 100 x [(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*. [6]. A total of 4.5 ml of test solution included in a 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.

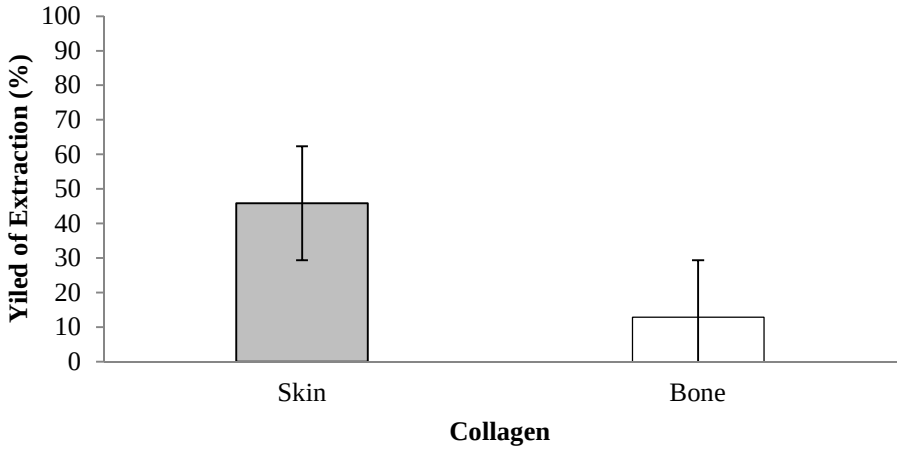
The antioxidant activity of each sample was expressed in percentage inhibition of free radicals which is calculated by the formula:

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

Reducing power
Reducing power was determined by the method of Oyaiza [7], the absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicates increasing reducing power.

RESULTS AND DISCUSSION

Yield of extraction
Extraction yield (w/w) for collagen from skin and bone fish found to be in order of 45.83 and 12.86%, respectively. Extraction yield of collagen from skin fish was higher than that of these collagen from bone fish (Fig. 1)



Degree of hydrolysis
The progression in DH during the hydrolysis of by bromelain enzyme shown in Fig. 2.

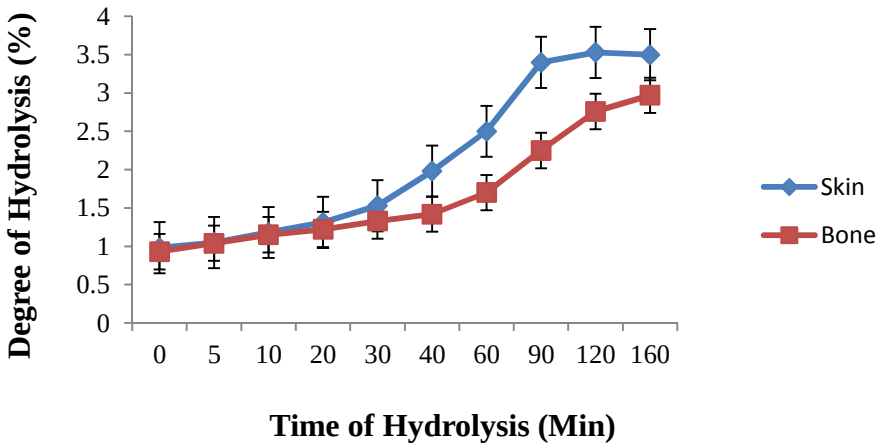


Figure 2. Degree of hydrolysis of collagen collagen hydrolysate from Skin and bone of Pangasius catfish by bromelain enzyme
Bars represent the standar deviation from triplicate determinations

Degree of hydrolysis of collagen hydrolysate from fish skin was higher than that of these hydrolysed collagen from fish bone (Fig. 1). Increasing of degree of hydrolysis linearly with time of hydrolysis. Degree of hydrolysis collagen from *Chanos chanos* treated with collagenase increased linearly with incubation time [8].

DPPH radical scavenging activity

DPPH radical scavenging activities of fish collagen with different time of hydrolysis and source of bromelain depicted in Fig. 3. The collagen peptide from skin exhibited the highest activity (61.67%) after 60 min incubation and collagen peptide from fish bone exhibited the highest activity (71.83%) after 120 min incubation (Fig. 3).

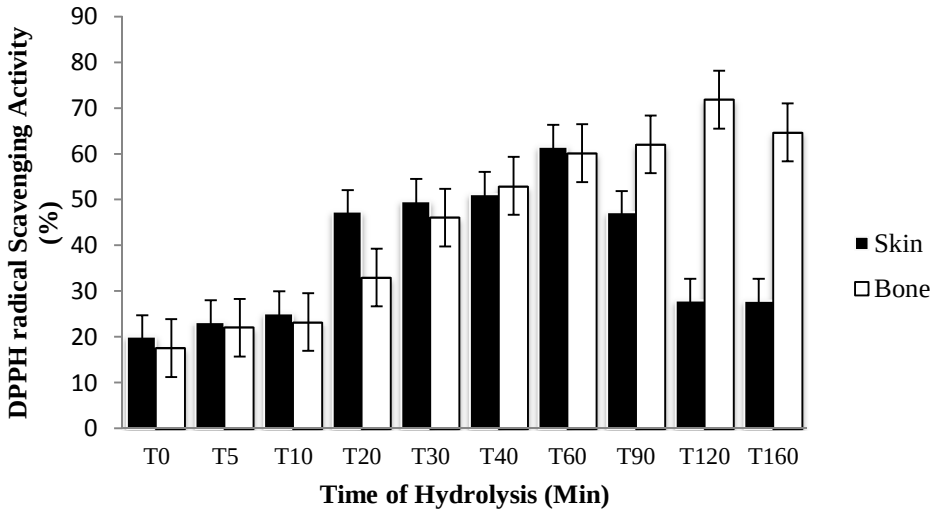


Figure 3. DPPH radical scavenging activity of collagen hydrolysate from Skin and bone of Pangasius catfish
Bars represent the standar deviation from triplicate determinations.

DPPH radical scavenging activities of fish collagen with different time of hydrolysis depicted in Fig. 1. DPPH radical scavenging activitiy of collagen hydrolysate from fish bone was higher (71.83%) than that of these hydrolysed collagen from fish skin (61.67%). Hydrolysate from skin fish can reduce the DPPH radical from 20,03% to 61.67% and the hydrolysate had the highest antioxidant activity by scavenging DPPH radical, which was 61.67% at the time of hydrolysis was 60 min. Hydrolysate from bone fish also can reduce the DPPH radical from 17.52% to 71.83% and the hydrolysate had the highest antioxidant activity, which was 71.83% at the time of hydrolysis was 120 min.

Reducing power

Fig 4 shows the reducing power activites (as indicated by the absorbance at 700 nm) of the collagen peptide hydrolysed from skin and bone from Pangasius catfish (*Pangasius pangasius*).

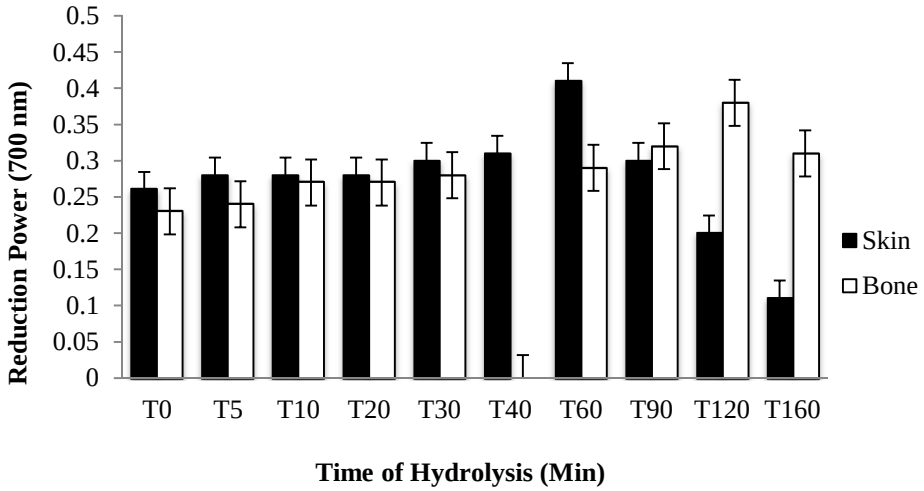


Figure 4. Reducing power of hydrolysate from Skin and bone of Pangasius catfish
Bars represent the standar deviation from triplicate determinations.

The collagen peptide hydrolysed from skin and bone fish exhibited the highest activity at time of hydrolysis of 60 min.the reducing power activites of collagen hydrolysate from fish skin was higher (0.41) than that of these hydrolysed collagen from fish bone (0.38). 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 [9].

CONCLUSION

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

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