# 01. Effect of Hydrolysis time and Papain Concentration on Some Properties of Apple Snail (Pilla ampullacea) Hydrolysate

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*Article*

# Effect of Hydrolysis time and Papain Concentration on Some Properties of Apple Snail (*Pilla ampullacea*) Hydrolysate

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**Abstract**

Soluble protein content and total peptide content were investigated in the non-farmed freshwater apple snail (*Pila ampullacea*) to understand its nutritional potential as alternative umami taste enhancer. Apple Snail samples with removed gut content were collected from a local snail non-farm in Surabaya City. Papain enzyme was used. The two variables, hydrolysis time (3, 6, 9, 12, 15, 18 h) and enzyme concentration (1%, 5%, 10%), was used to produce the apple snail hydrolysate. The result showed that total soluble protein was about 2.1%-7.3%, whereas total peptide content was 5.05-15.73mg/ml. The highest total soluble protein was achieved at 12 hour. Longer hydrolysis time significantly effect on total peptide content of apple snail hydrolysate.

**Keywords:** papain enzyme, protein hydrolysate, snail.

## 1. Introduction

Protein hydrolysates rich in bioactive compounds represent promising ingredients for food and industrial application. In addition, protein also affects the taste of food, especially amino acid component. Amino acid component give specific taste on food. It is well known that presence of umami taste to foods can increase in the acceptability for the sensory properties of those foods. Firstly, umami defined as the characteristic taste elicited by glutamates, and has since also been associated with monosodium glutamate (MSG). IMP (disodium 5'-inosine monophosphate), GMP (disodium 5'-guanosine monophosphate) and AMP (disodium 5'-adenosine monophosphate) was presumed responsible for presence of umami taste in food. [1].

Application of protease is often an attractive means for obtaining better functional properties of food proteins. Enzymatic hydrolysis of protein releases small peptides and free amino acids, and therefore, may contribute to increase the nutritional value of food proteins and an extensive review of enzymatic protein hydrolysates in human nutrition was reported in literature [2]. According to [3], and [4] properties of protein hydrolysate is influenced by type of enzyme, enzyme concentration, and hydrolysis time. Papain is a plant proteolytic enzyme found naturally in papaya (*Carica papaya L.*). It is also widely used in chemical and food industries. Papain possesses proteolytic activity that belongs to cysteine proteinase family. Papain is manufactured from the latex of raw papaya fruits or pineapple [5]. These enzymes showed broad specificity, cleaving peptide bonds of basic amino acids. That is why used as meat tenderizing and clarifying agent in food industries and in processing to confirm proper fading of leather [6].

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The Ampullariidae commonly known as apple snails, is a large family of freshwater snails, found across the tropics and subtropics of Asia, Africa, and the Americas. The family consists of nine extant genera and more than 150 recognised species. It includes the largest freshwater snails, with several species attaining sizes of more than 100 mm in shell height. Ampullariidae family consist of Pila, Pila ampullacea, Pila scutata, Pomacea maculata, Pomacea canaliculata genus. Until now, the value of apple snail is is still low whereas it have high protein content [7]. Apple apple snail protein hydrolysate have potential as an alternative can be source for flavor enhancer, ketchup, and other food ingredient. So, the current study is to assess total soluble protein and total peptide content of hydrolyzed apple snail obtained by the action of papain.

## 2. Material and Method

### 2.1. Sample preparation

Apple snail (*Pila ampullacea*) (AS) were obtained from traditional market at Pabean, Sidoarjo-Indonesia during June-Juli 2020. All the solvents and chemicals used in the study were of analytical grade purchased from Sigma Aldrich (St. Louis, MO, USA). Papain was bought from Merck Millipore (Burlington, Massachusetts, USA).

### 2.2. Protein hydrolysates preparation.

Apple snail were cleaned by using flowing water. Flesh of apple snail were cut into small pieces and then blanched using steam blanching with temperatures 93-94 °C for three minutes. After that, apple snail were crushed using a crusher and suspended in distilled water with ratio 1:1 (w/v) at room temperature. The slurry was hydrolysis using 1%, 5%, and 10% (w/w) papain enzyme. Hydrolysis time was done at room temperature (30°C) with interval time of 3h, 6h, 9h, 12h, 15h, 18h. The enzyme was inactivated by keeping the mixture in boiling water bath for 3 minutes. Supernatant and pellet were separated by centrifugation (3000 rpm, 30 min). The supernatant was transferred in a refrigerator until it was ready to be analyzed.

### 2.3. Soluble protein content

Soluble protein content of the sample, was measured by [8] with modifications. Aliquots of Aliquots of 1000µL of the AS hydrolyzed protein were mixed with 500µL of of TCA solution to obtain the soluble and insoluble fraction. After 30 minutes, the mixture was centrifuged at 3000 g. The amount of soluble protein in the filtrate was determined by the method of Lowry et al. 1954 with bovine serum albumin as the standard. Absorbance was measured at wavelength of 750 nm. The solubility was expressed as a percentage of total protein concentration.

### 2.4. Total Peptide Content

The supernatant of apple snail protein hydrolysate was mixed with 15% (W/V) trichloroacetic acid (volume ratio was 2:1) and reacted at 25 °C for 1 h afterwards. Then it was centrifuged at 12,000g for 10 min. Peptide concentration of apple snail protein-hydrolysate in supernatant was determined using the Folin-phenol method. The absorbance was read at 680 nm on a spectrophotometer [9].

## 3. Results and Discussion

Enzymatic hydrolysis is an excellent approach to enhance the biological activities of the proteins, with some peptides or fractions having stronger activity than others. The amino acid composition, the molecular weight of the protein hydrolysates, and the resulting biological activities are greatly affected by the protein substrate, the proteolytic enzymes, and the hydrolysis condition [10].

### 3.1. The Soluble Protein Content

Changes of the soluble protein content during the AS hydrolysis were investigated to understand the peptide release properties. As shown in **Fig. 1**, the soluble protein content of three concentration-treated hydrolysates was determined every 3 hours, from 3 hours to 18 hours. The Soluble protein content gradually increases as well as the longer time of hydrolysis.

The soluble protein content of AS hydrolysate treated with 1% increased from 2.1% to 4.64%, but decreased after 15 hours dan 18 hours. The same trend showed treated by 5% enzyme concentration; increased from 2.56% to 5.35%, but decreased after that at 15 hours and

18 hours. Also, the same trend showed treated by 10% concentration, increased from 2.87% to 7.3%, but decreased after that at 15 hours and 18 hours. The highest soluble protein content was recorded by 10% at 12 hours with 7.3%. The same result also reported by [3] and [4] where the soluble protein content was increased with time hydrolysis. Time hydrolysis have significantly influence on soluble protein content. The papain breaks down proteins into amino acids soluble. With increase of the hydrolysis time, these small-size peptides were further hydrolyzed to amino acids [3].

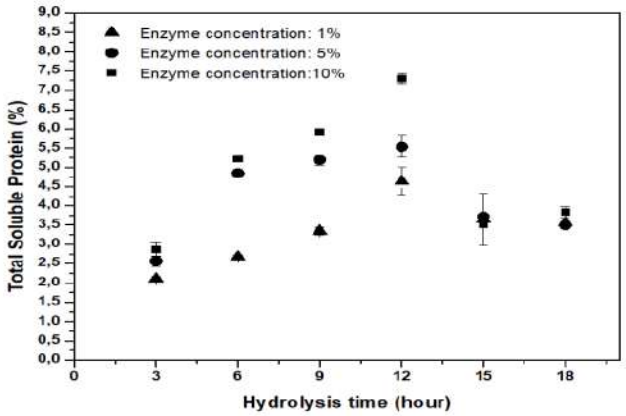


Fig. 1 Soluble protein content of AS hydrolysate with different papain enzyme concentration; 1% (▲), 5% (●), 10% (■)

The analysis of soluble protein content increased with increasing time during the first 12 hours. On the contrary, the soluble protein content decreased after 12 hours of hydrolysis. From this behavior, we concluded that it is not advisory to continue hydrolysis beyond 12 hours, because there was not a significant increase in rates of soluble proteins. So, it is not profitable to go to the end of the reaction to have an increase of 7.3% during 18 hours more. So, we can deduce the optimum conditions for enzymatic hydrolysis to produce the most interesting peptide fractions as: duration of 12 hours, and enzyme concentration of 10% and the yield of soluble protein content has a value of 7.3%.

### 3.2. Total Peptide Content

Fig. 2 shows the influence of hydrolysis time and enzyme concentration on total peptide content. Hydrolysis time and enzyme concentration have significantly influence on total peptide content. The result showed that total peptide content was 5.05-15.73mg/ml. The peptide content of AS hydrolysate treated with 1% increased with hydrolysis time from 5.05mg/ml to 9.56mg/ml. The same trend showed treated by 5%; increased from 5.95mg/ml to 12.1mg/ml. Also, the same trend showed treated by 10%; increased from 6.42mg/ml to 15.73mg/ml. The highest peptide content was recorded by 10% at 18 hours with 15.73mg/ml.

The activity of enzymes is affected by different conditions like temperature, pH as well as time. In this study, as hydrolysis time and concentration increased, the total peptides content gradually increase. The same result also reported by [4] where the peptide content was increased with time hydrolysis. Tsai [6] reported that the peptide content was influenced by the specific action of the enzyme used in the separation of peptide bond. This showed that the peptides of high molecular size gradually decomposed into small-sized peptides [11]. Generally, the peptide content of hydrolysate were increased with the increased of DH%.

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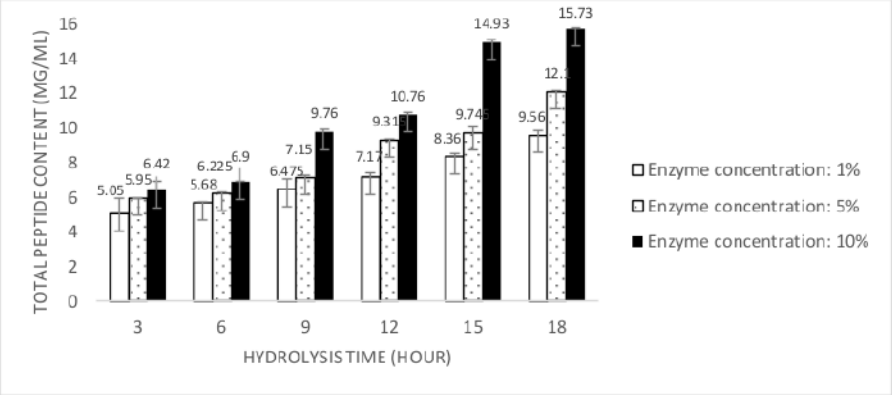


Fig. 2. Total peptide content (mg/ml) of AS hydrolysate with different papain enzyme concentration

In previous study, protein hydrolysates can have a spesific taste like bitter, sweet and umami. The spesific taste of protein hydrolysates associated to their hydrophobic or hydrophilic amino acid content. Protein hydrolysates showed excellent antioxidant potential, scavenging (DPPH) radical, inhibiting linoleic acid peroxidation and anti-hypertensive. Post-hydrolysis processes can also be used to modify and improve quality of protein hydrolysates. Advances technology of protein hydrolysates production have resulted in their use in biotechnology and fermentation in the future [12].

## 4. Conclusion

Total soluble protein and total peptide content of apple snail hydrolysate was significantly affected by enzyme concentration and hydrolysis time. Total soluble protein increased with increasing of enzyme concentration and hydrolysis time, but decreased after hydrolysis time at 12 hours. This study showed that the extent of hydrolysis time and enzyme concentration had greatly influenced total peptide content.

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