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Original Paper

Physicochemical Properties of Apple Snail Protein Hydrolysate (*Pila ampullacea*) and its Potential as Flavor Enhancer

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***Abstract*—Apple snail is a source of animal protein that has a high enough protein. Protein can be hydrolyzed by natural proteases, which one is bromelain. Enzymatic hydrolysis can produce hydrolysates containing peptides and amino acids that contribute to umami taste. The purpose of this study was to determine the effect of hydrolysis time and bromelain concentration on the physicochemical properties of apple snail hydrolysate. The optimal result in this study were used as natural flavor enhancer. This study used a completely randomized design with two factors. Factor I was the hydrolysis time (6 hours, 12 hours, and 18 hours) and factor II was the concentration of the bromelain (5%, 10%, and 15%). Based on the results showed that apple snail hydrolysate was influenced by hydrolysis time and bromelain concentration. The optimal result in this study was 18 hour hydrolysis treatment with 15% bromelain enzyme concentration had a yield 68.16%, degree of hydrolysis 72.09%, soluble protein 9.03%, total peptide 10.84 mg/mL, and glutamic acid 107.47 ppm. The application of apple snail protein hydrolysate give characteristics of flavor enhancers as follows: soluble protein 7.76%, glutamic acid 99.42 ppm, solubility 94.79%, water absorption 5.80 mL/g, hedonic test color 4.10 (neutral), aroma 5.05 (like slightly), and taste 5.10 (like slightly).**

Keywords*—Apple snail, protein hydrolysate, bromelain, flavor enhancers

I. INTRODUCTION

The apple snail (*Pila ampullacea*) is a freshwater mollusc that usually found in paddy fields, rivers, swamps, and lakes. Apple snails are often considered as pests that can reduce agricultural production in Indonesia. The damage due to snail pests can reach 10-40%, the snails need to be removed or managed further [1]. Even though it is considered as pest, apple snails have a high nutritional content as follows 10.67% protein, 76.32% water content, and 5.54% ash [2].

Hydrolysis process depends on the conditions during the hydrolysis. According to [3], physicochemical properties of protein hydrolysate are affected by the degree of hydrolysis, substrate types and protease enzyme. One of protease enzyme that can be used for protein hydrolysis is bromelain. Bromelain is a protease enzyme found in pineapple (*Ananas comosus* L.). Bromelain in pineapple flesh is exist from immature fruit until the fruit is ripe. Bromelain belongs to the sulfhydryl protease enzyme group which is able to decompose the molecular structure of proteins into amino acids [4]. The optimum conditions for the bromelain enzymatic reaction from ripe pineapple flesh were achieved at pH 6.5 at a temperature of 50°C [5]. In addition to enzymes, hydrolysis process is also influenced by time. The longer hydrolysis time can provide time for protease enzyme to hydrolysis the substrate (polypeptide) into peptides and free amino acids.

Protein hydrolysate can be used as flavor enhancer due to its short chain peptides and free amino acids content, especially glutamic acid which contribute to umami taste of protein hydrolysate. According to [6], glutamic acid was highly amount in all the mollusc species, based on the study shows that apple snail (*Pila globosa*) has glutamic acid content 5.1%. Several studies related to flavor enhancer products from protein hydrolysates have been carried out, such as flavor enhancer from jellyfish protein hydrolysates [7], common barb fish [8] and squid [9].

This study aims to determine the physicochemical properties of protein hydrolysate based on the effect of hydrolysis time and bromelain enzyme concentration. The best result on this study can be used to produce flavor enhancer from apple snail protein hydrolysate. The result of this study expected to enhance the economic value of apple snail and the flavor enhancer can be applied to other processed products.

II. MATERIALS AND METHODS

A. Materials

The materials used were apple snails (*Pila ampullacea*) obtained from Tebel, Sidoarjo, Indonesia, pineapple fruit, arabic gum, maltodextrin, salt, garlic powder, and sugar. The chemicals used were aquadest, bovine serum albumin (Merck), NaOH (Merck), trichloroacetic acid (Merck), $CuSO_4$ (Merck), HCl (Merck), phosphate buffer pH 7.5 (Merck), Na_2CO_3 (Merck), Na.K tartrate (Merck), ninhydrin reagent (Merck), Kjeldahl tablet (Merck), Follin Ciocalteu reagent (Merck), $FeCl_3$ (Merck), ascorbic acid (Merck), casein (Sigma Aldrich), L-glutamate (Sigma Aldrich), L-tyrosine (Sigma Aldrich), DPPH (Sigma Aldrich), 96% ethanol (Fulltime), methanol (Fulltime), and $K_3[Fe(CN)_6]$ (Pudak).

B. Procedure

Production of crude bromelain enzyme extract

Pineapple fruit was peeled to washed clean and cut into small pieces. Then, the flesh of the fruit was mashed using a blender with the addition of aquadest (1:1). The crushed pineapple was then filtered with a filter cloth. The filtrate was filtered again using filter paper to obtain crude bromelain enzyme extract and was analyzed on the enzyme yield [10] and protease activity [11].

Production of hydrolysate protein from apple snail

Apple snail meat was washed with flowing water and then weighed 100 g (Fig. 1). Next, the meat was mixed with aquadest (2:1) and mashed using a blender. Furthermore, bromelain enzymes (5%, 10% and 15%) were added to the mixture and hydrolyzed at 50°C for 6 hours, 12 hours, and 18 hours [41]. The enzyme was inactivated at 80°C for 15 minutes. Supernatant were separated by centrifugation at 3000 rpm for 30 minutes. The supernatant was stored in a refrigerator. The protein hydrolysate was analyzed on yield [12], degree of hydrolysis [8], soluble protein content [13], peptide concentration [14], and glutamic acid [15].

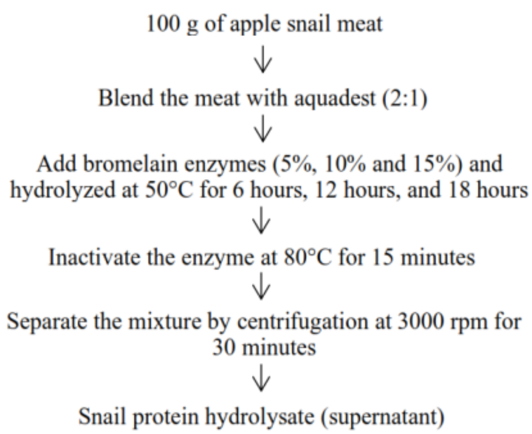

Fig. 1. Flowchart of snail protein hydrolysate production

Production flavor enhancers from protein hydrolysate (best result)

Take 80% of supernatant and mixed with 10% of the gum arabic and maltodextrin (1:4), 1% sugar, 0.5% salt, 0.5% garlic powder, and then mixtured by magnetic stirrer (6000 rpm, 20 minutes). Next, the mixture dried in cabinet dryer at 60°C for 4 hours to obtain flavor enhancer powder. Flavor enhancer was analyzed on soluble protein content [13], glutamic acid [15], solubility [16], water absorption [17], and organoleptic using hedonic test [18][19] .

C. Statistical Analysis

This study used a completely randomized design (CRD) with two factors. Factor I was the hydrolysis time (6 hours, 12 hours, and 18 hours) and factor II was the concentration of the bromelain (5%, 10%, and 15%). Each sample was measured in triplicate. The data were processed using ANOVA to determine the effect of each treatment. Significantly different results were further tested by *Duncan's Multiple Range Test* (DMRT) 5%.

III. Results and Discussion

A. Composition of Raw Materials

The main raw materials used in this study was snail flesh. Analysis on the snails flesh are proximate analysis which includes moisture content [20], ash content, and protein content [12]. The results of snail flesh analysis are shown in Table 1.

TABLE I. THE COMPOSITION OF SNAIL FLESH

Compounds	Composition (%)
Moisture	77.30 ± 0.43
Ash	3.15 ± 0.04
Protein	19.04 ± 0.07

The content of snails flesh obtained the following results: moisture 77.30%, ash 3.15%, and protein 19.04%. These results are slightly higher than [2] on the proximate composition of apple snail which moisture content was 76.32%, ash 5.54%, and protein of 10.67%. Differences result could be caused by species, age, season, nutritional values and environmental conditions that affect snail composition [21].

Higher moisture content may due to different size of snails, age, and environmental habits. Higher ash content may due to it habitat. According to [2], the aquatic snails absorb more minerals from water as rivers as chemical waste effluent to some industries may increase the absolute minerals in the water. Higher protein content may due to the feeding habits, environmental, and pre-treatment process. In this study, shells and flesh were separated by boiling in few minutes, while [2] using dehydrated process in an oven (60°C, 24 hours) which may causes protein denatured and the protein content became lower.

In addition to snail, the main raw material which important in this study is crude bromelain enzyme. Analysis of crude bromelain enzyme is a yield and protease activity. The results are shown in Table 2.

TABLE II. THE ANALYSIS OF CRUDE BROMELAIN ENZYME

Parameters	Results
Yield (%)	79.35 ± 0.39
Protease activity (Units/mL)	4.89 ± 0.06

The results showed that crude enzyme bromelain obtained a yield 79.35% and protease activity 4.89 Units/mL. These results are higher than [22] on the bromelain enzyme activity of pineapple flesh, which is 4.71 Units/mL. Differences in results were due to several factors, such as age and maturity level of pineapple flesh. [23] stated that the level of maturity greatly affects the proteolytic activity of crude bromelain enzyme from pineapple fruit. The more pineapple is ripe, the more the bromelain enzyme content will be. But otherwise, activity decreases at a certain level of maturity.

B. Degree of Hydrolysis

The degree of hydrolysis (DH) is an important parameter to control the hydrolysis process [40]. Correlation between hydrolysis time and bromelain enzyme concentration on DH of apple snail protein hydrolysates are shown in Fig 2.

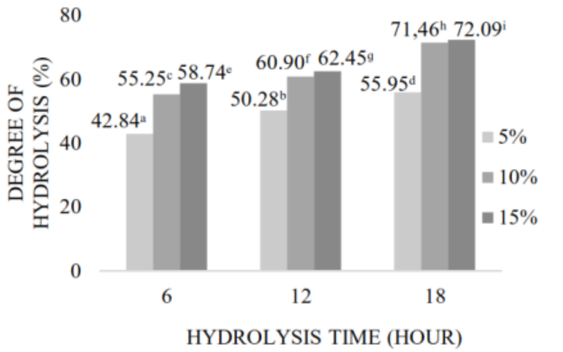

Fig. 2. Degree of hydrolysis of snail protein hydrolysate with hydrolysis time and bromelain enzyme concentration

DH indicates the percentage of peptide bonds that released during hydrolysis. The results of DH in this study were between 42.84 to 72.09%. The results were higher than [24] which apple snail hydrolyzed by papain enzyme have DH within 23.73 to 60.29%. The longer hydrolysis time can provide time for the bromelain enzyme to work optimally to break down protein into short chain peptides and amino acids so as to increase the DH.

[25] stated that degree of hydrolysis is influenced by the effectiveness of the enzyme to hydrolyze intermolecular protein peptides. DH increased when the concentration of bromelain enzyme increased, some peptides were released during hydrolysis by enzymes into smaller amino acids and peptides due to the increase of bromelain concentration [26].

C. Yield

Yield is a parameter that related to the effectiveness of the hydrolysis process which indicates the amount of hydrolysis product. Correlation between hydrolysis time and bromelain enzyme concentration on yield of apple snail protein hydrolysates are shown in Fig 3.

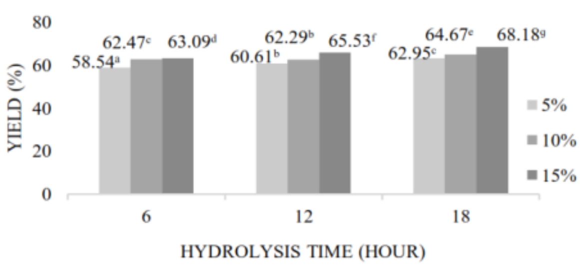

Fig. 3. Yield of snail protein hydrolysate with hydrolysis time and bromelain enzyme concentration

The yield in this study ranged between 58.54 to 68.8% which higher than [27] on golden apple snail hydrolysate using 2% of alcalase enzyme and 159 min of hydrolysis time has a yield 12.62%. The differences of the yield may due to the species of snails, type of enzyme and condition of hydrolysis. Each snail has different amount of protein content and the type of enzyme that related to its proteolytic activity and cleavage site. [18] stated that yield was influenced by substrate or protease type, pH and incubation temperature. The longer hydrolysis process and higher enzyme concentration used can increase the yield of protein hydrolysate. According to [28], the amount of protein in the raw material and condition of hydrolysis such as enzyme concentration and hydrolysis time can affect the yield of protein hydrolysate.

D. Soluble Protein Content

Soluble protein content is a parameter that shown the protein released after hydrolysis process which can be soluble in hydrolysate. Insoluble protein can be hydrolyzed into soluble protein by using protease enzyme to release short chain peptides which have hydrophilic side. Correlation between hydrolysis time and bromelain enzyme concentration on soluble protein of apple snail protein hydrolysates are shown in Fig 4.

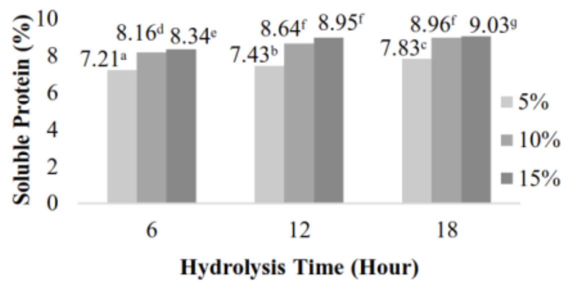

Fig. 4. Soluble protein content of snail protein hydrolysate with hydrolysis time and bromelain enzyme concentration

Based on the Fig 4. the soluble protein in this study were between 7.21 to 9.03%. The result in this study were higher than [29] on apple snail hydrolysate treated by papain enzyme (5% and 10% for 3-18 hours) have soluble protein 2.56 to 7.3%. The increase of hydrolysis time and bromelain enzyme concentration causes increase of the soluble protein content due to the release of hydrophilic peptides and amino acids. Hydrophilic amino acids can bind water, so the short chain proteins can be soluble in hydrolysate. [30] stated that protein hydrolysis can causes insoluble protein to become soluble protein. The longer incubation time gives the enzyme an opportunity to longer protein hydrolysis process, so that more protein was hydrolyzed into water-soluble peptides and amino acids.

E. Total Peptide Concentration

The longer hydrolysis process with the higher enzyme concentration causing the more peptides released during the hydrolysis due to the activity of bromelain enzyme which breaks the peptide bonds in the protein into smaller peptide molecules. Correlation between hydrolysis time and bromelain enzyme concentration on total peptide concentration of apple snail protein hydrolysates are shown in Fig 5.

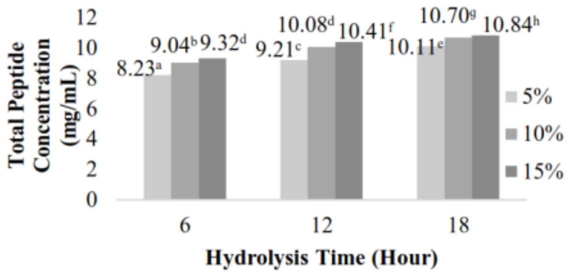

Fig. 5. Total peptide oncentration of snail protein hydrolysate with hydrolysis time and bromelain enzyme concentration

Based on Fig 5. the results of total peptide concentration in this study were 8.23 to 10.84 mg/mL. The results were higher than [14] on grass carp fish that treated by flavourzyme (5%, 6 hours) have peptide concentration within 4.8 to 7.2 mg/mL and higher than [28] on catfish that treated by alcalase and papain enzyme gave the result of peptide concentration within 0.40-7.19 mg/mL and 0.35-5.70 mg/mL.

The increase of peptide concentration is due to the cleavaged of peptides bond by bromelain enzyme during hydrolysis process and obtained small molecules of peptides. Total peptides are related to degree of hydrolysis. [31] stated that the longer hydrolysis and the higher protease enzymes used, the higher the rate of the enzyme reaction, which means more peptide bonds are hydrolyzed. According to [32], concentration of protein is related to the specific activity of protease enzymes. Higher specific protease activity obtain higher DH and peptide solubility, resulting in higher protein concentration.

F. Glutamic Acid Content

Hydrolysis process released the small peptides and amino acids, one of them is glutamic acid. Correlation between hydrolysis time and bromelain enzyme concentration on glutamic acid content of apple snail protein hydrolysates are shown in Fig 6.

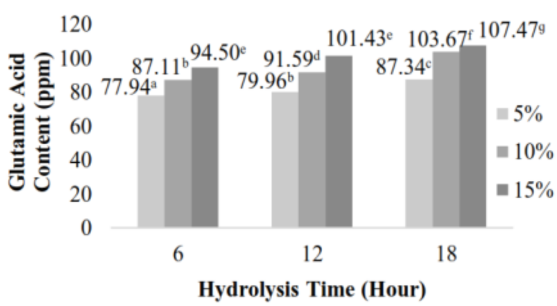

Fig. 6. Glutamic acid content of snail protein hydrolysate with hydrolysis time and bromelain enzyme concentration

The results of glutamic acid in snail protein hydrolysate are within 77.94 to 107.47 ppm. The results were slightly higher than [24] on apple snail hydrolysate treated by same hydrolysis time and papain enzyme (5% and 10%) have glutamic acid 77.95 to 102.08 ppm. The differences may due to the type of enzyme that has different proteolytic activity and specificity. [28] stated that the amino acid composition is affected by the protein substrate, proteolytic enzymes, and hydrolysis condition.

Increasing the hydrolysis time and bromelain enzyme concentration can help the process of breaking down more peptides and free amino acids, one of them is the glutamic acid. This is supported by [24] that increasing the enzyme concentration and hydrolysis time resulted more free glutamic acid in the hydrolysate. The longer hydrolysis time can help protein degradation to produce small fragments of peptides and free amino acids. The free glutamic acid in the hydrolysate may act as a savory and flavor enhancer. Protein hydrolysate can be develope as a flavor enhancer.

G. Physicochemical properties of flavor enhancer from apple snail hydrolysate

Based on the analysis of hydrolysate characteristics, the best results of the analysis were 18 hours hydrolysis time and 15% bromelain enzyme concentrations and used as a flavor enhancer powder. The physicochemical properties of flavor enhancer powder includes of soluble protein content, glutamic acid content solubility, water absorption, and hedonic test. The results of the physicochemical characteristics of flavor enhancer powder can be seen in Table 3.

TABLE III. PHYSICOCHEMICAL PROPERTIES OF FLAVOR ENHANCER

Parameters	Results
Soluble protein content (%)	7.76 ± 0.04
Glutamic acid content (ppm)	99.42 ± 0.77
Solubility (%)	94.79 ± 0.29
Water absorption (mL/g)	5.80 ± 0.10

Based on Table 3. the results of physicochemical properties of flavor enhancer was soluble protein 7.76%, glutamic acid 99.42 ppm, solubility 94.79%, and water absorption 5.80 mL/g. The soluble protein of flavor enhancer was 7.76%. The result was lower than [33] on flavor enhancer powder from inferior fish hydrolysate which has soluble protein levels ranging from 7.93 to 21.09%. The glutamic acid of flavor enhancer was 99.42 ppm, higher than [9] on flavor enhancer from squid hydrolysate which has glutamic acid 74.5 ppm. The differences may due to type of enzyme and protein content in substrate. Soluble protein and glutamic acid content on flavor enhancer was slightly lower than hydrolysate. The decrease in soluble protein and glutamic acid levels can be caused by the destruction of some peptides and amino acids by the drying process. According to [34] that heating process can damage amino acids where the resistance of protein by heat is closely related to its sequences, so this can cause decrease in protein content.

The solubility and water absorption, in this study were 94.79%, and 5.80 mL/g. Solubility in this study was lower than [9] which has a solubility 97.05%. Water absorption in this study compared to [35] on bony barb hydrolysate powder has a water absorption 3.1 mL/g. This parameters were related to the soluble protein content of the hydrolysate. The higher of soluble protein and the higher of solubility due to enzymatic hydrolysis causes the increase of hydrophilic group on the hydrolysate. According to [36] that enzymatic hydrolysis may reduce the molecular weight and release more hydrophilic groups and soluble peptide which contribute to high solubility.

The organoleptic characteristics of flavor enhancer powder was also carried out using a hedonic test. The hedonic test uses 7 scales (dislike very much, dislike, dislike slightly, neutral, like slightly, like, and like very much) and was assessed by 20 semi-trained panelists. The parameter in test includes color, aroma, and taste of flavor enhancer from apple snail hydrolysate. The results of the organoleptic analysis of flavor enhancer powder are shown in Table 4.

TABLE IV. HEDONIC TEST OF FLAVOR ENHANCER

Parameters	Results
Color	4.10 ± 1.02 (Neutral)
Aroma	5.05 ± 0.69 (Like slightly)
Taste	5.10 ± 1.02 (Like slightly)

Based on Table 4. the results of hedonic test was color of 4.10 (neutral), aroma 5.15 (like slightly), and taste 5.10 (like slightly). The value of preference for aroma and taste is higher than color because aroma and taste play an important role in flavor enhancers. This is in accordance with [37] which stated about definition of flavor enhancer based on Regulation of the Minister of Health of the Republic of Indonesia No. 722/Menkes/Per/IX/88 that food additives, flavorings and flavor enhancers are defined as additives that can provide, add, or emphasize taste and aroma.

The quality of flavor enhancer from apple snail hydrolysate is orange-brown in color, has a savory and slightly fishy aroma, and tastes umami, sweet and bitter slightly. Bitter slightly in flavor enhancers may due to the released of hydrophobic amino acids that contribute to bitter taste. [38] stated that the hydrophobicity is an important factor that affect bitterness in hydrolysate. The hydrolysis process produces peptides and amino acids, where one of the amino acids that contributes to the formation of a savory and umami taste is glutamic acid.

The formation of these characteristics can be caused by the Maillard reaction between peptides and reducing sugars from fillers during the drying process which produces a brown color and has a savory aroma and taste. According to [31] that during the hydrolysis process the peptide bond severance by the protease enzyme produces an amine group which is the precursor of the Maillard reaction, in which case the amine group of the protein reacts with the aldehyde or ketone group of reducing sugar, resulting in a brown color. Aroma can be formed from added sugar, free amino acids, peptides, nucleotides and organic acids which act as the main precursors in the formation of savory flavors in the resulting hydrolysate.

Based on this research, the optimal condition was 18 hours of hydrolysis with a concentration of 15% bromelain enzyme but the maximum time for the hydrolysis process is not yet known. According to [39], protein hydrolysate from three different mushroom (oyster, abalone, and shiitake) which hydrolyzed using 15% papain enzyme (6, 12, 18, and 24 hours) showed the maximum condition for oyster and abalone mushroom was 18 hours while shiitake mushroom was 24 hours which gave highest of DH and amino acids content. To find out the maximum condition in this research, it is necessary to do further research using hydrolysis time above 18 hours.

IV. CONCLUSION

Physicochemical properties of apple snail protein hydrolysate were significantly affected by hydrolysis time and bromelain enzyme concentration. The optimal treatment of apple snail protein hydrolysate was obtained at 18 hours of hydrolysis with a concentration of 15% bromelain enzyme. The flavor enhancer was produced with good physicochemical properties and high in glutamic acid content which play important role in umami taste of the product. Increasing hydrolysis time and enzyme concentration may have better result on amino acids content which contribute to umami taste of flavor enhancer.

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