Characteristics of candies made from Catfish (*Pangasius* sp.) skin collagen, gelatin and its hydrolysate

Mona Dianita Sari Putri[1], *Mala* Nurilmala[1*], and *Asadatun* Abdullah[2]

[1]Departement of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, IPB University, Jl Agatis Kampus IPB Dramaga, Bogor, West Jawa 16680, Indonesia

Abstract. Catfish skin is a by-product from the fishing industry that has potential as a source of collagen and gelatin. An enzymatic hydrolysis process was conducted using the commercial bromelain enzyme pineapple stem extract. The resulting collagen, gelatin and its hydrolysates were then used as basic ingredients in candy making. This study aims to determine the characteristics of candy made from collagen, gelatin and its hydrolysate; determine the effect of the type of raw material on the physicochemical properties of the candy; and determine the biological activity contained in it. The methods employed in this study consisted of the following stages: preparation of catfish skin, pre-treatment of catfish skin, extraction of catfish skin, hydrolysis of collagen and gelatin derived from catfish skin and candy formulation. The results showed that collagen hydrolysate produced through hydrolysis with pineapple stem extract is the best raw material for candy making. The hydrolysate had a pH value of 4.71±0.03; viscosity of 4.71±0.03 mPas; toxicity LD_{50} value of 1036.83 µg/mL; degree of hydrolysis of 57.42%; and molecular weight range of 9.8-12.7 kDa. The best characteristics were shown by collagen candy from hydrolyzate with pineapple stem extract. The candy produced a protein content of 64.03%; an ash content of 0.31%; a moisture content of 3.48%; and hedonic parameters appearance of 4.20, aroma of 4.73, flavor of 4.70, and texture of 3.00. Candy with the highest antioxidant activity was obtained from collagen hydrolyzed using commercial bromelain enzyme, which amounted to 98.62 µg/mL by the ABTS method and 382.33 µmol/g by the FRAP method.

1 Introduction

Catfish (*Pangasius* sp.) is one of the most economically important aquaculture commodities in Indonesia, with production increasing steadily each year. According to the Ministry of Marine Affairs and Fisheries, national catfish production reached 348,379 tons in 2024 [1]. Along with increased production, substantial amounts of by-products are generated, particularly fish skin from fillet processing industries. Fish skin has been widely recognized as a promising source of collagen due to its high protein content and well-developed connective tissue structure. Previous studies have reported that catfish skin contains approximately 30% collagen, highlighting its potential for conversion into high-value products such as collagen and gelatin [2]. Therefore, sustainable utilization of this by-product is essential to enhance its economic value and reduce processing waste.

Collagen is the most abundant structural protein in vertebrates and is characterized by a triple-helix structure rich in glycine, proline and hydroxyproline [3]. It can be extracted from

* Corresponding author: mnurilmala@apps.ipb.ac.id

various connective tissues, including skin, bones and scales and can be partially hydrolyzed to produce gelatin. Gelatin retains a similar amino acid composition to collagen and exhibits desirable functional properties, such as gelling, stabilizing, thickening and emulsifying capacities, which support its widespread application in food systems.

The use of collagen and gelatin derived from terrestrial animals has raised health and religious concerns, including risks associated with bovine spongiform encephalopathy and dietary restrictions. Consequently, fish skin has emerged as a safer and more widely accepted alternative source. Enzymatic hydrolysis further converts collagen and gelatin into low-molecular-weight peptides with improved digestibility and enhanced bioactivities, particularly antioxidant activity [4]. Proteolytic enzymes such as bromelain have been widely applied in collagen hydrolysis due to their effectiveness and stability under various processing conditions. Bromelain, a protease derived from pineapple, has been reported to exhibit superior hydrolytic performance and to enhance the antioxidant activity of collagen hydrolysates compared to other enzymes. Antioxidant peptides play an important role in scavenging free radicals and are increasingly valued in functional food with nutraceutical applications.

With the growing global demand for collagen- and gelatin-based functional products, the development of stable and economically feasible delivery systems is increasingly important. Hard candy represents a practical food matrix due to its long shelf life, ease of consumption, and low production cost. However, to the best of our knowledge, no previous studies have developed hard candy formulations incorporating collagen, gelatin and their hydrolysates derived from catfish (*Pangasius* sp.) skin. Therefore, this study aimed to evaluate the physicochemical characteristics and biological activities of hard candies formulated using different collagen-based raw materials from catfish skin.

2 Materials and methods

2.1 Equipments and materials

The equipment used in this study included a digital balance (Ohaus Corp., New Jersey, USA), cotton, calico cloth, pH indicator, electric stove, measuring cylinder (Pyrex, Asahi Glass, Thailand), Erlenmeyer flask (Pyrex, Asahi Glass, Thailand), water bath shaker (Depolab, Seoul, Korea), aluminum foil, thermometer, oven (Blue M, Jiangsu Zhengji Instruments, Jintan, China), universal pH indicator and blender (Miyako BL-151, Indonesia).

The main raw material used was catfish (*Pangasius* sp.) skin, obtained from PT. Adib Global Food (Indonesia). Chemicals used for collagen hydrolysate production included hydrochloric acid (HCl) (CV. Kimia Jaya Laboran, Cilacap Utara, Indonesia), sodium hydroxide (NaOH) (Merck KGaA, Germany), acetic acid (CH_3COOH) (Toko Central Kimia, Bogor, Indonesia), distilled water (Toko Central Kimia, Bogor, Indonesia), and bromelain enzyme $\geq$3 units (Sigma-Aldrich, USA). Additional ingredients used in the candy formulation were commercial sugar and pineapple stem obtained from a traditional market in Bogor, West Java, Indonesia. For hydrolysis degree analysis, trichloroacetic acid (TCA 20%) was used. Antioxidant activity was determined using the ABTS assay with 2,2-*azino-bis* (3-*ethylbenzothiazoline*-6-*sulphonic acid*) as the radical solution and $K_2S_2O_8$-ethanol as the blank.

2.2 Work procedure

The production of hard candy consisted of several sequential steps. The first stage was the preparation of catfish (*Pangasius* sp.) skin, followed by pre-treatment, extraction, enzymatic hydrolysis of collagen, gelatin and candy formulation.

2.2.1 Preparation of Catfish skin

The sample used was catfish (*Pangasius* sp.) skin that had been thoroughly cleaned. The skin was cut into square pieces and the purpose of this preparation was to remove residual fat. The skin was washed five times with water, followed by weighing according to the required sample amount.

2.2.2 Pre-treatment of Catfish skin

The pre-treatment process was carried out to remove non-collagenous components [5]. For collagen pre-treatment, catfish skin was soaked in 0.05 M NaOH solution at a ratio of 1:10 (w/v) for 1.5 hours. The solution was then neutralized to pH 6–7. Subsequently, the skin was immersed in 0.1 M acetic acid (CH_3COOH) solution at a ratio of 1:10 (w/v) for 2 hours at 4°C and again neutralized to pH 6–7. For gelatin pre-treatment, the skin was soaked in 0.05 M NaOH solution at a ratio of 1:10 (w/v) for 1.5 hours, followed by washing until neutral pH (6–7). The next step involved soaking the skin in HCl solution at a ratio of 1:5 (w/v) for 2 hours, after which it was washed until neutral pH (6–7) was achieved.

2.2.3 Extraction of Catfish skin

The extraction process was carried out in two stages, namely collagen and gelatin extraction from catfish (*Pangasius* sp.) skin [6]. Collagen extraction was performed using a water bath shaker at 40°C at a water–sample ratio of 1:1 (w/v) and maintained for 6 hours. Gelatin extraction was conducted at 70°C using the same proportion of 1:1 (w/v) and continued for 8 h. Following extraction, the solution was filtered using cotton and calico cloth. Collagen was dried in an oven at 40°C, while gelatin was dried at 50°C for 24 hours.

2.2.4 Hydrolysis of collagen and gelatin from Catfish skin

The hydrolysis process was conducted using an enzymatic method [7]. Collagen and gelatin (10 g) were hydrolyzed with pineapple core extract and commercial bromelain enzyme. Hydrolysis was conducted at 40°C and allowed to proceed for 3 hours, after which the enzyme was inactivated at 85°C for a duration of 15 minutes. The resulting liquid collagen and gelatin hydrolysates were subsequently oven-dried at 40°C for a period of 24 hours. The dried forms of collagen and gelatin hydrolysates were then ground into powder using a grinder.

2.2.5 Candy formulation

The candy formulation was adapted from the method of Wijaya [8] with modifications by incorporating 10 g of collagen, gelatin, and their respective hydrolysates. The candy preparation began with mixing the raw materials, including collagen powder, gelatin and their hydrolysates obtained from pineapple core extract and commercial bromelain enzyme for hydrolysis. Subsequently, 2 g of stevia sweetener and 88 mL of distilled water were added. The mixture was subjected to mild heating at 40°C for 90 minutes. The candies were dried in an oven at 40°C for 24 hours.

2.3 Analysis procedure

The analytical procedures in this study included sensory evaluation (hedonic test), determination of moisture, ash, protein levels, the extent of hydrolysis, viscosity, acidity, cytotoxicity using the Brine Shrimp Lethality Test (BSLT), antioxidant activity assays using ABTS and FRAP methods, as well as molecular weight analysis by SDS-PAGE.

2.3.1 Moisture percentage

Moisture percentage analysis [9] was conducted to quantify the water content present in the sample. Initially, a crucible was dried in an oven at 105°C for 1 hour, cooled in a desiccator for 15 minutes to reach room temperature and weighed. Approximately 3 g of the sample was placed in the crucible and dried in an oven at 105°C for 5 hours. The dried sample was then cooled in a desiccator to room temperature and weighed. Moisture content was calculated using the following equations:

$$\text{Weight loss (g)} = \text{Initial sample weight (g)} - \text{Final sample weight (g)} \quad (1)$$

$$\text{Moisture content (\%)} = \frac{Weight\ loss\ (g)}{Initial\ sample\ weight\ (g)} \text{ x } 100\% \quad (2)$$

2.3.2 Ash content

Ash content analysis [9] was performed to assess the mineral composition of the sample. A crucible was dried in an oven at 105°C for 1 hours, cooled in a desiccator for 15 minutes, and weighed. Approximately 1 g of the sample was placed in the crucible and incinerated over a Bunsen flame until no smoke was observed. The crucible containing the sample was then placed in a muffle furnace at 600°C for 6 hours. Following combustion, the crucible was allowed to cool to room temperature in a desiccator before being weighed. The ash content was then calculated using the following equations:

$$\text{Ash weight (g)} = \text{Final weight of crucible + sample (g)} - \text{Weight of empty crucible (g)} \quad (3)$$

$$\text{Ash content (\%)} = \frac{Ash\ weight\ (g)}{Sample\ weight\ (g)} \text{ x } 100\% \quad (4)$$

2.3.3 Protein content

Protein content was determined using the Kjeldahl method [9], which consists of three main stages: digestion, distillation and titration. Approximately 2 g of sample was placed into a 50 mL Kjeldahl flask, followed by the addition of 7 g K_2SO_4, 0.005 g HgO, 15 mL concentrated H_2SO_4, and 10 mL H_2O_2 added slowly, and left for 10 minutes in a fume hood. Digestion was performed at 410°C for approximately 2 hours until the solution turned yellowish green. The digested solution was diluted with 50 mL distilled water and 20 mL of 40% NaOH was added. The mixture was distilled at 100°C, and the distillate was collected in a 125 mL Erlenmeyer flask containing bromocresol green–methyl red indicator. Distillation was continued until 150 mL of green-colored distillate was obtained. Titration was performed as the final step using 0.1 N HCl until the endpoint (pink color) was reached. A blank solution was also analyzed. Protein content was calculated using the following formula:

$$\text{Protein content (\%)} = \frac{(VHCl - Vblank)\ x\ N\ x\ 14.007\ x\ F}{Sample\ weight\ (mg)}\ \text{x } 100\% \quad (5)$$

2.3.4 Degree of hydrolysis

The percentage of the degree of hydrolysis (DH) [9] was determined through protein fractionation using 20% trichloroacetic acid (TCA), which separates soluble and insoluble protein fractions at 10% each. A total of 500 µL of collagen hydrolysate sample was homogenized with 500 µL of 20% TCA and incubated at 4°C for 30 minutes. The mixture was then centrifuged at 3,000 g for 20 minutes. The supernatant was collected and its soluble protein content was analyzed using bovine serum albumin (BSA) as the protein standard. The degree of hydrolysis was calculated using the following formula:

$$\text{DH (\%)} = \frac{Nitrogen\ content\ in\ supernatant}{Total\ nitrogen\ content\ in\ sample}\ \text{x } 100\% \quad (6)$$

2.3.5 The degree of acidity

To determine the degree of acidity [9], a total of 1 g of sample was dissolved in 20 mL of distilled water, followed by the addition of 50 mL of distilled water, and homogenized thoroughly. The pH meter was turned on and allowed to stabilize before measurement. The electrode was immersed in the sample solution until a stable reading was displayed on the pH meter.

2.3.6 Antioxidant activity by ABTS method

To determine antioxidant activity, an ABTS stock solution [10] was prepared by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate at a 1:1 ratio and incubating in the dark at room temperature for 16 hours. The solution was then diluted with ethanol until an absorbance of 0.700 ± 0.02 at 734 nm was obtained. Subsequently, 1 mL of sample solution (1 mg/mL) was added to 2 mL of ABTS solution and incubated at room temperature for 10 minutes. The absorbance of the mixture was measured at 734 nm. Antioxidant activity was expressed as IC_{50} values, calculated from the regression curve of ABTS radical scavenging inhibition across different sample concentrations.

2.3.7 Antioxidant activity by FRAP method

The FRAP reagent [11] was prepared using 300 mM acetate buffer (8 mL CH_3COONa and 92 mL CH_3COOH, pH 3.6), 10 mM TPTZ (2,4,6-*tripyridyl-s-triazine*) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$. The working reagent was freshly prepared by mixing 35 mL acetate buffer, 3.5 mL TPTZ solution and 3.5 mL $FeCl_3 \cdot 6H_2O$ solution. For the assay, 500 µL of sample, 600 µL of distilled water and 3 mL of FRAP reagent were combined, vortexed and incubated in a water bath at 37°C for 30 minutes. The absorbance was measured at 593 nm. A standard calibration curve was constructed using ascorbic acid solutions at various concentrations.

2.3.8 Toxicity assay by Brine Shrimp Lethality Test (BSLT)

The BSLT method was used to evaluate the toxicity of the sulfated polysaccharide extracts [9]. Brine shrimp larvae were obtained by hatching 50 mg of shrimp eggs in 300 mL of seawater in a 500 mL Erlenmeyer flask. Hatching was carried out under continuous aeration and illumination with a 14 W white lamp for 48 hours at room temperature. Five serial dilutions of the test sample were prepared (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL) using seawater as the diluent. The assay was conducted in a 96-well plate, with each well containing 10 shrimp larvae and the respective test concentration. Each concentration was tested in triplicate. The plates were incubated under a 14 W white lamp at room temperature for 24 hours. After incubation, the number of surviving larvae was recorded. A negative control was prepared using the same procedure without the extract. Toxicity (IC_{50}) was calculated using probit analysis based on the following formula:

$$\text{Mortality (\%)} = \frac{NNumber\ of\ dead\ larvae}{Total\ larvae} \text{ x } 100\% \quad (7)$$

2.3.9 Sensory evaluation

Sensory evaluation was conducted using human senses to assess the quality attributes of the candy samples based on several indicators, including appearance, color, aroma, taste, and texture. A semi-trained panel of 30 participants was recruited. Panelists evaluated the samples using a 5-point hedonic scale: (1) dislike very much; (2) dislike; (3) neutral; (4) like; (5) like very much.

2.3.10 Viscosity

A sample of 3.33 g was dissolved in 50 mL of distilled water and homogenized. The viscosity of the solution [9] was measured using a Brookfield Synchro-Lectric Viscometer at room temperature with a shear rate of 60 rpm. Spindle No 1 was used in this assay and the viscosity values were expressed in mPas.

2.3.11 Molecular weight determination (SDS-PAGE)

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used to determine molecular weights [12]. Gelatin samples (2 mg) were dissolved in 1 mL of 5% SDS solution and heated at 85°C for 1 hour. The solution was centrifuged at 12,400 × g for 5 minutes and 20 µL of sample buffer was collected. The sample was reheated at 85°C before being loaded into the gel wells. For gel preparation, 5 mL of separating gel containing 12.5% acrylamide was prepared, followed by the addition of 1 mL distilled water. The gel was incubated at room temperature for 30 minutes until solidified and the excess water was removed. Subsequently, 1 mL of stacking gel was added and incubated at room temperature for 2 hours. Denatured protein samples and molecular weight marker 245 (BIO-RAD) were loaded into the gel wells (5 µL each). Electrophoresis was carried out at a constant voltage of 100 V for 3 hours. After electrophoresis, the gel was removed from the cast, stained with 25 mL *Coomassie Brilliant Blue* for 2 hours, rinsed with water and destained with 50 mL destaining solution until the protein bands became clearly visible.

2.4 Data analysis

The experimental design used in this study was a factorial Completely Randomized Design (CRD) consisting of two stages, namely:
Stage 1
Evaluation of raw material quality of hydrolysates consisting of six treatments, i.e.:

1. C = Collagen
2. G = Gelatin
3. CHP = Collagen hydrolysate (pineapple core extract hydrolysis)
4. GHP = Gelatin hydrolysate (pineapple core extract hydrolysis)
5. CHB = Collagen hydrolysate (commercial bromelain hydrolysis)
6. GHB = Gelatin hydrolysate (commercial bromelain hydrolysis)

Stage 2
Evaluation of candy product quality was carried out on six types of raw materials used in Stage 1, consisting of six treatments, i.e.:

1. P1 = Collagen candy
2. P2 = Gelatin candy
3. P3 = Collagen hydrolysate candy obtained from pineapple core extract hydrolysis
4. P4 = Gelatin hydrolysate candy obtained from pineapple core extract hydrolysis
5. P5 = Collagen hydrolysate candy obtained from commercial bromelain enzyme hydrolysis
6. P6 = Gelatin hydrolysate candy obtained from commercial bromelain enzyme hydrolysis

Each treatment in both stages was replicated three times. Data analysis was performed separately for each stage using Analysis of Variance (ANOVA). If a significant difference was observed ($P<0.05$), Duncan's Multiple Range Test (DMRT) was applied at a 95% confidence level. Sensory data were analyzed using a non-parametric test, namely the Kruskal–Wallis test. If significant differences were found, a Multiple Comparison Test was performed using the Mann–Whitney U test.

3 Results and discussion

3.1 Degree of acidity of collagen, gelatin and its hydrolysates

The measurement of acidity was conducted to determine the acid–base level of the samples. Acidity of collagen- and gelatin-based products is a key parameter in assessing their stability across various application fields. The acidity values of collagen, gelatin and its hydrolysates derived from catfish skin are presented in Fig. 1.

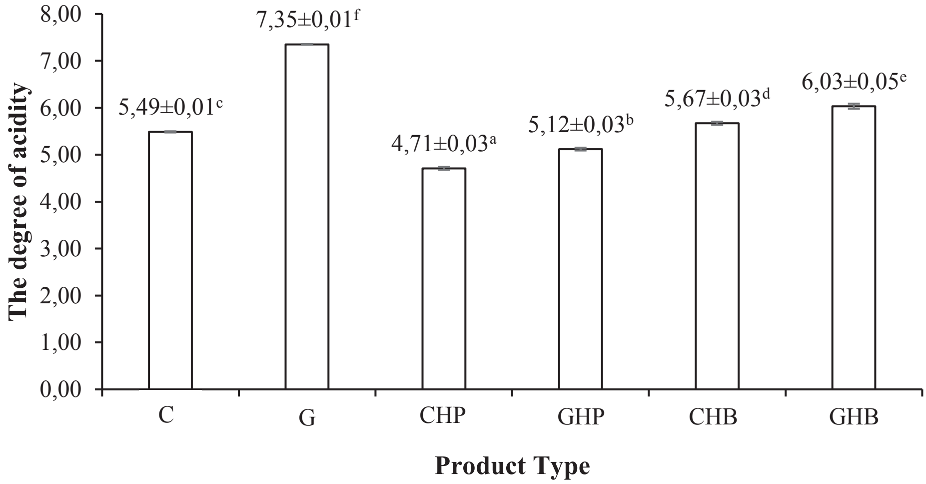

Fig.1. The degree of acidity of Collagen (C); Gelatin (G); Collagen Hydrolysate with pineapple extract (CHP); Gelatin Hydrolysate with pineapple extract (GHP); Collagen Hydrolysate with bromelain enzyme (CHB); Gelatin Hydrolysate with bromelain enzyme (GHB).

In the degree of acidity measurements shown in Figure 1, values ranged from 4.71 to 7.35. Significant differences were observed among the treatments (ANOVA, $P<0.05$). These results indicate that collagen and gelatin derived from catfish skin are suitable for use as ingredients in confectionery applications. The degree of acidity values of collagen obtained in this study tended to be slightly below neutral. This condition may be attributed to the soaking process using acetic acid solution (CH_3COOH) during the swelling stage of catfish skin. The hydrogen ions (H^+) from acetic acid are not fully absorbed into the collagen matrix. Repeated neutralization of the skin cannot completely eliminate residual acid retained within the collagen fibril network.

3.2 Viscosity of collagen, gelatin and its hydrolysates

Viscosity is one of the key parameters for assessing the physical properties of collagen-based materials, gelatin, collagen hydrolysate, as well as gelatin hydrolysates. The viscosity values for collagen, gelatin and their hydrolysates obtained from catfish skin tissue are presented in Fig. 2.

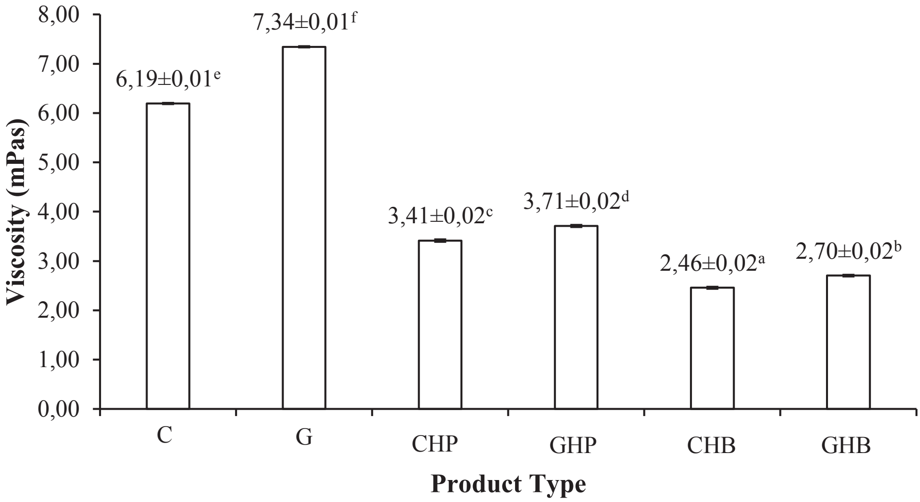

Fig.2. Viscosity of Collagen (C); Gelatin (G); Collagen Hydrolysate with pineapple extract (CHP); Gelatin Hydrolysate with pineapple extract (GHP); Collagen Hydrolysate with bromelain enzyme (CHB); and Gelatin Hydrolysate with bromelain enzyme (GHB).

Gelatin exhibited higher viscosity values compared to collagen extracted from catfish skin (Fig. 2). Different raw material types significantly influenced the viscosity values (ANOVA, $P<0.05$). Variations in viscosity between collagen, gelatin and their hydrolysates obtained from catfish skin tissue may be attributed in part to soaking duration. Longer soaking times tend to decrease the viscosity of the final product. This phenomenon is likely associated with the breakdown of polypeptide chains in gelatin into shorter peptide chains.

3.3 Toxicity of collagen, gelatin and its hydrolysates

Toxicity testing is essential to ensure the safety of a product. The Brine Shrimp Lethality Test (BSLT) is widely employed as an initial bioassay method because it is practical, cost-effective and capable of providing a preliminary indication of the presence of toxic compounds. The toxicity results of collagen, gelatin and its hydrolysates from catfish skin are summarized in Table 1.

Table 1. Toxicity of collagen, gelatin and its hydrolysates

Sample type	LC_{50} value (µg/mL)	Category
Collagen	1091.86	Non-toxic
Gelatin	1421.87	Non-toxic
Collagen hydrolysate (pineapple extract)	1036.83	Non-toxic
Gelatin hydrolysate (pineapple extract)	1138.94	Non-toxic
Collagen hydrolysate (bromelain enzyme)	1073.89	Non-toxic
Gelatin hydrolysate (bromelain enzyme)	1229.27	Non-toxic

The toxicity results revealed that all treatments were categorized as non-toxic and safe for consumption. A product is considered highly toxic when $LC_{50} < 30$ ppm, toxic when LC_{50} ranges between 31–200 ppm, mildly toxic when LC_{50} falls within 201–1000 ppm and non-toxic when LC_{50}>1000 ppm. An increase in the lethal dose (LC_{50}) value indicates a reduced toxic effect on larval mortality of *Artemia salina*. The BSLT method offers several advantages compared to other toxicity testing approaches. It requires a relatively short time to perform, is cost-effective, practical, does not necessitate aseptic techniques or special maintenance, requires only a small amount of sample and does not involve the use of animal serum. The findings indicate that collagen and gelatin possess potential to be developed into nutraceutical candy products. This is attributed to the non-toxic nature of catfish skin collagen and gelatin proteins within the human body.

3.4 Degree of hydrolysis of collagen and gelatin

The degree of hydrolysis (DH) is a crucial indicator used to assess the extent to which protein peptide bonds are cleaved during the hydrolysis process. DH has a major influence on determining the functional properties and biological activities associated with collagen and gelatin hydrolysates. The results of DH measurement are presented in Fig. 3.

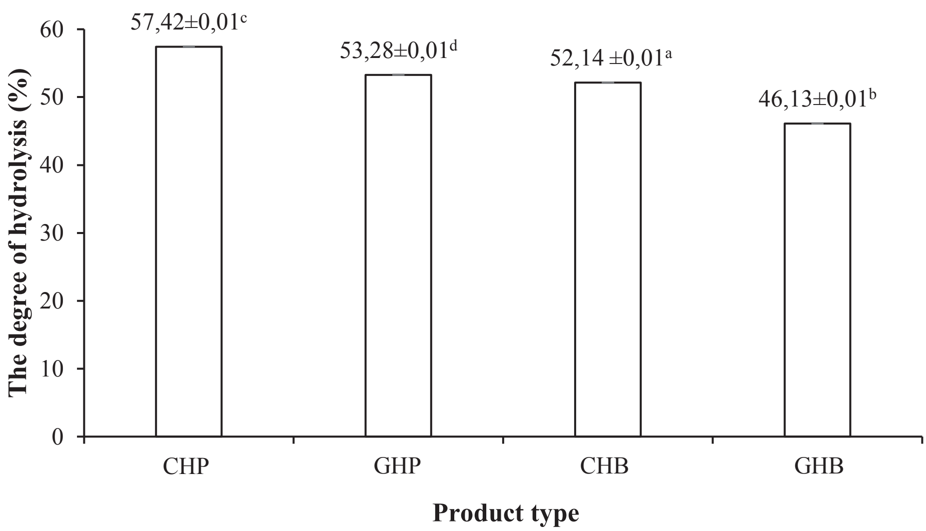

Fig.3. The degree of hydrolysis of Collagen Hydrolysate with pineapple extract (CHP); Gelatin Hydrolysate with pineapple extract (GHP); Collagen Hydrolysate with bromelain enzyme (CHB); and Gelatin Hydrolysate with bromelain enzyme (GHB).

The DH values displayed significant differences among treatments ($P<0.05$). The highest DH value was observed in CHP (collagen hydrolysate obtained from pineapple core extract hydrolysis), reaching 57.42%, while the lowest was found in GHB (gelatin hydrolysate obtained from commercial bromelain enzyme hydrolysis) at 46.13%. These results demonstrate that collagen substrates produced hydrolysates with higher DH values compared to gelatin. Variations in DH values may be affected by factors such as acidity, processing temperature and enzyme concentration, which directly influence enzymatic efficiency. Higher DH values indicate a more extensive protein hydrolysis process.

3.5 Molecular weight (SDS-PAGE)

The molecular weight profiles of collagen, gelatin and their hydrolysates were determined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). This method separates protein biomolecules according to their molecular size and electrical charge, allowing visualization of protein band patterns as indicators of protein degradation resulting from enzymatic hydrolysis. The molecular weight profiles of the raw materials are illustrated in Fig. 4.

Catfish skin collagen (C) had molecular weights of 227.59 kDa (α1), 180.40 kDa (α2) and 93.81 kDa (β), while gelatin (G) had molecular weights of 229.62 kDa (α1), 192.67 kDa (α2) and 91.38 kDa (β). The molecular weights of pure collagen and gelatin exhibited different values compared to their hydrolysates, which had undergone the hydrolysis process. The molecular weights of the hydrolysates ranged between 9.8–34.2 kDa. Variations in molecular weight resulting from hydrolysis can affect the level of antioxidant activity. Compounds with lower molecular weights tend to have higher antioxidant activity. The SDS-PAGE profiles confirmed the existence of β, α1 and α2 chains in collagen as well as gelatin derived from catfish skin. The presence of two α chains observed in this study further confirmed that the collagen sample belonged to type I collagen, which is the dominant structural collagen commonly found in connective tissues. Type I collagen is characterized by its high molecular weight, consisting of α-chain monomers and β-chain dimers [13].

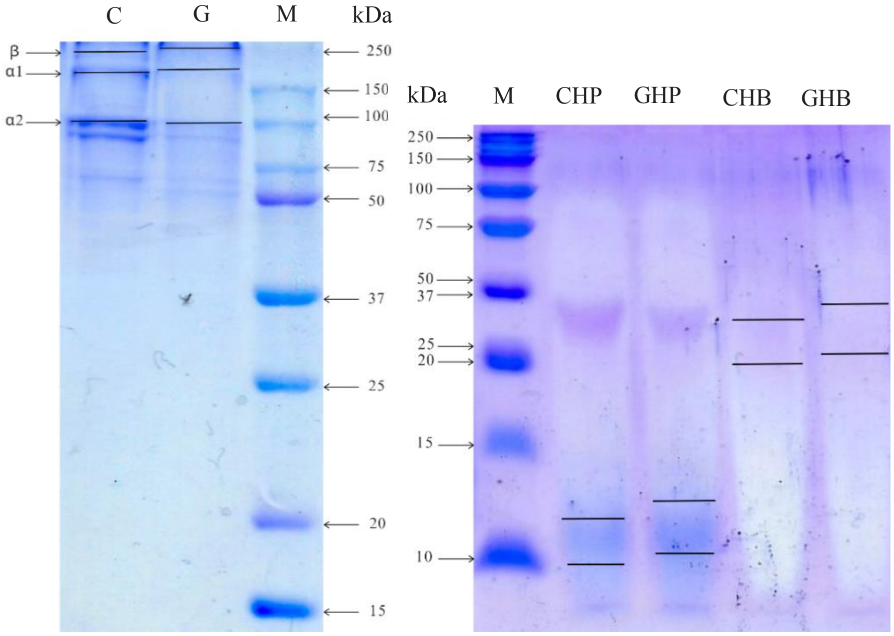

Fig.4. SDS-PAGE Profile of Collagen (C); Gelatin (G); Collagen Hydrolysate with pineapple extract (CHP); Gelatin Hydrolysate with pineapple extract (GHP); Collagen Hydrolysate with bromelain enzyme (CHB); and Gelatin Hydrolysate with bromelain enzyme (GHB). Standard molecular weight markers are shown in lane M.

3.6 Protein, ash, and moisture composition of candy

Proximate analysis aims to identify the chemical composition of a food product. The determination of protein, ash and moisture contents was performed to assess the nutritional value, quality and stability of the final product. The results of the candy chemical composition analysis are summarized in Table 2.

Table 2. Protein, ash and moisture composition of candy in accordance with SNI 3547.1:2008.

Parameter (%)	P1	P2	P3	P4	P5	P6	SNI (%)
Protein content	67.47 ± 0.06	67.33 ± 0,11	64.03 ± 0.16	65.70 ± 0.16	62.29 ± 0.01	60.75 ± 0.01	-
Ash content	0.48±0.03	0.40±0.06	0.31±0.12	0.44±0.09	0.34±0.01	0.47±0.04	Max. 2.0
Moisture content	3.17±0.03	3.23±0.04	3.48±0.04	3.41±0.04	3.37±0.01	3.30±0.02	Max. 3.5

Note: P1: Collagen candy; P2: Gelatin candy; P3: Collagen hydrolysate candy obtained from pineapple extract hydrolysis; P4: Gelatin hydrolysate candy obtained from pineapple extract hydrolysis; P5: Collagen hydrolysate candy obtained from bromelain enzyme hydrolysis; P6: Gelatin hydrolysate candy obtained from bromelain enzyme hydrolysis

Appearance is among the parameters used to assess the visual quality of candy products. The highest hedonic score for appearance was obtained in treatment P3 (candy formulated with collagen hydrolysate obtained using pineapple extract). This finding indicates that the hydrolysis of collagen using pineapple extract was able to enhance the visual appearance of the product. The next parameter is aroma. Treatment P3 received the highest hedonic score for aroma, with a value of 4.73. These findings indicate that both the raw material type and the hydrolysis method have a significant role in influencing the aroma quality of candy, where hydrolysis using pineapple extract contributes positively to aroma enhancement, whereas hydrolysis with bromelain enzyme tends to produce a less favorable aroma.

Flavor is another key factor in determining consumer acceptance of food products. The quality of flavor is not solely influenced by the composition of the raw materials, but is also affected by the processing method. The most preferred candy flavor was observed in treatment P3, which obtained a score of 4.70. The hedonic test results for candy color showed significant differences among treatments, with scores ranging from 3.00 to 4.30. The highest acceptance level for color was also found in treatment P3. Furthermore, texture is another crucial parameter that determines the overall quality of a product. Table 2 shows that the texture of each treatment varied significantly. Treatment P5 (candy formulated with collagen hydrolysate obtained using bromelain enzyme) received the highest preference score for texture, with a value of 4.57. The texture characteristics of candy are affected by the composition of the raw materials and the candy-making process.

3.7 Antioxidant activity of candy

The antioxidant activity of the hard candy samples was evaluated using two analytical methods, namely the ABTS and FRAP assays. The principle of ABTS is to evaluate the change in color of radical compounds after reacting with antioxidants, as determined by absorbance values using spectrophotometry. The antioxidant activity of the candies as measured by the ABTS method is presented in Fig. 5.

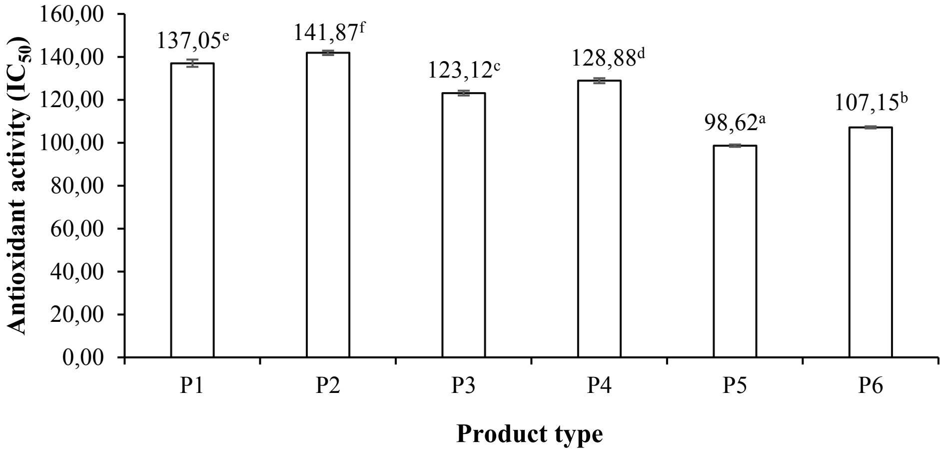

Fig.5. Antioxidant activity of candies; Collagen candy (P1), Gelatin candy (P2), Candy made from collagen hydrolysate obtained from pineapple extract hydrolysis (P3), Candy made from gelatin hydrolysate obtained from pineapple extract hydrolysis (P4), Candy made from collagen hydrolysate obtained from bromelain enzyme hydrolysis (P5), Candy made from gelatin hydrolysate obtained from bromelain enzyme hydrolysis (P6).

The IC_{50} values of antioxidant activity in the candies ranged from 98.62 to 141.87 µg/mL. The candy with the strongest antioxidant activity (lowest IC_{50} value) was the collagen hydrolysate candy obtained through enzymatic hydrolysis using bromelain, which exhibited an IC_{50} of 98.62 µg/mL. A higher IC_{50} value indicates weaker antioxidant activity in a product. There were significant differences (ANOVA, $P<0.05$) among treatments with respect to IC_{50} values of antioxidant activity. Hydrolysis of collagen and gelatin, whether by pineapple extract or bromelain enzyme, was found to enhance the antioxidant activity of the candy products. Variations in antioxidant activity can be affected by various factors, including the source of raw materials, amino acid composition, type of enzyme applied and extraction solvent used [14].

The Ferric Reducing Antioxidant Power (FRAP) assay operates based on the principle of reducing Fe^{3+} within the Fe^{3+}–TPTZ complex to the Fe^{2+}–TPTZ form through electron donation. The intensity of the resulting complex color is measured using spectrophotometric analysis and serves as an indicator of the reducing capacity of antioxidant compounds. The results of antioxidant activity measurements of both raw materials and candy products are presented in Fig. 6. The antioxidant capacity of raw materials prior to candy production ranged from 393.33 to 450.33 µmol/g. The data indicates that hydrolysis treatments, particularly using pineapple extract, enhanced antioxidant capacity compared to pure collagen or gelatin. Significant differences existed among the treatments with respect to the antioxidant activity of the raw materials (ANOVA, $P<0.05$). Likewise, antioxidant capacity measurements of the candy samples demonstrated statistically significant variations among treatments (ANOVA, $P<0.05$). The measured antioxidant capacity of candies measured using the FRAP method ranged from 335.33 to 382.33 µmol/g. All treatments exhibited strong antioxidant capacity, falling within the standard range of 100–400 µmol/g. The highest antioxidant capacity was observed in treatment P5, reinforcing the notion that hydrolysis plays a crucial role in breaking peptide bonds into smaller, bioactive fragments, thereby generating strong antioxidant activity [15].

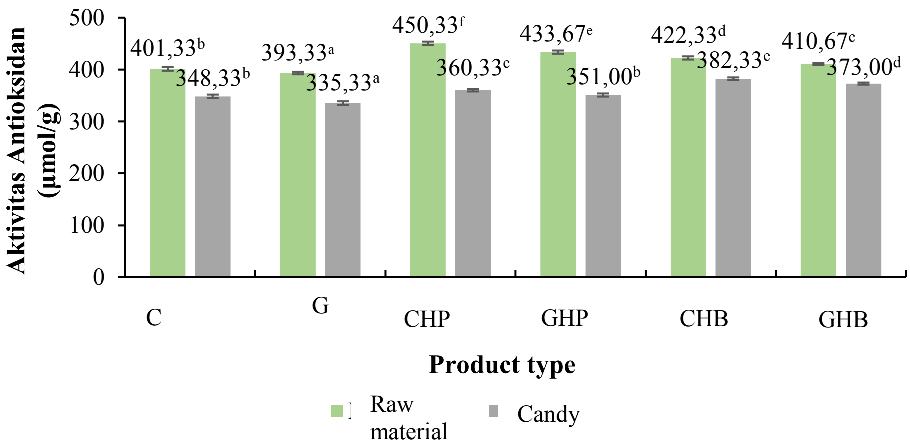

Fig.6. Antioxidant activity (FRAP) of raw materials and candy products Collagen (C); Gelatin (G); Collagen Hydrolysate with pineapple extract (CHP); Gelatin Hydrolysate with pineapple extract (GHP); Collagen Hydrolysate with bromelain enzyme (CHB); and Gelatin Hydrolysate with bromelain enzyme (GHB).

4 Conclusion

The differences in raw materials, namely collagen, gelatin, collagen hydrolysate and gelatin hydrolysate, had a significant influence on the characteristics as well as the antioxidant properties of the candy. Collagen hydrolysate produced through enzymatic hydrolysis with pineapple core was identified as the most appropriate raw material for candy formulation, as it demonstrated physicochemical properties that support overall product quality and safety in the finished product. The most favorable candy characteristics were observed in collagen based candy derived from this hydrolysate. Meanwhile, the strongest antioxidant activity was observed in collagen hydrolysate produced using commercial bromelain enzyme.

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