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Effects of Papain Concentration and Hydrolysis Time on Degree of Hydrolysis and Glutamic Acid Content of Apple Snail Hydrolysate

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ABSTRACT

Umami taste enhances the high palatability in cuisine by the presence of high level of glutamic acid content. This savoriness compound could get from fermentation and hydrolysis process. Generally, consumer perception thinks preferable natural sources due to food safety issues. Apple snail (*Pila ampullacea)* is known as abundant wildlife containing high protein source. The most popular protease in Indonesia is papain, which was used to generate the apple snail hydrolysate. The objective of this research is to define the specified condition based on papain concentration and hydrolysis time to produce umami taste from apple snail. Those two variables showed a significant effect on the degree of hydrolysis and glutamic acid content of apple snail hydrolysate, therefrom the data were several various values in the lowry method and ninhydrin colorimetry, respectively. The established condition resulting in a degree of hydrolysis and glutamic acid content was as high as 56.56 ± 1.65% and 95.34 ± 0.13 ppm. The study concluded that papain within certainly condition denotes free amino acid in releasing of umami taste. Furthermore, this research will be applied as flavour enhancers, commonly known as monosodium glutamate (MSG). Also, this study promotes a natural source as safer flavor enhancers, utilization of potential local commodity, and can compete with the commercial ones.

Keywords: Pila ampullacea, umami, flavour enhancer, papain, and hydrolysate

Introduction

Flavour enhancer is a substance used to boost the taste of a food or drink. Recently, umami is defined as the fifth taste, after sweetness, sourness, bitterness, and saltiness. This savoriness substance has become most explored as a flavor in the food industry, especially in Asia due to incredibly used in Asian food as food additives to satisfy the consumer. Nowadays, umami as a flavor enhancer is described as monosodium glutamate (MSG) and usually is produced by chemical and microbial fermentation. However, there is some issue that side effects that cause consumers to prefer to choose from natural sources.

The earliest reports on umami taste were screened derived from protein hydrolysate of soybean (Arai et al., 1972). Recently, most studies about flavor enhancers were collected from peptides, such as deamidated wheat gluten hydrolysate (Schlichtherle-Cerny & Amadò, 2002), peanut hydrolysate (Su et al., 2012), Jinhua hams, parma hams (Dang et al., 2014), rohu head hydrolysate (Bruno et al., 2019), and straw mushroom (Xu et al., 2019). As we know that peptides are a protein component composed of two or more amino acids bonded in a chain known as peptide bonds, which is naturally present in foods. Therefore, exploration of flavour enhancers from food sources attracts a big research interest for researches who work in the field of food science and technology.

Snail has become a popular organism that is used in many products, such as food and cosmetics. Archaeological evidence has found that snails are the main edible meals in the prehistoric era

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(Lubell, 2004). Although the diet has been changing along with new era, we are still seeing some society consuming this food due to their culture and behavior. This commodity also has a high protein content. Therefore, this abundant staple raw food is a promising sources and diversification strategy to explore their potential raw material as a flavour enhancer.

The umami taste requires protein hydrolysis which releases specifically glutamic acid, peptides, and the other free amino acids that contributes to enhancing savoriness (Rastogi & Bhatia, 2019). Protease plays a key role in umami taste production from natural sources by enzymatic hydrolysis methods, such as pepsin, trypsin, papain, pronase, biprase, rapidase, and other proteases. However, papain is a proteolytic enzyme that is extracted from any part of papaya plants (*Carica papaya*), such as latex, leaves, tree trunk, and fruit. The statistic shows that the production of papaya in Indonesia has increased from 2010 to 2019 (Hirschmann, 2020). This benefit is an advantage to utilize papaya into other derivative products, as well as papain.

As far as we know, no study on flavour enhancers derived apple snail (*Pila ampullacea*) have been reported. Therefore, this research aims to find out the specific establish treatment based on papain concentration and hydrolysis time towards the degree of hydrolysis and glutamic acid content from hydrolysate of apple snail. This preliminary research would be used as a flavor enhancer for umami taste. Hopefully, this study could be used as a food ingredient that has nutritional benefits and adds an *umami* flavor to dishes.

Material and Methods

Materials and chemicals

Apple snails were obtained from Soponyono traditional market, Surabaya city, East Java Province, Indonesia in July 2020. Papain was bought from Merck Millipore (Burlington, Massachusetts, USA). All chemical reagents used in this study were analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA).

Proteolytic hydrolysis of apple snails

The flesh of apple snails was separated from its shell and the flesh was cleaned using flowing water. Distilled water was added to the flesh of apple snails with a ratio of 2:1 (v/w) and grinding of the flesh was conducted using a Philips HR2115 blender (Amsterdam, Netherlands). The proteolysis conditions for papain hydrolysis of apple snail flesh were observed based on various of enzyme concentrations (1, 5, and 10%) and hydrolysis time (3, 6, 9, 12, 15, and 18 h). The proteolysis reactions were incubated at 54 ^{o}C. The hydrolysis reaction was stopped using a high temperature at 90 C for 10 min. Supernatant and pellet were separated by centrifugation (3000 rpm, 30 min) and the supernatant was transferred into fresh tubes.

Degree of hydrolysis determination

The degree of hydrolysis (DH) was analyzed according to the method developed by Morais *et al*. (2013) with slight modification. The apple snail flesh hydrolysates of 1 mL were mixed with 10% of TCA solutions, followed by incubation for 30 min. The mixture was centrifuged at 3000 rpm for 15 min. The supernatant was analyzed the soluble protein content by the method of Lowry et al. (1951). The DH number was determined based on the following equation:

$$DH\ (\%) = [\text{soluble protein content in 10\% TCA} / \text{total protein content}] \times 100$$

Quantification of glutamic acid

The glutamic acid analysis was determined by the method of Khokhani et al. (2012) with slight modification. The mixture solutions containing 5 mL of apple snail flesh hydrolysates were added with 0.5 mL ninhydrin reagent and 2.5 mL of 40% ethanol. The sample solutions were mixed using

a vortex mixer. The homogenized sample solutions were heated in a water bath for 20 min, followed by analyzed their absorbances using a spectrophotometer with a wavelength at 570 nm, individually. The obtained absorbance values were calculated using an equation from a standard curve of glutamic acid.

Results and Discussion

Effects of different papain concentrations and hydrolysis time on the degree of hydrolysis

The DH shows the number of peptide bonds of protein in hydrolysate that cleaved by protease activity. Protein hydrolysate refers to products that have highly purified protein content from sources. However, we define that hydrolysate was obtained from the whole part of apple snail flesh. This method aims to cover all substances that contribute to enhancing the umami taste, although only the supernatant (aqueous fraction) was collected from proteolysis condition. To assess the effects of hydrolysates digested by various papain concentration and hydrolysis time on DH, the analysis was conducted using the method described by Morais et al. (2013). The DH of apple snail flesh hydrolysed by papain with various enzyme concentrations and hydrolysis times are shown in Fig. 1.

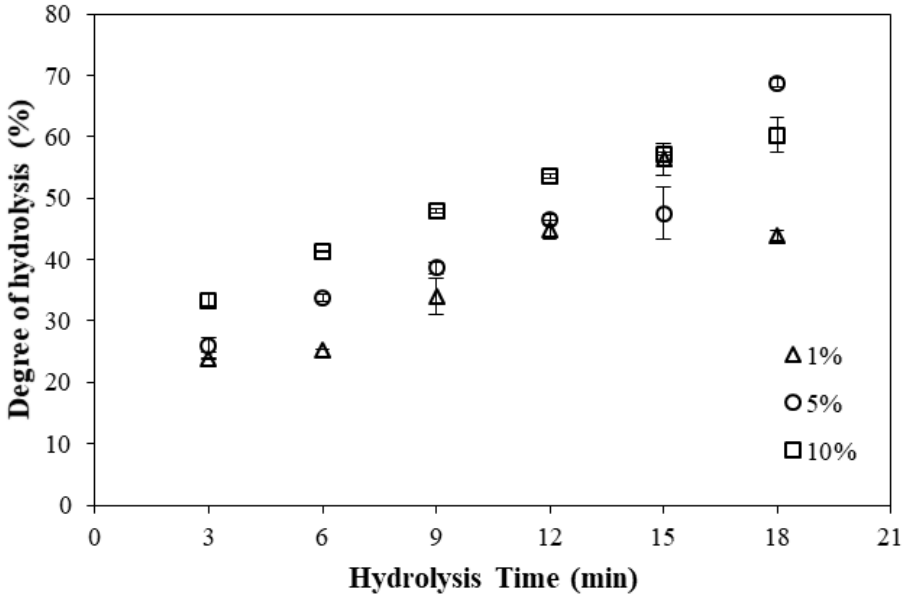

Figure 1. Degree of hydrolysis of apple snail flesh hydrolysates obtained by proteolysis of papain with different concentrations of 1% (Δ), 5% (○), and 10% (□).

DH number of hydrolysates were obtained within the range of 23.73 ± 0.12 to 60.29 ± 2.79% and exhibited considerably different ($P < 0.05$) among others. Papain has broad specificity and prefers to leave the peptide bonds with basic amino acids, such as leucine and glycine. Hydrophilic residues reside in outer the surface area and hydrophobic residues incline be located inside the molecule (Amri & Mamboya, 2012). Those indicate that solubility in the aqueous phase helps proteolysis reaction. The higher concentration and the longer hydrolysis time generated a higher number of DH, as shown in Fig. 1. A similar effect of proteolysis time using papain for goat milk and cow milk had been reported that the time provided increasing the DH level of hydrolysates (Shu et al., 2018).

Effects of different papain concentrations and hydrolysis time on the glutamic acid content

The results of glutamic acid concentration in hydrolysates obtained from triplication are of 53.76 ± 1.65 to 102.08 ± 6.91 ppm. Statistical analysis shows substantially differ ($P < 0.05$) from the two independent variables on glutamic acid contents. Higher papain concentration and hydrolysis time generate the higher content of free glutamic acid in hydrolysates, as shown in Fig. 2. A longer time of hydrolysis facilitates the degradation of protein resulting in a small fragment of peptides and free amino acids. This parameter is related to DH of hydrolysates due to its values associated with the activity of the enzyme.

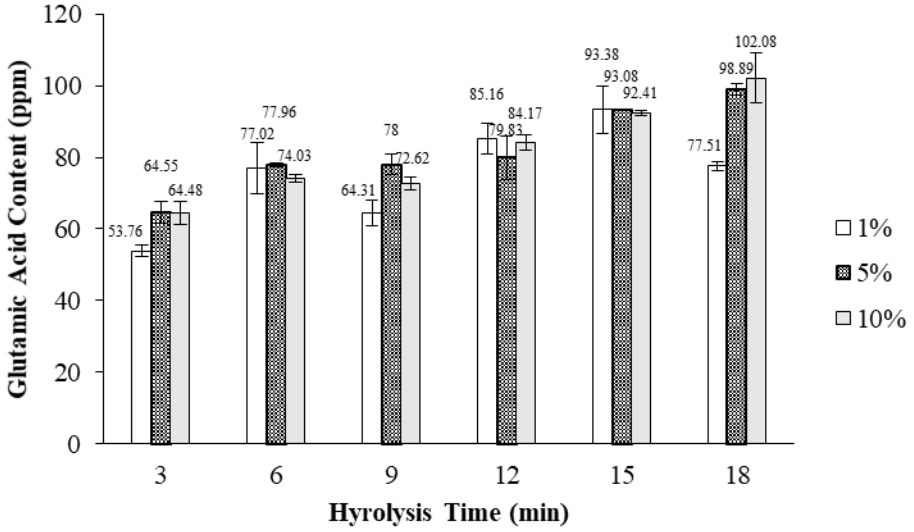

Figure 2. Glutamic acid content of apple snail flesh hydrolysates obtained by proteolysis of papain with different concentrations of enzyme.

The previous study about papain-hydrolyzed beef meet has been reported that identified umami taste from octapeptide, specifically the sequence is KGDEESLA (Yamasaki and Maekawa, 1978). Earlier studies about the application of papain showed effectiveness to degrade bitterness substances of hydrolysates and increase umami flavor from bovine muscle and porcine plasma (Fu et al., 2018). Papain is classified as endopeptidase, amidase, and esterase activities. Not only the proteins are hydrolyzed, but also the lipids are digested by this enzyme. The formed free fatty acids in hydrolysates would contribute to boosting the umami feeling. The high taste of umami properties is required in hydrolysates as flavor enhancers from any substances, even though this study only was focused on glutamic acid content.

Conclusion

The properties of papain have many advantages for proteolysis application in umami taste production as flavor enhancers due to the widespread utilization of these products.

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