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CHARACTERISTICS OF COLLAGEN HYDROLYSATE FROM PANGASIUS (*Pangasius* sp.) SKIN USING CRUDE BROMELAIN EXTRACTED FROM PINEAPPLE CORE

KARAKTERISTIK HIDROLISAT KOLAGEN KULIT IKAN PATIN (*Pangasius* sp.) MENGGUNAKAN ENZIM BROMELAIN KASAR DARI BONGGOL NANAS

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ABSTRACT

Pangasius (*Pangasius* sp.) skin, a by-product of fillet processing, is a potential alternative source of collagen. This study investigated the effect of varying concentrations of crude bromelain enzyme, extracted from pineapple core, on the characteristics and antioxidant activity of Pangasius skin collagen hydrolysate. Collagen was extracted and hydrolyzed from Pangasius skin using this enzyme. The activity unit and specific activity of the crude bromelain enzyme were 3.10 ± 0.32 U/mL and 24.04 ± 2.23 U/mg, respectively. The pH of the resulting collagen hydrolysate ranged from 5.57 ± 0.08 to 6.43 ± 0.04, the degree of hydrolysis ranged from 50.37 ± 2.95 to 66.03 ± 7.77, and solubility ranged from 76.7 ± 0.79 to 79.5 ± 2.23. The optimal enzyme concentration was determined to be 3%, yielding collagen hydrolysates with a low molecular weight (15.9–11.6 kDa) and very strong antioxidant activity (IC_{50} value of 33.99 µg/mL). Analysis of functional groups revealed the presence of Amide A, B, I, II, and III peaks. The ratio of the Amide III peak to the C-H group bending vibration peak was 0.91, indicating that the collagen hydrolysate retained the triple-helix structure and had not been denatured into gelatin.

Keywords: business feasibility, development strategy, fish processing MSMEs, smoked catfish

ABSTRAK

Salah satu sumber alternatif kolagen yang cukup potensial dapat berasal dari produk sampingan produk filet yang berupa kulit ikan patin (*Pangasius* sp.). Tujuan penelitian ini adalah untuk penentuan pengaruh konsentrasi dari enzim bromelin kasar hasil ekstrak dari bonggol nanas terhadap karakteristik dan aktivitas antioksidan hidrolisat kolagen kulit ikan patin. Penelitian ini menggunakan metode ekstraksi dan hidrolisis kolagen dari kulit ikan patin. Unit aktivitas dari enzim dan aktivitas spesifik enzim bromelin kasar dari bonggol nanas masing-masing bernilai 3,10 ± 0,32 U/mL dan 24,04 ± 2,23 U/mg. Kadar pH dari hidrolisat kolagen kulit berasal dari ikan patin yang dihidrolisis dengan menggunakan enzim yang berasal bromelin kasar bonggol nanas berkisar 5,57 ± 0,08 sampai 6,43 ± 0,04; derajat hidrolisis berkisar 50,37 ± 2,95 sampai 66,03 ± 7,77, dan kelarutan berkisar 76,7 ± 0,79 sampai 79,5 ± 2,23. Konsentrasi terbaik yang didapatkan berada pada 3%, dari hasil hidrolisis enzimatis kolagen dengan enzim bromelin kasar dan menghasilkan hidrolisat kolagen dengan bobot molekul rendah (15,9–11,6 kDa), serta nilai IC_{50} aktivitas antioksidan tergolong sangat kuat (33,99 µg/mL). Puncak Amida A, B, I, II, dan III dihasilkan dari analisis gugus fungsi hidrolisat kolagen. Nilai rasio puncak Amida III dan puncak getaran pembengkokan gugus C-H adalah 0,91, yang mengindikasikan hidrolisat kolagen mengandung struktur *triple-helix* dari kolagen dan belum mengalami denaturasi menjadi gelatin.

Kata kunci: enzim bromelin, hidrolisat kolagen, kulit ikan patin, nanas

INTRODUCTION

The consumption of Pangasius (*Pangasius* sp.) and processed Pangasius products has become increasingly diverse and in demand due to changing lifestyles. Pangasius is commonly sold either fresh or as fillets. Vietnam, recognized as the world's leading producer of Pangasius, reported significant increases in exports of frozen whole Pangasius fillets in January 2021, with growth rates of 54% and 16%, respectively (GSO 2021). Similarly, Indonesia experienced an increase in Pangasius aquaculture production, reaching 348,379 tons in 2024 (KKP 2022). The fillet form is preferred because it is easy to process and suitable for whole consumption. Pangasius fillets are value-added products produced by separating the flesh from bones, skin, and other unwanted parts, followed by frozen storage (Rathod *et al.* 2018). This process generates Pangasius skin as a by-product.

Fish are a source of type 1 collagen, the most abundant protein in the human body (Subhan *et al.* 2017). Fish skin is the best source of collagen among other fish by-products (Rajabimashhadi *et al.* 2023). Extracted collagen lacks bioactivity, and its bioactive properties can be achieved through enzymatic hydrolysis (Ahmed *et al.* 2020). Collagen hydrolysates have a relatively low molecular weight but high biological activity (Leon-Lopez *et al.* 2019). Bromelain is a protease enzyme that has been widely used in fish collagen hydrolysis. The bromelain enzyme produces an umami taste in the hydrolyzed product, which can suppress the bitter taste (Rhyu and Kim 2011). The results of collagen hydrolysis from milkfish skin with the bromelain enzyme showed high ability to capture free radicals, so it has high antioxidant properties, namely 80.17 ± 0.18% DPPH radical scavenging activity (Hartina *et al.* 2019). Research conducted by Coscueta *et al.* (2021) also reported the cod hydrolysis with a 0.5% bromelain enzyme, which produced an antioxidant activity of 514 µmol Trolox Equivalent/g protein with the ORAC (Oxygen Radical Absorbance Capacity) test. Based on Yanti *et al.* (2022), the collagen antioxidant activity derived from Pangasius skin using acid extraction and the addition of bromelain enzyme was 20.45 ferrous sulfate/g.

Crude enzymes extracted from these sources can be a low-cost alternative and have the potential for further research into their effects on enzymatic hydrolysis. Several previous studies have reported that collagen derived from Pangasius skin (Atma *et al.* 2021), sturgeon skin (Hou and Chen 2023), salmon skin (Vazquez *et al.* 2021), and yellowfin tuna skin (Nguyen *et al.* 2021) has high quality for development into collagen hydrolysate products with good bioactivity. This study focuses on the characteristics of collagen hydrolysates and aims to analyze the effect of crude bromelain concentration from pineapple cores on the characteristics and antioxidant activity of the hydrolysate of Pangasius skin collagen.

METHODS

Materials and tools

The key component for generating collagen is derived from Pangasius skin, sourced from PT Adib Global Food. For collagen extraction, the materials used include NaOH (Merck KGaA, Germany), distilled water, and CH_3COOH (Central Kimia Store, Bogor, Indonesia). Crude enzyme extract from pineapple cores was prepared using corms purchased from local markets in Bogor City, West Java. The degree of hydrolysis test employed Trichloroacetic Acid (20% TCA). The molecular weight of the collagen hydrolysate was assessed using SDS-PAGE, which involves separating and stacking gels, Coomassie Brilliant Blue (CBB), and buffer solutions. Antioxidant activity was determined using the ABTS test with 2.2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) and $K_2S_2O_8$, with ethanol serving as blanks.

This research utilized a variety of tools including cutting instruments, electric stoves, ovens, blenders, knives, spoons, Erlenmeyer flasks (from Pyrex, Asahi Glass, Thailand), measuring cups (also from Pyrex, Asahi Glass, Thailand), water bath shakers (Depolab, Seoul, Korea), centrifuges (VWR Mega Star 1.6 General), and digital scales. pH measurements were conducted using a pH meter (WalkLAB HP9010, Singapore). SDS-PAGE analysis was performed using a shaker and a spectrophotometer. The assessment of antioxidant activity was carried out using a microplate reader (Labsystems, Multiscan Ex and Champaign, USA).

Research procedures

This research encompassed multiple phases within the methodology. The initial phase involved the extraction of crude bromelain enzyme from pineapple cores sourced from a local market in Bogor City, West Java. The extraction process for the crude bromelain enzyme was adapted from the study conducted by Ramli and Munir (2022). Pineapple cores were cleaned and blended using an electric mixer to

obtain crude bromelain extract. The resulting pineapple extract underwent centrifugation at 4,000 rpm for 30 minutes to eliminate insoluble materials, after which the supernatant was retained as the crude bromelain extract.

Collagen was isolated from Pangasius skin and subsequently hydrolyzed. The skin was cleaned, cut into approximately 1×1 cm pieces, and soaked in 0.1 M NaOH for 12 h at 4 °C to remove non-collagenous proteins. The samples were rinsed with tap water until neutral, then soaked in 0.1 M acetic acid for 2 h at 4 °C, followed by washing.

Collagen extraction was performed using distilled water at 40 °C for 6 h. The extracted collagen solution was hydrolyzed using crude bromelain extract following a modified method of Hartina *et al.* (2019). Enzyme concentrations of 1%, 2%, and 3% (v/v) were applied, and the mixtures were incubated at 40 °C for 90 min. Enzyme activity was terminated by heating at 80 °C for 10 min, followed by cooling at room temperature. The resulting hydrolysate was dried at 40 °C for 24 h.

Enzyme activity was determined according to Maryam *et al.* (2019) using 1% casein in 0.05 M Tris-HCl buffer (pH 8). The reaction mixture was incubated at 37 °C for 20 min after pre-incubation, terminated with 10% TCA, and centrifuged at 5,000 rpm for 20 min. The absorbance of the supernatant was measured at 280 nm.

The degree of hydrolysis was determined following Nurilmala *et al.* (2020). Collagen hydrolysate (20 mL) was mixed with 20% (w/v) TCA, incubated for 30 min, and centrifuged at 6,000 × g for 30 min. The nitrogen content of the supernatant was analyzed using the Kjeldahl method.

For pH measurement, 1 g of dried hydrolysate was dissolved in 20 mL of distilled water and measured using a digital pH meter (Nurilmala *et al.* 2019). Solubility was determined following Sun *et al.* (2017) by dissolving 0.5 g of the sample in 5 mL of distilled water, centrifuging at 32,000 × g (22 °C, 10 min), drying the supernatant at 130 °C, and weighing the residue.

Functional groups were analyzed using FTIR (IRPrestige-21, Shimadzu, Japan) following Ahmad *et al.* (2010). Samples were mixed with KBr (1:9) and scanned over 400–4,000 cm^{-1} at 2 cm^{-1} resolution. Molecular weight distribution was determined using SDS-PAGE (Nurilmala *et al.* 2020) with 3% stacking gel and 15%/17.5% separating gels. Samples were treated with SDS, heated, centrifuged, loaded, and electrophoresed at 13 mA and 100 V for 3 h. Gels were stained with Coomassie Brilliant Blue, destained, and analyzed. Antioxidant activity was evaluated using the ABTS radical scavenging assay (Sae-leaw and Benjakul 2018). ABTS solution (7.4 mM) was reacted with 2.6 mM potassium persulfate and incubated in the dark for 18 h, then diluted to an absorbance of 1.1 ± 0.05 at 405 nm. The sample (100 µL) was mixed with ABTS solution (200 µL), incubated for 15 min, and measured at 405 nm.

Data analysis

The concentration of crude bromelain enzyme (1%, 2%, and 3% v/v of the collagen solution volume) was the only variable in this study's completely randomized design (CRD). Three replicates were conducted for each treatment. After analyzing the data using analysis of variance (ANOVA) with a 95% confidence level (α = 0.05), Duncan's test was performed.

RESULTS AND DISCUSSION

Crude bromelain enzyme activity of pineapple core

This enzyme's ability to cleave peptide bonds at the carbonyl group is made possible by the presence of cysteine and histidine groups in its active site (Masri 2013). Pineapple core has less bromelain with an optimal pH range of 3–8 and an isoelectric point of 4.6 (Maurer 2001). The proteolytic activity of crude bromelain extracted from pineapple cores is strongly influenced by the degree of ripeness (Dzulqaidah *et al.* 2021). Table 1 displays the crude pineapple core extract's enzyme activity.

Table 1. Enzyme activity and specific activity of crude bromelain from pineapple core.

Samples	Activity Units (U/mL)	Specific Activities (U/mg)
Crude bromelain from pineapple cores*	3.10 ± 0.32	24.04 ± 2.23
Crude bromelain from pineapple fruit[1]	5.37	0.52
Bromelain powder from pineapple stem[2]	43.09 ± 0.01	26.02 ± 0.003

Note: *Research data, [1]Wuryanti (2004), [2]Sari dan Zaini (2022)

With a value of 3.10 ± 0.32 U/mL, the crude bromelain enzyme activity unit derived from pineapple core was comparatively low. The specific activity value (24.04 ± 2.23 U/mg) of pineapple core bromelain was comparable to that of the enzyme. Temperature, pH, and extraction technique can all affect bromelain enzyme activity. Enzyme hydrolysis was carried out in this extraction procedure at a pH of 5.0 and a temperature of 40 °C. As a result, the enzyme activity value was low. The activity of the bromelain enzyme increased with increasing temperature. Wuryanti (2004) stated that the number of activity units per milligram of protein is a measure of an enzyme's purity.

Degree of hydrolysis of collagen hydrolysate

Using a 20% TCA solvent, the total dissolved nitrogen in the sample was calculated to determine the degree of hydrolysis. The average degree of hydrolysis of Pangasius skin collagen hydrolysate ranged from 37.28 to 66.03 (Figure 1).

ANOVA analysis showed that enzyme concentration significantly affected the DH values ($P < 0.05$). The treatment with 1% bromelain resulted in the highest DH (66.03%), whereas the control (without enzyme) showed the lowest value (37.28%). Interestingly, increasing enzyme concentration (2% and 3%) resulted in a decrease in DH.

This phenomenon may be attributed to enzyme saturation and substrate limitation, where excessive enzyme concentration does not correspond to increased hydrolysis due to limited available substrate. In addition, high enzyme concentrations may promote aggregation or competition among enzyme molecules, reducing their effective interaction with the substrate. Another possible explanation is product inhibition, in which the accumulation of smaller peptides interferes with enzyme activity, thereby lowering the overall degree of hydrolysis.

The DH values obtained in this study were higher than those reported by Azizah (2020), who observed a DH of 49.76% for bromelain-hydrolyzed Pangasius skin collagen. Similarly, tuna skin gelatin hydrolyzed using crude fisin enzyme showed a DH of 42.78% (Ismed *et al.* 2023).

Acidity (pH) of collagen hydrolysate

The pH standard used in the acidity test complies with SNI 8076:2020 for crude collagen from fish scales or skin. The pH measurement results of the collagen hydrolysate are presented in Figure 2.

Figure 2 demonstrates that the acidity (pH) of each treatment varied significantly ($P < 0.05$). The findings of the statistical analysis indicate that the 3% bromelain treatment produced the lowest pH of 5.57, whereas the control treatment (which did not include any enzymes) produced the highest pH of 7.64. When collagen was hydrolyzed using the enzyme bromelain from crude pineapple stems in the 2% bromelain treatment, the optimal pH value was 6.43.

Masri (2014) stated that pH 6 is the optimal activity of the crude bromelain enzyme produced by pineapple stems, whereas pH 7 is the optimal activity of the crude bromelain enzyme produced by pineapple cores. Because collagen degradation is indicated by a low or acidic pH value, the pH of collagen hydrolysate varies after each enzymatic treatment. This finding is consistent with Hidayat *et al.* (2016), who reported that numerous acid cations are still trapped in ossein following collagen hydrolysis, causing the polymer to continue to break down and the pH to decrease.

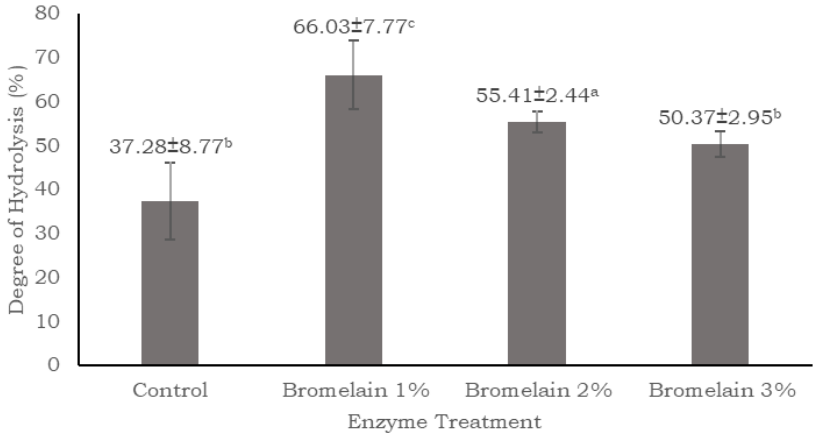

Figure 1. Degree of hydrolysis (DH) of Pangasius skin collagen hydrolysate at different bromelain concentrations.

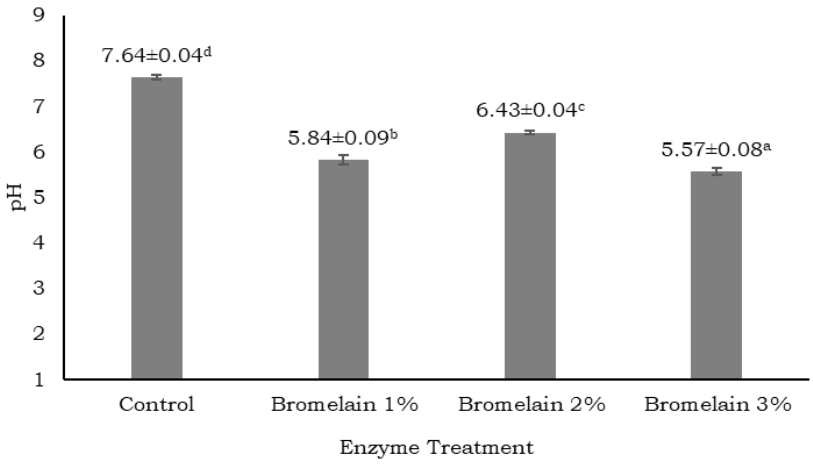

Figure 2. pH of Pangasius skin collagen hydrolysate at different bromelain concentrations.

Collagen hydrolysate solubility

The Pangasius skin collagen hydrolysate solubility was measured by comparing the weight of the residue and the sample dissolved in distilled water. The results of the solubility measurement of Pangasius skin collagen hydrolysate are presented in Figure 3. The results show that the highest solubility value was found in the 1% crude bromelain enzyme treatment (79.5%), while the lowest value was in the control treatment (36.36%). According to the ANOVA analysis, there was no significant difference between the enzyme concentration treatments of 1%, 2%, and 3% on the solubility value of the collagen hydrolysate ($P > 0.05$). Nurilmala *et al.* (2019) reported that the solubility results were smaller than those of tuna skin collagen hydrolysate hydrolyzed with the papain enzyme, which was found at 78.67–95.27%. The solubility value of the collagen hydrolysate was higher than that of the control collagen.

The enzyme used in hydrolysis and the degree of hydrolysis also affect the solubility and bioactivity of the hydrolysate (Barzideh *et al.* 2014). This is demonstrated by the treatment with 1% crude bromelain, which exhibited the highest degree of hydrolysis and resulted in the greatest solubility compared to other treatments. The hydrolysate results with a smaller size, more polar residue, and enhanced capability to form hydrogen bonds with water and increase its solubility are attained from an advanced degree of hydrolysis (Gbogouri *et al.* 2004).

The type I collagen main structure consists of three α helices, namely two identical α1(I) helices and one α2(I) helix, each containing roughly 1,000 amino acids and molecular weights of roughly 130–140 kDa and 110–120 kDa, independently. The collagen motes have three helix sections and two non-helix sections at both ends, called telopeptides. Electrophoresis results of Pangasius skin collagen and hydrolysate produced from hydrolysis using the bromelain of crude pineapple stems (Figure 4). In this study, the molecular weight of the β chain of Pangasius skin collagen was 212 kDa, while the molecular weights of the α1 and α2 chains were 160 and 95 kDa, respectively, which are classified as Type I collagen. Collagen has the characteristic of consisting of two analogous α chains and a β chain. The presence of the β chain indicates that collagen contains intermolecular relations; the presence of a γ chain in the gel indicates that the three collagen chains are cross-linked intramolecularly (Chi *et al.* 2014).

Crude bromelain extracted from pineapple has also been used for the extraction of collagen from Pangasius skin, with molecular weights of α1, α2, β, and γ being 131.51 kDa, 110.48 kDa, 202.48 kDa, and 243.93 kDa (Yanti *et al.* 2022). The β and γ factors indicate the presence of collagen motes that have experienced cross-linking (Astiana and Nurjanah 2016). Pineapple stem bromelain is an endoprotease that cleaves peptide bonds in protein molecules (Arshad *et al.* 2014), and it helps increase the possibility of carrying low-molecular- weight hydrolysates with lesser bioactivity. In addition, bromelain can produce low-molecular-weight composites because it cuts carbonyl groups, including tyrosine, alanine, lysine, and glycine. Glycine is an amino acid that's abundant in fish collagen (Wani and Mashru 2014).

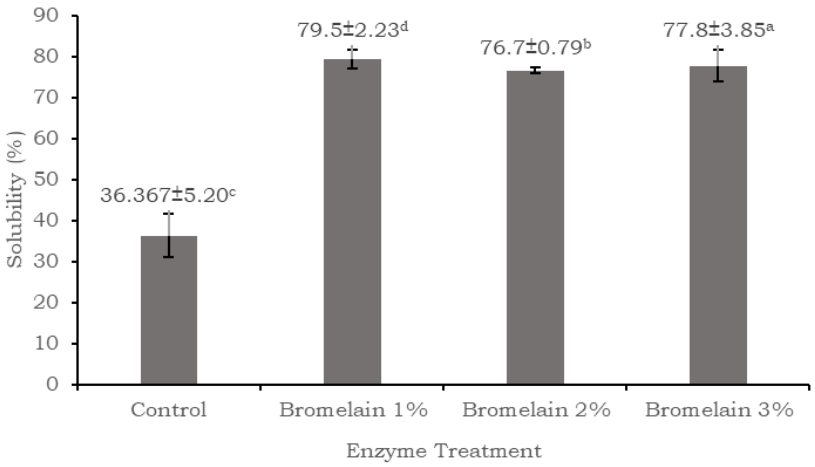

Figure 3. Solubility of Pangasius skin collagen hydrolysate at different bromelain concentrations.

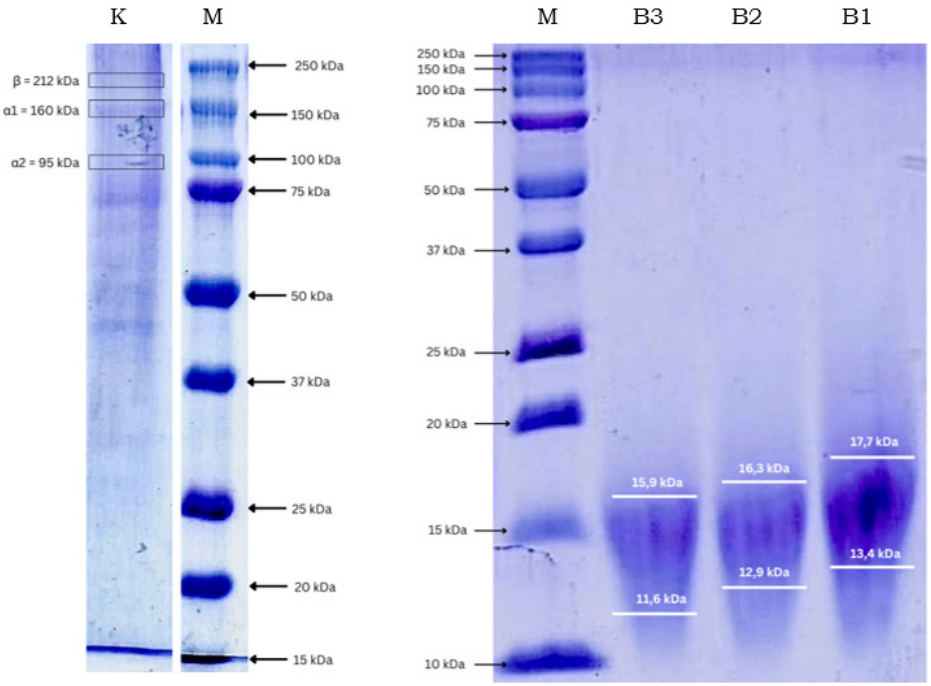

Figure 4. SDS-PAGE profiles of collagen (a) and collagen hydrolysate (b) from Pangasius skin under different bromelain treatments (M = marker, K = control, B1 = 1% bromelain, B2 = 2% bromelain, B3 = 3% bromelain).

Antioxidant activity of collagen hydrolysate

The antioxidant activity of Pangasius skin collagen hydrolysate is expressed as an IC_{50} (inhibition concentration 50) value. IC_{50} refers to the concentration of a sample required to inhibit 50% of free radicals (Prastyo *et al.* 2020). Figure 5 shows the results of the IC_{50} measurements of Pangasius skin collagen hydrolysate.

The analysis showed that antioxidant activity differed significantly among enzyme-treated samples and the non-enzyme control. The IC_{50} values of Pangasius skin collagen hydrolysate ranged from 33.99 to 61.24 µg/mL, while the IC_{50} value of collagen before hydrolysis was 54.88 µg/mL. Antioxidant activity decreased as the bromelain enzyme concentration increased from 1% to 3%.

The antioxidant activity (IC_{50}) value varied significantly between all treatments, according to the ANOVA analysis ($P < 0.05$). A crude bromelain enzyme content of 3% with an IC_{50} value of 33.99 µg/mL was the best treatment outcome. The study's antioxidant activity was shown to be higher than the 38 µg/mL of tilapia skin collagen hydrolysate digested with the papain enzyme (Prastyo *et al.*

2018). In this investigation, Pangasius collagen hydrolysate's antioxidant activity value was rated as strong to extremely strong. The activity of the collagen hydrolysate can be influenced by amino acids, the degree of hydrolysis, the size of the polyhydrolysate, and the kind of enzyme utilized in the hydrolysis process (Sarmadi *et al.* 2011).

Analysis of collagen functional groups and collagen hydrolysates

FTIR (Fourier Transform Infrared Spectroscopy) analysis in the range of 4,000–500 cm^{-1} showed that collagen and collagen hydrolysate from the four treatments exhibited similar absorption bands with slight differences in peak intensities (Figure 6). The FTIR spectra of the four treatments differed based on their absorption band characteristics.

The location of the amide band peak from each treatment did not significantly change, according to the FTIR results of collagen and collagen hydrolysate of Pangasius skin; nevertheless, the addition of crude bromelain enzyme concentration increased the shift in peak amplitude. With amine stretching vibrations, the amide A band peak was located between 3,400 and 3,440 cm^{-1} (Doyle *et al.* 1975).

The stretching vibration of free amines was shown by the peak of the amide A band of Pangasius skin collagen hydrolysate, which was found in the region of 3,420–3,429 cm^{-1} (Table 2). Sai and Babu's (2001) study found that the frog skin collagen band has an amide bond with a free amine group and no hydrogen bonding, with a value of 3,437 cm^{-1}. Amide B is distinguished by the stretching of the carbonyl group and has a wavenumber between 2,933 and 2,940 cm^{-1}. With a value of 2,930 cm^{-1}, this value is nearly identical to the pepsin-soluble collagen of bigeye tuna skin.

Amide I in the hydrolysate of Pangasius skin collagen exhibits a wave number range of 1,647–1,675 cm^{-1}, indicative of extended carbonyl groups. The wave number of amide I spans from 1,700 to 1,600, reflecting the hydrolysate's secondary structure and indicating hydrogen bonds among C=O stretches (Riaz *et al.* 2018). The amide II band of collagen hydrolysate from Pangasius skin has a wave number between 1,536 and 1,541. These findings are nearly the same as the wave number of pepsin-soluble collagen from squid skin, which falls within the range of 1,541–1,544 (Veeruraj *et al.* 2015). The inclusion of crude bromelain enzyme concentration leads to a decreased Amide II wave number of collagen hydrolysate in comparison to the treatment without the enzyme. The research of Chuaychan *et al.* (2015) supports this statement, indicating that pepsin-soluble collagen exhibits a low amide II wave number and demonstrates a greater proportion of hydrogen bonds. Reduced polymer regularity may lead to the weakening and loss of a specific type of hydrogen bond (Djaenudin *et al.* 2019).

The Pangasius skin's hydrolyzed collagen exhibits an amide III peak ranging from 1,333 to 1,336. This value falls within the amide III absorbance range of 1,200–1,400, featuring bending in the amine group and stretching of the cyanide group (Veeruraj *et al.* 2015). Studies on hydrolyzed Pangasius skin collagen show an amide III ratio and a C-H group bending vibration peak of 0.91, indicating that the collagen hydrolysate retains the triple-helix structure of collagen.

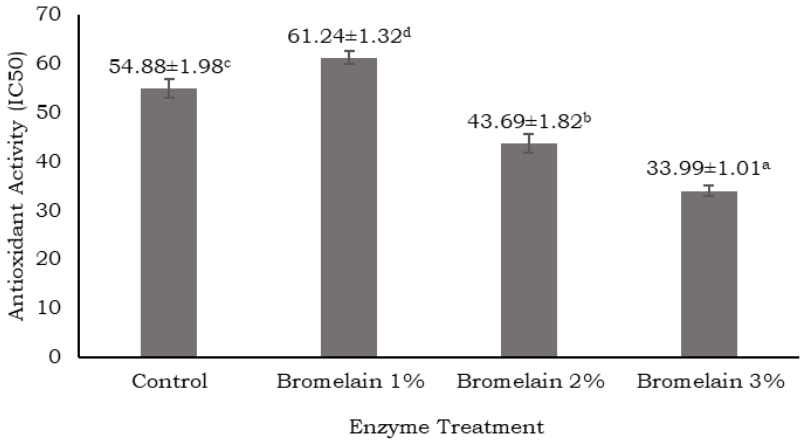

Figure 5. Antioxidant activity (IC_{50}) of Pangasius skin collagen hydrolysate at different bromelain concentrations.

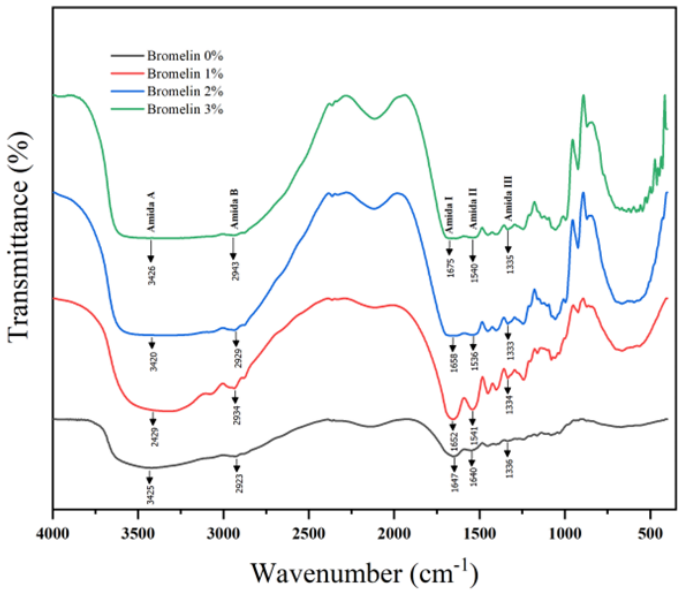

Figure 6. FTIR spectra of collagen and collagen hydrolysate from Pangasius skin at different bromelain concentrations, showing characteristic amide bands (A, B, I, II, and III).

Table 2. FTIR spectral characteristics of functional groups in Pangasius skin collagen hydrolysate.

Amida	Wave number (cm^{-1})	Absorption Area (cm^{-1})	Notes	References
A	3,420–3,429	3,400–3,440	Stretch N-H	Doyle *et al.* (1975)
B	2,923–2,940	3,080–2,889	Asymmetric strain CH_2	Riaz *et al.* (2018)
I	1,647–1,675	1,700–1,600	Stretch C=O	Riaz *et al.* (2018)
II	1,536–1,541	1,541–1,544	Bending N-H with a stretch C-N	Veeruraj *et al.* (2015)
III	1,333–1,336	1,200–1,400	Cluster stretching C-N dan Cluster stretching N-H	Riaz *et al.* (2018)

CONCLUSION

The level of crude bromelain enzyme greatly influences the properties and antioxidant activity of collagen hydrolysate derived from Pangasius skin. Fish skin collagen hydrolysate exhibits properties such as pH values (5.57 ± 0.08 to 6.43 ± 0.04); hydrolysis degree (50.37 ± 2.95 to 66.03 ± 7.77), and solubility (76.7 ± 0.79 to 79.5 ± 2.23). The enzymatic hydrolysis of collagen using crude bromelain enzyme yielded the optimal concentration of 3% and generated collagen hydrolysate with a low molecular weight (15.9–11.6 kDa). The antioxidant effect of Pangasius skin collagen hydrolysate was rated as strong to very strong (33.99 ± 1.01 to 61.24 ± 32) with the rise in crude bromelain enzyme levels. Evaluation of the functional groups in collagen hydrolysates reveals Amide A, B, I, II, and III peaks, indicative of the triple-helix configuration of collagen that remains undenatured into gelatin.

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