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Original Research Article

Characteristics of Natural Collagen of Freshwater Snail Flesh (*Pomacea paludosa*) Extracted with Bromelain Enzyme and Acid-Hydro-Extraction Method

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ABSTRACT

Freshwater snails (*Pomacea paludosa*) are aquatic biota abundantly available as a source of collagen. Collagen isolation can be done chemically and enzymatically with bromelain. This study aims to study the isolation of collagen enzymatically using bromelain enzymes and hydroextraction acid using acetic acid and to determine the physicochemical characteristics of freshwater snail flesh collagen. Collagen isolation is carried out in two stages: pretreatment and hydrolysis enzymatically and chemically using an acid solution. The stages include pretreatment (a) chemically using 0.1 M and 0.15 M NaOH at ratios of 1:8 and 1:10 and biologically using 10% rice husk charcoal with a soaking time of 24 hours. (b) Freshwater snail flesh collagen is extracted with 0.1 and 0.3 M acetic acid for 2 and 3 hours and hydrolysed with distilled water for 12 hours. (c) Enzymatic extraction with 3% and 4% bromelain for 3 and 4 hours. The best non-collagen protein results in chemical pretreatment came from using 0.1 M NaOH (1:8) at 105.88 ppm and 10% rice husk charcoal at 110.48 ppm. The research found that using 3-4% bromelain enzyme for extraction gave better collagen yield, protein content, and viscosity results than the acid hydro-extraction method. The FTIR test results indicate that swamp snail meat has collagen with a strong hydrogen triple helix structure in its bonds, which include amides A, B, I, II, and III. In the hydroextraction acid, the detected functional groups were amides A, B, and III.

Keywords: Natural collagen, Freshwater snails (*Pomacea paludosa*), Bromelain enzyme, Hydro acid extraction

Introduction

Collagen is a polypeptide and the main component of animal extracellular matrix (ECM), abundantly available in bones, skin, and other by-products in the flesh industry.[1,2] (Collagen is considered one of the most useful biomaterials in various industrial applications.[3] The main characteristic of collagen is the presence of a stable triple-helix structure that has three α polypeptide chains connected by hydrogen bonds, where each polypeptide chain contains one or more amino acid residues (Gly-XY); glycine (Gly) accounts for about 1/3 of all amino acids; X and Y are mostly proline and hydroxyproline.[4] Currently, most commercial collagen comes from mammals, especially cows and pigs, but both collagens are sometimes not accepted by some religious and ethnic communities, so their use is limited. In addition, there are biological contaminants such as bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), and foot and mouth disease (FMD).[5,6] Animal collagen is made up of large molecules curcumin and green chiretta.[8,9] that are stuck together with proteoglycans, glycoproteins, and other substances. The right extraction method for that material's specific bonds is needed to get the collagen out of the raw material. The method of pretreatment has a significant influence on the characteristics, physical, and chemical properties of collagen.[7,8]. Collagen extraction can be done chemically or as a combination of chemical and enzymatic.[9] Chemical extraction is usually done through an acid or base process. The acid process is more appropriate for raw materials that have a collagen structure with little cross-linking, such as pork and fish skin, while the base process is generally used for raw materials that have denser and more complex cross-linking, such as bones and cowhide.[10]. Furthermore, biological collagen extraction with the addition of enzymes is more promising in producing high-nutrient products. [11] In addition, enzymatic collagen extraction produces little waste, and the process is shorter but more expensive. The success of collagen extraction depends on pretreatment aimed at removing intra- and intermolecular cross-links, mainly involving lysine and hydroxylysine residues, ester bonds, and other bonds with saccharides.[12] Commonly used raw materials are basic solutions, such as sodium hydroxide (NaOH), lasting from several days to several weeks. [13] NaOH can penetrate thicker and more aggressive raw materials such as bovine ossein or shavings.[14] NaOH is better for pretreatment because it causes significant swelling, facilitating collagen extraction by increasing the mass transfer rate in the tissue matrix. Pretreatment aimed at removing non-collagenous proteins using NaOH at 0.01 mol/L OH and 0.1 mol/L OH concentrations. Deproteinisation of the skin and bones of bigeye snapper (*Priacanthus tayenus*) using 0.1 N NaOH 1:10 (w/v) for 6 hours followed by defatting with 10% butyl alcohol 1:10 (w/v) for 18 hours[8,15], then extracted with CH_3COOH 0.5 M 1:30 (w/v) for 24 hours.[16] Collagen extraction from aquatic animals has been widely carried out, including collagen extraction from sea cucumbers with acetic acid, producing a collagen yield of 1.50% (w/w).[17] Collagen from snakehead fish skin produced a yield of 16% (DM)[18], yellow tail fish skin extraction produced a yield of 18.4%

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(DM).[19] and mangrove snails (*Telescopium telescopium*) using acetic acid produced a yield of 1.08 + 0.21% and proline amino acids 9.21%. [20] Yield collagen of 11.91% was obtained from golden snails (*Pomacea canaliculata*) when extracted with sulphuric acid. [21] Different yield results are influenced by the extraction method and conditions used[22], so extraction optimisation is needed to produce collagen with the highest yield. Research information on collagen from freshwater snails is still minimal, especially for the *Pomacea paludosa* species. Freshwater snail flesh (*Pomacea paludosa*) is one of the abundant sources of collagen, and its use has not been optimal. This snail was chosen for collagen extraction because its population in swamp waters was abundant, and it reproduces quickly with a production cycle of 4-5 times a year, an egg-laying period of 18-128 days, and an egg-hatching rate of almost 80%.[23] The flesh of a freshwater snail is made up of rigid tissues, which makes it harder to extract collagen. First, the snail flesh needs to be treated with a base solution, and then it needs to be broken down with an acid or enzyme solution. Collagen extraction uses the acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) methods. This study aims to investigate the characteristics of freshwater snail collagen using a combination of the ASC method and hydro-extraction. The hydro-extraction method has several advantages, including shorter time, requiring little laboratory equipment, being produced continuously, having a high yield, little waste, and lower production costs. [24] The hydro-extraction method has been carried out to extract collagen from tilapia scales by soaking the scales in aquabidest at temperatures of 20 or 50 °C using a water bath for 2 hours.[25] The extraction of freshwater snail flesh with bromelain (BSC) was considered because bromelain works more actively on animal protein.[26] Few studies have been done on freshwater snail collagen from swamps, and the ones that have been done have only used acetone to extract collagen from *Achatina fulica* snails.[27] This study aims to isolate collagen enzymatically using bromelain enzymes and hydroextraction acid using acetic acid and to determine the physicochemical characteristics of freshwater snail flesh collagen. Research on the extraction and characterisation of freshwater snail collagen is important; besides contributing to science, it also aims to minimise the population of freshwater snails in the waters.

Materials and Methods

Animals and experimental protocol

The research stages include preparing, isolating, and characterising freshwater snail collagen *(Pomacea paludosa).* The freshwater snails used were 8 months old with an average freshwater snail weight of 110-120 g per head. The snails used in the study were obtained from the swamp waters of South Kalimantan (Borneo); geographically, South Kalimantan is between 114°19’33’’ - 116°33’28” East Longitude and 1°10”14” South Latitude, Indonesia which are pests for agricultural land. Before pre-treatment, the freshwater snails were cleaned and soaked in water at a temperature of 60℃, and then the snail meat was removed from the shell and cleaned.

Ethical approval

Ethical approval for the study was given by the Animal Care and Use Committee of Islam Kalimantan Muhammad Arsyad Al Banjary University, No. 12-KEP-UNISA.PPJ-2024

Sample Preparation and Pretreatment.

The freshwater snail flesh was separated from its shell, frozen, and transported to the laboratory. The snail flesh was cleaned, cut into approximately 1.5x1.5 cm sizes, and stored at -20ºC until the flesh was used. The collagen isolation method is based on a modified method. [8,28] It has three main steps: biological pretreatment with rice husk charcoal, chemical pretreatment with an alkali solution (NaOH), and biological pretreatment with NaOH. (1) Chemical pretreatment with NaOH removes minerals, fats, smells, colours, and non-collagen proteins. NaOH aims to eliminate non-collagen proteins and other impurities such as minerals, fats, odours, and pigments. The flesh of a mangrove snail was soaked in a 1:8 (w/v) NaOH solution with concentrations of 0.1 M and 0.15 M for 12 hours, with a new NaOH solution added every 2 hours. The sample was then washed with aquabidest until it was neutral, and the pH was found using a pH meter, Kromameter CR-310 (Minolta CR-310 Tokyo Japan). [29] The entire procedure was carried out at a temperature of 4°C. Also, it was decalcified with 0.5 M EDTA at pH 7.4 for 24 hours with a 1:8 (w/v) ratio and stirred all the time to eliminate any present minerals. The sample was then washed with aquadest before extraction. The sample underwent a biological pretreatment, which involved soaking it in rice husk charcoal at a concentration of 10% for 24 hours. Before being extracted, both the flesh and the pretreatment solution were tested using the Bradford method and BSA as a standard for dissolved protein[30].

Collagen extraction using a combination of acid-hydro-extraction and enzymatic methods.

Acid hydro-extraction

This method has been modified to use a 1:8 acetic acid solution and the ASC method to extract the flesh of a freshwater snail treated first (w/v). The concentrations of acetic acid used were 0.1 M and 0.3 M, with soaking times of 2 and 3 hours[31]. The flesh of the freshwater snail soaked in acetic acid was neutralised with aquabidest. It was then extracted using hydroextraction, which involves putting the sample and aquadest in a water bath at 40°C for 5 hours. The extraction results were filtered using filter paper to obtain the filtrate. The filtrate obtained was centrifuged for 30 minutes at a speed of 6000 rpm, and the extraction results were dried at a temperature of 40°C for 2 x 24 hours.

Enzymatic extraction. Freshwater snail flesh that had been treated was put into a culture bottle. A bromelain enzyme solution with 3% and 4% concentrations was added in ten millilitres. The sample was left to soak for three to four hours at 55°C in a shaker incubator. It was then put into a water bath at 80°C to stop the enzyme from working. The sample was then put into a propylene tube, and cold EtOH was added for precipitation. All samples were centrifuged at 10,000 rpm at a temperature of 4°C for 10 minutes.

The design used a nested RAL pattern where the concentration was nested in the soaking time, and the soaking time was nested in the pre-treatment.

Collagen yield: Collagen yield was obtained from the comparison of the dry weight of the collagen produced with the weight of the freshwater snail flesh that has been pretreated using the equation[32]:

$$\text{Collagen Yield (\%)} = \frac{\text{collagen dry weight}}{\text{Weight of pretreated snail flesh}} \text{ x } 100 \quad (1)$$

Nutrient content includes protein. The Bradford method measured non-collagen and collagen protein levels in freshwater snails.[30] Bovine serum albumin (BSA) was used for protein standards. Preparation steps: 100 µL of NaOH was placed into a test tube. Then, 5 mL of Bradford reagent was mixed with it and allowed to stand at room temperature for 5 minutes. After that, the absorbance was measured using a spectrophotometer at 610 nm. Standard solutions with concentrations of 0, 400, 500, 600, 800, 1,000, and 1,200 µg/mL were used for the measurements. Standard protein measurements and samples were carried out in triplicate. The ash and fat content were measured using AOAC. [33] Amino acid composition was measured by HPLC. [34]

Viscosity. Freshwater snail collagen was dissolved in an acetic acid solution with 0.1 M and 0.3 M concentrations. The viscosity of the homogeneous collagen solution was then measured using a viscometer (LVT Model Brookfield, USA) with spindle No. 2 at 100 rpm. The measurement results were multiplied by a conversion factor and expressed in centipoise units (cP).[35] Functional groups with FTIR[38]. Infrared spectrum analysis uses wave numbers ranging from 4000 to 400 cm^{-1} using an infrared spectrophotometer (Nicolet, Thermo Electron, USA). Samples of snail meat collagen were mixed with potassium bromide (KBr) in a ratio of 1:30 and moulded into pellets. The resulting FTIR spectrum shows the wavenumber absorption peaks of the test sample. The functional group of the test sample was identified by observing the absorption peak in the wave number related to the protein’s functional group.

Results and Discussion

Freshwater snail flesh *(Pomacea paludosa)* has a dry matter of 36.51±0.48% to 39.47±1.45%. Mangrove snails *(Telescopium telescopium)* have a dry matter of 19.93%±2.25.[20] Red sea snails

(Cerithidea obtusa) have a dry matter of 24.56±0.48% [37], and gamma sea cucumber flesh has a dry matter of 6.16±0.10%.[17] The nutrient content of freshwater snail flesh before pretreatment is presented in Table 1. The results of the proximate test on freshwater snail flesh contained 67.35% DM crude protein and 11.55 ± 0.08% ash content. This analysis result is higher than the results of the study on *Pila ampulacea* snails containing 22.70% dry matter, 19.04% protein, and 3.15% ash, and 19.04% protein[38], and *Pomacea canaliculata* with a dry matter content of 23.68%, 10.67% crude protein, and 5.54% ash.[39] This difference is due to differences in species, the age of the snails, the season of sampling, and the habitat of the snails used. The ash content of freshwater snail flesh was higher in this study, indicating that the mineral content of freshwater snail flesh was relatively high.

The concentration of non-collagen protein in freshwater snail flesh after being soaked using NaOH solution and rice husk charcoal flour is presented in Table 2. The non-collagen protein of freshwater snail flesh that was soaked for 12 hours in 0.1 M base solution, a 0.15 M solution with a 1:8 and 1:10 (w/v) ratio, or a rice husk charcoal solution changed significantly. Soaking in NaOH solution for 12 hours was chosen because that was the best time to break down non-collagen protein.[40] Soaking in rice husk charcoal solution for 24 hours was the best time for breaking down collagen protein.[41] Pertiwi's study from 2006 showed that soaking something in a 0.1 M NaOH solution for 12 hours can lower the amount of dissolved protein. Soaking using NaOH, if done for more than 12 hours, can cause collagen protein loss.[42] The NaOH pretreatment solution's protein content decreased with increasing soaking time. This proves that non-collagen proteins break down faster in NaOH solution as the soaking time increases.[43] High NaOH concentrations with long soaking times will increase the amount of dissolved protein. [44]

Table 1. Nutritional content of freshwater snail flesh (*Pomacea paludosa*) before pre-treatment

Nutrition content	Composition (dry basis %)
Dry matter (%)*)	92.12±0.03
Ash (%)*)	11.55±0.08
Crude Protein (%)*)	67,35±0.04
Crude fiber (%)*)	1.74±0.04
Crude fat (%)*)	3.20±0.09
Fosfor (%)	0.46±0.03
Calsium (%)	1.22±0.04
Methionine (mg/kg)**)	12226.39±0.02
Lysine (mg/kg)**)	6554.98±0.04

Description:(*Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry, Brawijaya University, Malang Indonesia, 2024 **) Healthy Animal Laboratory, Malang, Indonesia, (2024)

Table 2. The protein content of freshwater snail flesh after treatment using NaOH and rice husk charcoal flour.

Pretreatment	Concentration of non-collagen protein (ppm)
NaOH 0.1 M (1:8), 12 hours	105.88±[d]
NaOH 0.1 M (1:10) 12 hours	63.82[a]
NaOH 0.15 M (1:8), 12 hours	94.91[c]
NaOH 0.15 M (1:10) 12 hours	84.04[b]
Freshwater snail flesh: Rice husk charcoal (10% from material), 24 hours	110.48[d]

Description: Numbers followed by different letters indicate significant differences at DMRT 5% (1: 8 and 1: 10 are the ratios of freshwater snail flesh: NaOH solution).

The use of alkaline solutions, both NaOH solutions and rice husk charcoal, aims to remove non-collagen components, including enzymes, fibrinogen, fat, minerals, pigments, and odours. Because NaOH and rice husk charcoal are alkaline, so they swell the flesh of freshwater snails. This makes breaking down non-collagen proteins in the flesh easier,[45] and protects the collagen content from the effects of endogenous proteases during extraction.[46] It has been shown that NaOH can break down the telopeptide region of collagen molecules before they are treated.[47] So, swelling occurs when snail flesh mixes with NaOH, breaking apart the OH group attached to the protein. The development of freshwater snail flesh is caused by the telopeptide region of the collagen molecule becoming open so that water can remove non-collagen proteins from the collagen matrix. The migration of non-collagen proteins causes the increasingly turbid NaOH soaking water.[48] The swelling of the freshwater snail flesh caused by NaOH can increase the tissue matrix's mass transfer rate in the extraction process and assist the extraction process enzymatically and chemically.[8] The alkaline solution functions to dissolve non-collagen proteins and saponify fat bound to collagen fibres so that it will come out of the snail flesh.[15] The snail's flesh will swell, breaking the tropocollagen fibre structure into procollagen by breaking non-covalent bonds. This makes it easier for the collagen to dissolve during the extraction process.

The collagen yield shows the effectiveness of the extraction process. In this study, the extraction (chemical) used the acetic acid hydro extraction method and enzymatically used the bromelain enzyme (Table 3). The amount of collagen extracted from freshwater snail flesh using bromelain enzyme at a concentration of 3% to 5% for 3 hours was higher than the amount extracted chemically using acetic acid (ASC hydroextraction method) at 29.39 ± 0.05% to 30.72 ± 0.50%. This indicates that enzymatic extraction has advantages over chemical extraction. Enzymes are specific in breaking peptide bonds to produce bioactive peptides with a higher probability.[49] The pepsin enzyme (PSC) combination of 0.7 M acetic acid to extract Nilem Fish Skin made 6.18% collagen[16]. Similarly, a study found that using PSC to extract skin from a sharpnose stingray (Dasyatis zugei) made 34.84±1.26% collagen, while the ASC method only made 20.48±4.41% collagen.[32] There was a higher collagen yield (9.79±0.12%–14.48±0.06%) when acetic acid solutions of 1:8 and 1:10 (w/v); 0.1 M and 0.3 M were used for 2 and 3 hours, compared to that which used snails from the *Pomacea canaliculata* species and hydrochloric acid, phosphoric acid, and acetic acid at concentrations of 2.5%, 5%, and 10%, respectively, mineralised for 12, 24, and 36 hours; the collagen yield was 0.94–11.91%. [21]. Chemical collagen extraction (hydro-extraction acid) at an acetic concentration of 0.3 M in the study consistently produced higher yields of freshwater snail collagen both at extraction times of 2 hours or 3 hours (12.48 ± 0.22% - 14.48 ± 0.06%). The results of this study are in line with the research of [21], where the higher the solvent concentration, the higher the yield of collagen from snail flesh. More H+ ions in the solvent break up hydrogen bonds and cause the yield to rise.[50,51,52] The more H^+ ions that cause damage to the hydrogen bonds of collagen, the more it will be broken, so the higher the collagen stretching and thermal breaking of the peptide bonds. This is thought to be because there are more H^+ ions, which break down collagen. The longer the extraction, the more collagen breaks down because H_2O molecules denature the hydrogen bonds in tropocollagen, which makes the triple-helix molecule less stable. When collagen coils are loose, heat spreads evenly during extraction, breaking more peptide bonds. The more peptide bonds are broken, the more yield is obtained. In addition, collagen that is increasingly stretched will get a longer collagen biopolymer chain. Collagen quality can be seen from its viscosity value.

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Table 3. Chemical composition, yield collagen and viscosity of freshwater snail flesh (*Pomacea paludosa*) with different pretreatment and extraction methods

Pretreatment	Methods Extraction	Soaking times extraction (hours)	Concentration	Yield collagen (dry basis %)	Viscosity (mPa.S)	Crude Protein (dry basis %)	Ash (%)	Fat (dry basis%)
Freshwater snail flesh: NaOH 0.1 M (1:8), soaking for 12 hours	Acid Soluble Collagen (ASC) hydro extraction	2	0.1 M	10.32±0.09^{a}	12.67±0.47^{b}	57.54±0.33^{b}	17.58±2.56^{cd}	1.27±0.13
			0.3 M	13.35±0.07^{ab}	11.00±0.82^{a}	56.37±0.97^{b}	18.74±0.41^{d}	1.40±0.18
		3	0.1 M	10.52±0.26^{a}	11.33±0.47^{a}	53.82±0.53^{ab}	17.05±0.32^{cd}	1.42±0.13
			0.3 M	14.49±0.16^{b}	13.33±0.47^{cd}	51.70±0.25^{a}	20.04±1.52^{e}	1.04±0.05
Freshwater snail flesh: NaOH 0.15 M (1:8) 12 jam	Acid Soluble Collagen (ASC) hydro extraction	2	0.1 M	9.79±0.12^{a}	13.33±0.47^{cd}	57.72±0.06^{b}	21.06±0.50^{e}	1.22±0.14
			0.3 M	12.48±0.22^{a}	13.00±0.82^{c}	58.62±0.37^{b}	18.62±0.37^{d}	1.70±0.17
		3	0.1M	14.48±0.06^{b}	14.67±0.47^{d}	59.51±0.23^{b}	19.83±0.28^{e}	1.83±0.28
			0.3M	14.47±0.38^{b}	12.00±0.82^{ab}	57.77±0.32^{b}	16.87±0.46^{c}	1.21±0.02
Freshwater snail flesh: Rice husk charcoal (10% from material)	Bromelain Soluble Collagen (BSC)	3	3%	30.72±0.50^{d}	16.00±0.82^{e}	67.57±0.28^{c}	14.12±0.09^{b}	1.15±0.41
			4%	29.39±0.05^{d}	13.67±0.47^{cd}	65.21±0.09^{c}	15.71±0.36^{bc}	1.02±0.06
		4	3%	20.61±0.01^{c}	14.33±0.47^{cd}	64.33±0.27^{c}	14.21±0.57^{b}	1.46±0.39
			4%	19.59±0.61^{c}	13.67±1.25^{cd}	61.63±0.28^{c}	12.05±0.20^{a}	1.07±0.02

Description: Numbers followed by different letters indicate significant differences at DMRT 5%.

Table 4. Amino acid composition of freshwater snail flesh collagen

Amino Acids	Quantity (%)			
	A Chemical method Acid-Hydro Extraction with acetic acid 0,3 M; extraction time 3 hours)	B Chemical method Acid-Hydro Extraction with acetic acid 0,3 M; extraction time 4 hours)	C Enzimatical Extraction with bromelain extraction time 3 hours, concentration 3%	D Enzymatical Extraction with bromelain extraction time 4 hours, concentration 3%
Lysin	5.23	5.28	5.41	5.50
Arginine	6.12	6.11	6.05	5.96
Aspartat	5.23	4.80	4.46	3.76
Glutamate	9.74	9.84	9.94	9.26
Serin	5.64	5.69	5.73	5.50
Threonine	5.23	5.28	4.78	5.41
Glycine	12.48	13.41	15.78	15.58
Tyrosine	4.19	3.66	3.95	4.12
Proline	8.13	8.21	8.60	8.97
Valin	3.62	2.60	3.82	2.93
Leucine	6.44	6.18	6.37	5.96
Isoleusin	2.58	2.11	3.18	1.83

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Penilalanin	1.61	1.63	2.23	1.19
Histidine	6.85	7.72	7.96	6.42
Alanine	13.29	14.63	14.23	16.50
Methionine	3.62	2.85	3.51	3.11

Description:
A. Pre-treatment Freshwater snail flesh: NaOH 0.1 M (1:8), soaking 12 hours; Extraction time 4 hours, concentration 0.3 M.
B. Pre-treatment Freshwater snail flesh: NaOH 0.15 M (1:8), soaking 12 hours; Extraction time 3 hours, concentration 0.3 M
C. Freshwater snail flesh: Rice husk charcoal (10% from material)
D. Freshwater snail flesh: Rice husk charcoal (10% from material)

Table 5. Characteristic of collagen functional groups

Functional groups	Sample/treatment				Absorption area	Characteristic Amide
	A Chemical method Acid-Hydro Extraction with acetic acid 0,3 M; extraction time 3 hours)	B Chemical method Acid-Hydro Extraction with acetic acid 0,3 M; extraction time 4 hours)	C Enzimatical Extraction with bromelain concentration 3%, extraction time 3 hours,	C Enzimatical Extraction with bromelain , concentration 3% extraction time 4 hours		
Amida A[42]	3196.05	3203.29	3298.23	3275.13	3500-3100	NH *stretching*
Amida B[62]	2920.87	2335.12	2935.07	2929.60	2935-2915	CH2 *asymmetric stretching*[2]
Amida I[62]	Not detected	Not detected	1665.25	1631.78	1690-1600	C=O *stretching*
Amida II[62]	Not detected	Not detected	1568.41	1535.34	1575-1480	NH *bending*, CN *stretching*
Amida III[62]	1242.16	1247.00	1265.63	1242.16	1301-1229	NH *bending*, CN *stretching*[3]

Description:
E. Pre treatment Freshwater snail flesh: NaOH 0.1 M (1:8), soaking 12 hours; Extraction time 4 hours, concentration 0.3 M
F. Pre-treatment Freshwater snail flesh: NaOH 0.15 M (1:8), soaking 12 hours; Extraction time 3 hours, concentration 0.3 M
G. Freshwater snail flesh: Rice husk charcoal (10% from material)
Freshwater snail flesh: Rice husk charcoal (10% from material)

The freshwater snail flesh collagen had the highest viscosity when extracted with 3% bromelain enzyme for 3 hours (16.00 ± 0.82 cP). On the other hand, the best viscosity was found when it was pretreated with 0.15 M (1:8) NaOH for 12 hours, extracted for 2 hours, and treated with 0.1 M acetic acid (14.67 ± 0.47 cP). The difference in viscosity value in the amino acids in fish flesh causes this phenomenon. The lower the proline amino acid, the lower the viscosity value produced. Proline amino acid can affect the mechanical properties of freshwater snail flesh, namely gel strength and melting point.[53] The viscosity of freshwater snail flesh collagen is higher than that of gamma sea cucumber meat collagen, 5.37 ± 0.06 cP[43]. However, it is significantly lower than the viscosity of mackerel scad (17,65 cP)[70].

The acid composition of freshwater snail flesh is presented in Table 4. The results showed that the amino acid proline of freshwater snail flesh extracted with a bromelain enzyme was higher than the amino acid of freshwater snail flesh extracted chemically. Proline amino acid functions to help fish skin and bones undergo the formation of collagen.[54.] Viscosity is closely related to the length of the amino acid chain. The longer the amino acid chain, the higher the viscosity value. Freshwater snail flesh collagen comprises polypeptide chains with amino acids typical of the collagen structure, namely glycine, proline, and alanine (Table 4). The study results showed that collagen has low levels of tyrosine and histidine and does not contain tryptophan and cysteine[5]. Amino acids of collagen from freshwater snail flesh are presented in Table 4. Collagen consists of three polypeptides twisted together to form a triple helix composed of three primary amino acids: glycine, proline, and alanine. Glycine is almost a third of the collagen structure; the rest are other amino acids. [56] The amino acid glycine affects hydrogen bonds and forms the alpha triple helix chain in collagen. [57] Glycine is found in the third position of the collagen triple helix amino acid structure (Gly-XY). Position X is proline, and position Y is hydroxyproline, forming a triple helix. [58] Glycine plays a role in reducing steric hindrance and triggering hydrogen bond interactions in the helical chain. The proline content of flesh snail collagen is 8.13-8.97%, relatively lower than proline in mangrove snails (9.21%).[20] Proline is an amino acid that plays a role in maintaining the integrity of the collagen structure [59]; high proline levels can increase thermal stability.[60] The proline and hydroxyproline amino acid rings help stabilize the triple helix at high temperatures and keep the polypeptide chain from changing shape. [24] The amino acids glycine and proline are found in high amounts in type 1 collagen, while tyrosine and histidine are found in low amounts. [61] Collagen from fish that live in cold places has lower melting points and is less stable at high temperatures. It also has less proline and hydroxyproline than collagen from fish that live in warmer areas.[16]

The FTIR investigation found five large bands characteristic of amides (amides A, B, I, II, and III) in freshwater snail flesh. FTIR analysis was performed to show that collagen has been formed. Analysis of the presence of collagen using FTIR provides five typical significant absorptions, namely the presence of amide A in the region of 3500-3100 cm^{-1}. Amide B at 2935-2915 cm^{-1}, amide I at 1690-1600 cm^{-1}, amide II at 1575-1480 cm^{-1}, and amide III at 1301-1229 cm^{-1} .[42,62,63,64] The characteristics of freshwater snail flesh collagen with bromelain enzyme and acetic acid-hydro extraction are presented in Table 5.

Soluble collagen of freshwater snail flesh extracted with bromelain enzyme at concentrations of 3% and 4% contains amide group I. Amide group I is characterised by amino acids glycine and proline in the collagen triple helix. [65.] Amide group II is characterised by NH groups bond paired with CN stretching groups. [66] Amide group II is in the absorption region with a wave number of 1,575-1,480 cm^{-1} [63]. Freshwater snail flesh extracted with bromelain enzyme has amide II groups with wavelengths of 1568.41 cm^{-3} and 1535.34 cm^{-3} (Figure 3 and 4). Collagen from freshwater snail flesh extracted with 0.1 and 0.3 M acetic acid was found with the amide III group instead of the amide group. The collagen produced contains an amide III group, according to the standard. Amide III group with a wave absorption region of 1301-1229 cm^{-1}.[63] While the amide II group is identified by CN paired with NH, the amide III group is the same. It is connected to the triple helix structure of collagen. The form of collagen structure was determined in amide III, at an absorption of 1301-1229 cm^{-1}, which is collagen with a triple helix structure[64]. This is in line with the absorption of research samples, which appeared in almost all treatments, both enzymatic and chemical extraction, which indicate a research sample being collagen with a triple helix shape. The amide group that wasn't found could be because of the chemicals in the freshwater snail flesh, the acetate concentration used, or how it was treated before extraction. Based on the five absorptions found in the FTIR spectrum of the research sample, the sample was confirmed as collagen or has collagen.

The results of the isolation of collagen from freshwater snail flesh, both enzymatically and chemically, with the ASC hydro-extraction method, contained amide A at the absorption peaks of 3274.13 cm^{-1} and 3298.23 cm^{-1} (enzymatic extraction) and 3196.05 cm^{-1} and 3203.29 cm^{-1} (chemical extraction) with an absorption region (3350-3550 cm^{-1}) that has the characteristics of NH stretching.[67] The position of amide A is changed by OH group components, which proves that water molecules are actively involved in collagen.[67] In this research, amide A showed up at wave numbers 3196.05 cm^{-1} and 3203.29 cm^{-1} for collagen precipitation using the 0.3 M acid hydro-extraction method and an extraction time of 3–4 hours (figure 1 and figure 2).

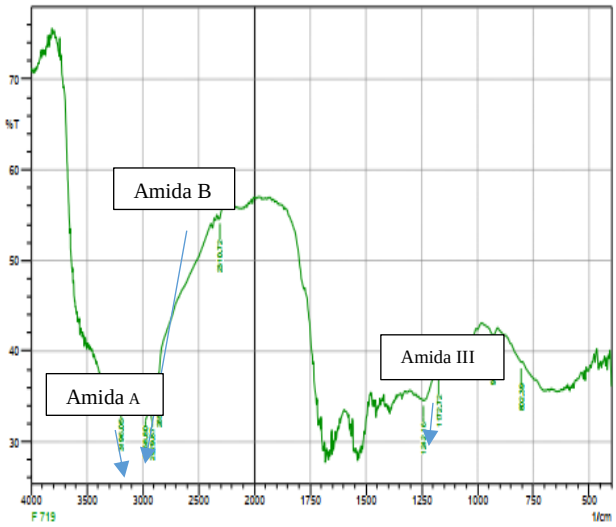


Figure 1. FTIR spectra of collagen from freshwater snail flesh using the acid hydroextraction method (0.3 M acetic acid, extraction time 3 hours)

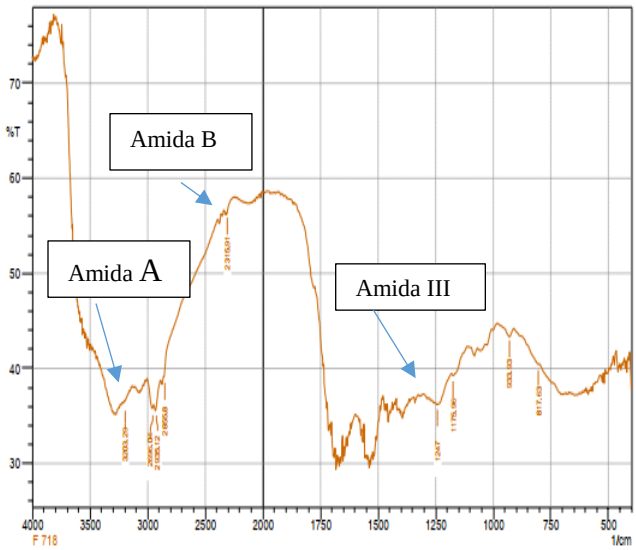


Figure 2. FTIR spectra of collagen from freshwater snail flesh using the acid hydroextraction method (0.3 M acetic acid, extraction time 4 hours)

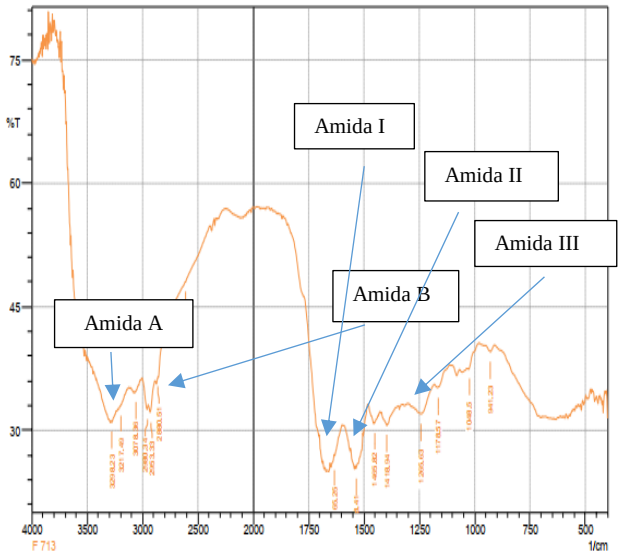
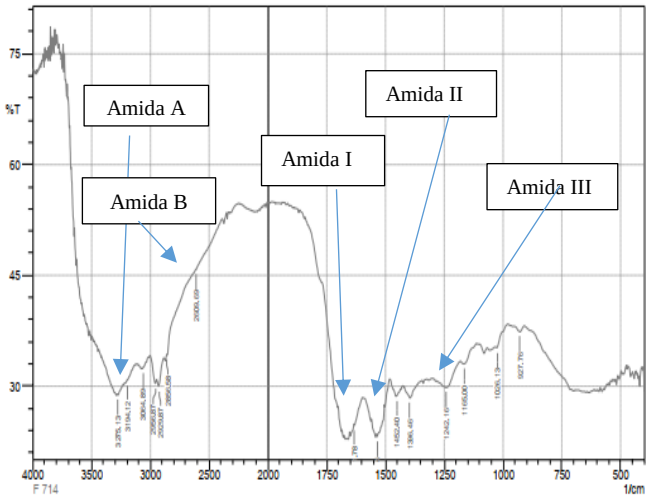


Figure 3. FTIR spectra of collagen from freshwater snail flesh using the enzimatic method (3% bromelain enzim, extraction time 3 hours)

While the enzymatic extraction of freshwater snail flesh collagen with 3% bromelain enzyme with an extraction time of 3 hours and 4 hours, precipitation occurred at waves 3298.23 cm^{-1} and 3275.13 cm^{-1}. The presence of OH group components changes the position of amide A. This shows that water molecules are actively involved in collagen. Amide B in the research results showed an absorption number of waves in the 2920.87-2935.12 cm^{-1} range. [62.] The peak of amide B wave number absorption was 2935-2915 cm^{-1}, which has the characteristics of asymmetric CH_2 stretching CH_2. In the isolation of collagen from the skin of parang-parang fish using the hydro-extraction method, amide A shifted to the right or a lower frequency. The NH group could cause this in the peptide involved in hydrogen bonds in the extraction process with water[68], but amide did not show a significant difference. All collagen samples have amide B peaks, indicating the interaction of -NH groups between peptide chains. The shift and difference in amide wave numbers are influenced by differences in treatment and extraction time.[35.] Figure 1 and Figure 2 show that amide B shows up at wave numbers 2920.87 cm^{-1} and 2335.12 cm^{-1} when collagen is precipitated using the 0.3 M acid hydro -extraction method and an extraction time of 3 to 4 hours for each. The extraction of freshwater snail flesh using the bromelain enzyme results in collagen precipitation at waves 2935.07 cm^{-1} and 2929.60 cm^{-1} (figures 3 and 4).



We use wave 2925 cm^{-1} as the standard for the collagen C=O group, which is a typical functional group of collagen components. [63] Thus, it can be concluded that the secondary structure as a marker of collagen is possessed by golden snail collagen. The results of the FTIR tests are similar to collagen from mammals in that they both have aromatic functional groups like OH, CH, C=O, NH, and CH.[69]

Conclusion

The research results, both chemically using the acid hydroextraction method and enzymatically using the bromelain enzyme, show that freshwater snail flesh contains collagen with the absorption of amide III as a marker for the presence of collagen. The highest yield was obtained from collagen extracted using the bromelain enzyme at a 3-4% concentration with an extraction time of 3 hours, producing a yield of 29.39 ± 0.05 to 30.72 ± 0.50 yield. Overall, the physicochemical properties of freshwater snail collagen depend on the pretreatment of freshwater snail flesh before being extracted into collagen.

Conflict of interest

The author reports no conflicts of interest in this work.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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