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Original Paper

The Physicochemical Properties of Flavor Enhancer Made from Different Types of Snail Protein Hydrolysates

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***Abstract*—Golden apple snails, apple snails, and freshwater snails are mollusks found in shallow waters with mud on the bottom, such as rice fields, swamps, and riverbanks. Snail has a high protein content of 14%-19%, but its utilization is still limited. In addition, certain types of snails, such as golden apple snails and apple snails, are pests to rice plants, so they have not been used optimally. One alternative is using snail as a flavor enhancer. Flavor enhancers can be made through enzymatic hydrolysis using bromelain enzymes. This study aimed to determine the effect of snail type and hydrolysis time on the physicochemical properties of flavor enhancer made of snail protein hydrolysate. This experimental study used a completely randomized design with two factors. Factor I was the type of snail (golden apple snail, apple snail, and freshwater snail), and factor II was the hydrolysis time (3 hours, 6 hours, and 9 hours). Data were analyzed using ANOVA with α 5%. When significant difference was identified, DMRT test at 95% significance level will be conducted. The result has showed that the flavor enhancer made of snail protein hydrolysate is influenced by different snail types and hydrolysis time. The apple snail with 9-hour hydrolysis produces the best flavor enhancer characterized by 3.96% water content, 14.73% yield, 92.29% water solubility, oil absorption of 1.15 ml/g, glutamic acid content of 107.23 ppm, and brownish yellow tone.**

Keywords* — *snail, flavor enhancer, protein hydrolysate, glutamic acid

I. INTRODUCTION

Snails are mollusks that usually live in shallow, mud-based waters such as rice fields, swamps, and riverbanks. Snails are one of the food creatures with high protein content. The protein content of snails varies to large extent. For example, golden apple snail has 15.09% protein [28], apple snail contains 10.67% protein [32], and freshwater snail offers 10.4% protein [34]. In addition to high protein, the price of snails is relatively cheap, and they are quite abundant. To date, snails are only traded in the form of fresh whole, fresh peeled, satay, and smoked [17]. Even some types of snails, such as golden snails and rice snails, are known as pests because they eat newly planted rice stalks so that they hamper rice growth [31]. The amount of damage to rice plant caused by golden apple snails can reach 10%-40%, so that golden snails need to be removed or managed further [27].

Innovations in snail processing will increase its economic value and utilization. One of the potential uses of products made of snails is to use them as the ingredients of flavoring products because of their high amino acid content, especially glutamic acid which contributes to the umami taste [30]. This is based on research by [34] claiming that glutamic acid is the largest non-essential amino acid content in golden apple snails and garden snails. Another study [12] shows that the glutamic acid content in golden apple snails is 8.4%, while in freshwater snails it reaches 7.2% [34].

Snail flavor enhancers can be made through enzymatic hydrolysis using the bromelain enzyme [60]. Enzymatic hydrolysis is the breakdown of protein polypeptide bonds into short chain peptides and free amino acids by proteases [16]. The formation of amino acids, especially glutamic acid, causes protein hydrolysate to have a good taste [33]. Bromelain is an endopeptidase enzyme that requires long incubation time to produce protein hydrolysate with high amino acids or short-chain peptides [11]. According to [48], the longer incubation time, the longer the opportunity for the enzyme to hydrolyze protein, resulting in more protein hydrolyzed into amino acids.

The aforementioned studies point out that snails have high protein content, but low economic value. Flavor enhancer can be one alternative to address the underexplored economic potential. Nevertheless, the study of flavor enhancer made of the snail is still limited. This study aims to investigate the physicochemical properties of flavor enhancers made from three types of snails and different duration of hydrolysis. The research aims to shed light on the use of flavor enhancers made of snails applicable to various kinds of processed products.

II. MATERIALS AND METHODS

A. Materials

The materials used in this study were golden apple snails, apple snails, and freshwater snails obtained from Perak, Jombang Regency, Indonesia, and natural protease enzymes from pineapples (crude bromelain). Additional ingredients used included distilled waters, gum arabic, maltodextrin, salt, powdered sugar and garlic powder. The reagents used in this study were glutamic acid standard, bovine serum albumin (BSA) standard, trichloroacetic acid (TCA), $CuSO_4$, sodium carbonate, Na.K tartrate, Lowry reagent, Follin Ciocalteu reagent, 96% ethanol, and cooking oil [58].

B. Research Design

This study employed a completely randomized design (CRD) with 2 factors: the first factor was the type of snail (golden apple snails, apple snails, and freshwater snails), and the second factor was the hydrolysis time (3, 6, and 9 hours). The data obtained were processed using ANOVA to determine the effect of each treatment. Any identified significant difference led to DMRT (*Duncan's Multiple Range Test*) with α 5%.

C. Procedure

The production of crude bromelain enzyme extract

Pineapple was peeled, washed, and cut into small pieces. Then, these pieces were crushed using a blender with the addition of distilled water (1:1). Furthermore, the pineapple juice was filtered using a filter cloth. The filtrate obtained was then filtered again using filter paper to obtain crude bromelain enzyme extract.

The production of flavor enhancer from snail protein hydrolysate

Golden apple snail, apple snail, and freshwater snail flesh (Factor I) weighed 100 g were washed in flowing water. Distilled water was added to the snail flesh in a ratio of 2:1 and mashed using a blender. Crude bromelain enzyme at 10% was added to the slurry. Hydrolysis conditions were set at 50^o C for 3, 6, and 9 hours (Factor II). The hydrolysis was stopped using a high temperature of 90^o C for 10 minutes. Pellet and supernatant were separated by centrifugation at 3000 rpm for 30 minutes at room temperature. The supernatant with 80% concentration was mixed with 2% powdered sugar, 0.5% garlic powder, 0.5 g salt, and 10% encapsulated gum Arabic, and maltodextrin in ratio of 1:4. The mixing was carried out using a magnetic stirrer at 6000 rpm for 20 minutes), and the results of the mixing were dried using a cabinet dryer at a temperature of 60^o C for 6 hours. The results of drying were mashed to obtain flavor enhancer powder.

D. Observation Paramaters

The observation parameters were focused on snail flesh as raw materials, including water, ash, and protein content [5], coupled with protease activity of crude bromelain [56]. The protein hydrolysate parameters represented the degree of hydrolysis [42], soluble protein content [43], and glutamic acid [20]. Parameters of the physicochemical properties of flavor enhancers were water content [5], yield [29], water solubility [18], oil absorption [1], glutamic acid [20] and color [3][59].

III. RESULTS AND DISCUSSION

The analysis of chemical composition in raw material

The chemical composition tested included water content, protein content, and ash content. The test results of the raw materials can be seen in Table 1.

Table 1. Chemical composition of raw material

Chemical composition	Golden apple snail	Apple snail	Freshwater snail
Water (%)	78.62 ± 0.38	76.81 ± 0,10	79.38 ± 0,25
Ash (%)	2.29 ± 0,12	2.83 ± 0,13	4.17 ± 0,88
Protein (%)	14.94 ± 0,11	19.11 ± 0,04	14.35 ± 0,10

Different types of snails resulted in different chemical compositions (Table 1). The moisture of golden apple snail was 78,62%, which was lower than that reported by [14] for golden apple snail (83,85%). The difference may due to snail size and environmental condition. The golden apple snail in [14] was obtained from Pahang in Malaysia, while in this study it was obtained from Jombang, Indonesia.

The protein content of apple snail measured in this study was 19,11%. A study by [32] reported lower protein content in apple snail (*Pila ampullacea)* at 10,67%. Higher protein content may result from different environmental factors and treatment before analysis. In [32], flesh and shells were separated and dried in an oven at 60^o C for 24 hours, while in this study only flesh and shells were separated by boiling for 3 minutes without any drying process. As in [50], protein content decreased with each addition of drying time and temperature because heating could damage amino acids in protein. This is supported by [57] who point out that extensive heating at excessive temperature causes protein to be denatured.

Ash content indicates the presence of minerals in the material. The ash content in freshwater snail was found at 4,17%, which is fairly similar to that reported by [34] at 4,29%. The mineral content in snails comes from plants and their environment. Each organism has different ability to absorb minerals, so this affects the value of the ash content. According to [32], because aquatic snails live in muddy environments, such as rivers, they absorb minerals from river water. The river usually serves as massive deposit of chemical waste from industries, so this can increase the absolute mineral content in the water. In line with [14], different environmental factors, soil type, age, species type and feeding habit affect snail composition.

The protease activity of crude bromelain enzyme

Protease activity of crude bromelain enzyme in this study was 5.20 U/ml, which was lower than that discovered in [53] at 5.84 U/ml. This can be caused by different level of maturity and the type of pineapple used as the source of enzymes. [55] The maturity significantly affects the proteolytic activity of the crude bromelain enzyme from pineapple fruit. The high level of maturity obstructs the bromelain enzyme reactivity. This is due to the formation of certain compounds during fruit ripening. In this case, enzymes as proteins may be used in these compounds so that some of the enzyme structures will be damaged, resulting in decreased enzyme activity [54].

The chemical characteristics of snail protein hydrolysate

The degree of hydrolysis is an important parameter to monitor the hydrolysis process. The degree of hydrolysis shows the percentage of peptide bonds released due to the hydrolysis process. The degree ranged from 26.60% to 69.84% (Table 2). This finding was higher than that in [41]. The degree of hydrolysis from golden apple snail hydrolysate was 12.62% with alcalase enzyme (2%, 2.65 hours). The hydrolysis degree is influenced by the enzyme effectiveness to cut peptide bonds between protein molecules [38]. In addition, the type of substrate and hydrolysis duration also affects the degree of hydrolysis. The degree of hydrolysis of fish protein hydrolysate may vary due to different fish species, fish parts, types of enzymes used, and hydrolysis conditions [19].

Table 2. The chemical characteristics of snail hydrolysate

Treatments		Degree of hydrolysis (%)	Soluble protein content (%)	Glutamic acid content (ppm)
Type of snails	Hydrolysis time			
Golden apple snail	3 hours	38.21 ± 0.28^c	8.55 ± 0.11^d	62.39 ± 0.38^c
	6 hours	56.87 ± 0.05^e	8.87 ± 0.11^e	75.71 ± 0.77^e
	9 hours	68.34 ± 0.81^g	9.52 ± 0.11^g	99.89 ± 0.38^h
Apple snail	3 hours	35.36 ± 0.51^b	5.66 ± 0.04^c	59.40 ± 0.77^b
	6 hours	60.96 ± 0.11^e	9.04 ± 0.09^e	80.60 ± 0.77^f
	9 hours	69.84 ± 0.75^h	9.92 ± 0.09^h	109.67 ± 0.38^h
Freshwater snail	3 hours	26.60 ± 0.60^a	4.63 ± 0.04^a	54.51 ± 0.77^a
	6 hours	51.93 ± 0.38^d	5.57 ± 0.09^b	68.10 ± 0.77^d
	9 hours	63.74 ± 0.44^f	9.15 ± 0.07^f	90.11 ± 0.38^g

The mean values followed by the same letter are not significantly different. ($p \leq 0,05$).

Based on Table 2, the lowest results are found in freshwater snails with 3-hour hydrolysis time, while the highest results were marked in apple snails with 9-hour hydrolysis time.

Insoluble proteins can be hydrolyzed by using protease enzymes that break the bonds in protein polypeptides into simpler peptides [10]. The soluble protein content of the snail protein hydrolysate ranged from 4.63% to 9.92% (Table 2). The results of soluble protein content in this study were higher than that in [37] as discovered in apple snail protein hydrolysate at 5.91%, resulting from papain enzyme treatment (at 10%, 9 hours). The soluble protein content is related to the degree of hydrolysis. The higher the degree of hydrolysis, the more short-chain peptides resulting from hydrolysis, which results in higher levels of soluble protein. According to [16], longer hydrolysis leads to further protein degradation and higher degree of hydrolysis, implying greater soluble protein content.

The umami taste occurs due to the glutamic acid. The glutamic acid content of snail hydrolysate was obtained within the range of 54.51 ppm to 109.67 ppm. The glutamic acid levels found in this study were lower than those in [22] discovering glutamic acid at 285.28 ppm from chicken hydrolysate treatment with flavor protease (at 0.1%, 1.5 hours) and natural protease (at 0.25%, 2.5 hours).

Longer hydrolysis caused more peptide bonds to break, so more short chain peptides and free amino acids are released. [37] state that a longer hydrolysis facilitates the degradation of protein resulting in a small fragment of peptides and free amino acids. This parameter is related to the degree of hydrolysis of hydrolysate associated with enzyme activity. According to [51], of all amino acid components, glutamic acid is the most abundant nonessential amino acid produced in protein hydrolysis. This is influenced by raw material type, source of enzyme, and the hydrolysis conditions.

The physicochemical properties of snail flavor enhancer

1) Moisture

The moisture of snail flavor enhancer is presented in Figure 1.

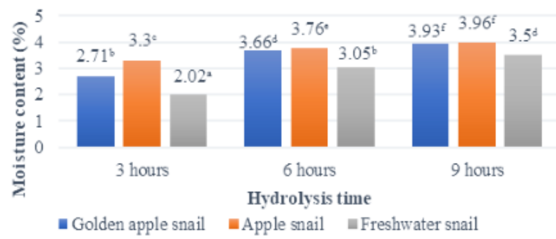

Fig. 1. Moisture content of flavor enhancer from snail hydrolysate

Figure 1 shows that the higher soluble protein content in snail hydrolysate and the longer hydrolysis produce a flavor enhancer with higher water content. The highest water content is obtained from apple snail flavor enhancer with a 9-hour hydrolysis . Hydrolysis increases soluble protein content in the hydrolysate, resulting in increased short chain peptides, especially those with hydrophilic groups. [35] The higher soluble protein content increases hydration and the amount of water content. [9] The hydration occurs in the hydroxyl group (–OH) of serine and threonine or in the amide group ($-CONH_2$) of asparagine and glutamine. In addition, hydration can also occur in the hydrophilic groups of proteins, especially the groups positively charged with lysine and arginine side chains and the negatively charged group with aspartic acid and glutamic acid.

The water content of flavor enhancers from three types of snails are different because it is influenced by the soluble protein content produced during hydrolysis (see Table 2). In protein hydrolysis, polypeptide bonds are broken into peptides with lower molecular weight and become easily soluble. The higher soluble protein content enhances hydration level and the amount of water content. Study by [4] demonstrates that the difference in the water content of protein hydrolysate from tilapia, milkfish, and mackerel is caused by differences in fish species and habitats affecting the nutritional content in fish meat. In addition, variations in water content are also influenced by climate, fat content, age, and fish growth.

2) Yield

The yield of snail flavor enhancer is presented in Figure 2.

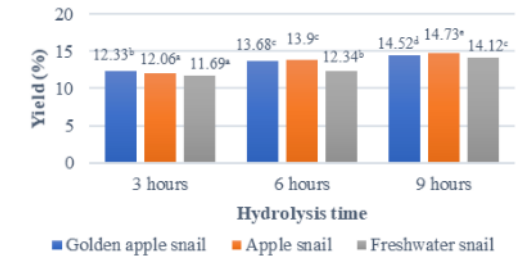

Fig. 2. Yield of flavor enhancer from snail hydrolysate

The higher soluble protein content in snail hydrolysate and longer hydrolysis produce higher yield of flavor enhancer (Fig. 2). This is because the longer hydrolysis and longer contact between protease and substrate result in higher chance for the protein to be broken down into short-chain peptides and soluble amino acids. As a result, more supernatant is produced, so the yield of the flavor enhancer increases. [29] The longer hydrolysis, the more peptide chains broken into short peptide chains. The resultant short-chain peptides can bind water. As a result, the free water in the material evaporates and the yield increases in drying process. [21] state that yield increases along with the extension of hydrolysis, before eventually reaching a stationary phase.

Yield value of the three types of snails was different. This difference is caused by the different species of snails used in this study. Different snail species produce different soluble nutrient components during the hydrolysis. [36] The hydrolysis causes nutritional components such as minerals, proteins, and fats to be soluble, so this affects the yield of protein hydrolysate. Raw material or substrate used can affect the chemical composition of the hydrolysate. This is caused by several factors that affect the hydrolysis, including pH, time, temperature, enzyme activity and specificity, the proportion between enzymes or substrates, and interactions between nutrients in the raw materials during hydrolysis [8].

3) *Solubility*

The solubility of powder products determines how easy it is to use the product. The greater the solubility value, the better the product because it will dissolve faster when mixed with water, so the flavor enhancer will be absorbed by the dish more quickly [45]. The solubility of snail flavor enhancer is presented in Figure 3.

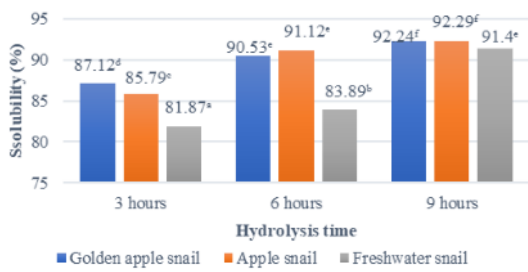

Fig. 3. Solubility of flavor enhancer from snail hydrolysate

The longer hydrolysis and the higher soluble protein in the hydrolysate produce a flavor enhancer with high water solubility. Increased solubility follows the degree of hydrolysis because longer hydrolysis increases soluble protein content. High levels of soluble protein indicate a large number of polar (hydrophilic) peptides and amino acids. Amino acids with hydrophilic groups combine with water to form hydrogen bonds and increase the solubility. [49] report on enzymatic hydrolysis which increases the interaction of hydrophilic groups with water molecules by reducing the size of the peptide, thereby increasing protein solubility. During the hydrolysis, short chain peptides with low molecular weight are generated from the cleavage of peptide bonds in complex proteins. This will produce a polar group peptide or amino acids that can bind water through hydrogen bonds [13].

Snail flavor enhancers obtain high solubility values ranging from 81.87% to 92.29% (Fig. 3). High solubility values are very important for flavor enhancer because the umami component must be released when in contact with saliva so that interactions can occur between the umami component and the umami receptor on the tongue [15]. In accordance with [52], the protein hydrolysate in golden apple snail has more dominant hydrophilic amino acid (at 20.21%) compared to hydrophobic amino acid (at 9.54%). This property makes it easier to bind water. [45] flavor enhancers from boiled water of three different types of fish, such as silver shark-babe, pomfret, tread fin, and bream fish, produce different solubility values because the nutritional content of each fish is different.

4) *Oil absorption*

The oil absorption of snail flavor enhancer is presented in Table 3 and 4.

Table 3. Oil absorption of flavor enhancer from snail hydrolysate with type of snail treatments

Type of snails	Oil absorption (ml/g)
Golden apple snail	1.28 ± 0.13^{z}
Apple snail	1.2 ± 005^{a}
Freshwater snail	1.37 ± 0.13^{b}

The mean values followed by the same letter are not significantly different. ($p \leq 0,05$).

The oil absorption between snail types is significantly different because it is influenced by soluble protein content. Higher soluble protein content indicates lower hydrophobic groups than hydrophilic groups, which results in lower oil absorption (Table 3). [39] High levels of soluble protein content indicate low hydrophobicity, so the oil binding ability is low.

Table 4. Oil absorption of flavor enhancer from snail hydrolysate with hydrolysis time treatments

Hydrolysis time	Oil absorption (ml/g)
3 hours	1.38 ± 0.13^{b}
6 hours	1.28 ± 0.08^{a}
9 hours	1.18 ± 0.06^{a}

The mean values followed by the same letter are not significantly different. ($p \leq 0.05$).

The longer hydrolysis causes higher soluble protein content in the hydrolysate, so the oil absorption ability decreases (Table 4). This is supported by [47] reporting that the hydrophobic group is related to the oil absorption capacity. If the soluble protein content is high, the number of hydrophobic groups is small. [24] Oil binding capacity is correlated with surface hydrophobicity. Protein hydrolysate develops this hydrophobicity on account of the hydrolysis which cleaves the protein chain, resulting in the exposure of more internal hydrophobic groups.

5) *Glutamic acid content*

The glutamic acid content of snail flavor enhancer is presented in Figure 4.

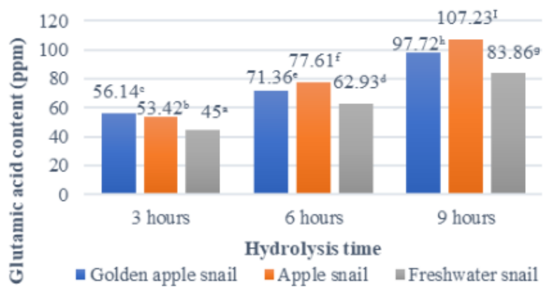

Fig. 4. Glutamic acid content of flavor enhancer from snail hydrolysate

The higher protein content of the snail raw material and the longer hydrolysis time results in higher glutamic acid in the flavor enhancer (Fig. 4). The high protein content of the raw material indicates the availability of abundant substrate, so that more polypeptide bonds in the protein will be broken down into short-chain peptides and free amino acids by protease enzymes. [25] Different types of protein substrates affect the functional properties and flavor profiles of protein hydrolysate. The cutting side of peptide bonds will be better on protein substrates that contain ample amino acids [6]. [40] explain that longer hydrolysis facilitates the degradation of protein resulting in a small fragment of peptides and free amino acids.

6) *Color*

Color is an important parameter affecting consumer preference because dark-colored products have limited application in food [46]. The color of snail flavor enhancer is presented in Figure 5.

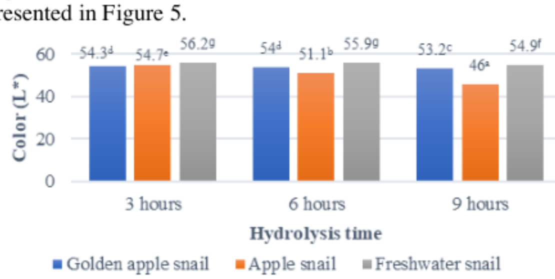

Fig 5. Color/brightness of flavor enhancer from snail hydrolysate

The darker snail and longer hydrolysis produce a flavor enhancer with lower brightness intensity (L* value) (Fig 5). In the hydrolysis, the bromelain enzyme breakdown proteins into low molecular weight peptides. The longer the hydrolysis time, the more peptide hydrolyzed into amino acids, thereby increasing soluble protein content. This is in accordance with [44] who point out that increased protein content will produce a dark brown solution. [23] mention that the enzymatic hydrolysis reaction contributes to reduced brightness and gives a darker tone to the hydrolysate.

Maillard reaction also affects the color of flavor enhancers. The longer hydrolysis, the more soluble protein produced, subsequently increasing the amine group of protein that interacts with the reducing sugar. This darkens the color of the hydrolysate product as the hydrolysis time increases. [2] The longer hydrolysis extends the contact between the reducing sugar and the amino group, so more melanoid in compounds is formed due to the Maillard reaction. The interaction between amino groups and reducing sugars will decrease the brightness of hydrolysate.

The type of snail affects the color of flavor enhancer from snail protein hydrolysate. To that direction, the apple snail flesh appears dark brown, while the golden apple snail and freshwater snail flesh turn out light brown. This color difference affects the protein hydrolysate produced. [7] The color difference of the hydrolysate produced results from different materials, enzymes, and hydrolysis conditions. [15] state that brownish shiitake raw materials can turn out yellow after being processed into flavor enhancers.

IV. Conclusion

Physicochemical properties of flavor enhancer are significantly affected by snail type and hydrolysis time. Using snails as raw material effects the chemical composition of hydrolysate. Different snail types produce different soluble nutrient components during the hydrolysis. These differences further influence the characteristics of snail flavor enhancer. This research has showed that longer hydrolysis produces a flavor enhancer with good characteristics and high levels of glutamic acid as an umami ingredient. The research findings have corroborated that the best flavor enhancer is made of apple snail with 9-hour hydrolysis.

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