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*Article*

# Influence of Enzyme Concentration and Hydrolysis Time on Soluble Protein Content of Protein Hydrolysate Prepared from Apple Snail (*Pila ampullacea*)

**Andre Yusuf Trisna Putra[1], Dedin Finatsiyatull Rosida[1*], Anugerah Dany Priyanto[1]**

[1] Department of Food Technology, Faculty of Engineering, UPN "Veteran" Jawa Timur
Jl. Rungkut Madya No.1, Gn. Anyar, Kec. Gn. Anyar, Surabaya, Indonesia
*Corresponding author: dedin.tp@upnjatim.ac.id | Phone number: -

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**Abstract**

The objective of this study was to evaluate soluble protein content of protein hydrolysates obtained by enzymatic hydrolysis of apple snail using a trypsin enzyme. Apple snail were collected from traditional market at Pabean-Sidoarjo. Trypsin enzyme was used in enzymatic hydrolysis. The two variables, enzyme/substrate (E/S) ( 0.01, 0.05, 0.1) ratio and hydrolysis time (3 h, 6 h, 9 h, 12 h, 15 h, 18 h) and was used to produce the apple snail hydrolysate. The result showed that soluble protein content was about 2.3%-4.52%. The increase E/S ratio and hydrolysis time, the higher soluble protein content values was. The highest total soluble protein was achieved E/S 0.1 ratio at 12 h, 4.52%. But, after 12 h hydrolysis time, soluble protein was decreased. Optimum treatment to hydrolyzing apple snail using trypsin enzyme was E3H4 treated (E/S 0.1 ratio and 3 h)

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

## 1. Introduction

*Pila ampullacea* are freshwater snails belonging to the family *Ampullariidae*. Apple snail (AP) *(Pilla ampullaceal)* is known as a parasite in the field throughout the Southest Region and some of islands in the Pacific. This animal has become a serious agricultural parasite causing damage in rice and other crops[1].

*Pila ampullacea* meat contains several nutrients, such energy, protein, and minerals. 100 g contains energy 209.49 kcal, protein 10.67 g, lipids 0.06 g, calcium 129 mg, magnesium 31.19 mg, potassium 71.13 mg, zinc 1.31 mg and iron 10.9 mg. The protein contents of 10.40% of *Pila ampullacea* is still comparable to values with another livestock [2].

As future protein source, apple snail have been studied and processed into food products. For examples, Ihsani, et al [3] reported that *Pila ampullacea* protein could be used as an instant baby porridge. The protein content of instant baby porridge, complementary feeding with the substitution of freshwater snail flour ranged from 10.41-18.97% per 100 g. Broto et al [4], apple snail can be processed into cracker. The protein content of this cracker is 10.67%

Solubility is an important factor because it can affect the functional properties of protein. Enzymatic hydrolysis can make protein more soluble. Besides that, hydrolyzed protein can play role as bioactive compounds such as antihypertensive, antioxidant, antimicrobial, anticoagulant, and chelating agent. Generally, bioactive peptides compound are a result of enzymatic hydrolysis of protein in the gastrointestinal tract. But, they can also be produced by enzymatic hydrolysis using

commercial protease enzyme such as papain, bromelain, trypsin, alcalase, and flavourzyme [5].

Characteristics of hydrolyzed protein can be influenced by enzyme concentration, type of enzyme and hydrolysis time. For the hydrolysis of two pea protein isolates, Pisane and Propulse isolates by trypsin, higher degree hydrolysis values were observed at ascending E/S ratios. An increase in the E/S ratio to 35 mAU/ gave a degree of hydrolysis value of 11.5% [6]. Trypsin works by hidrolysing proteins and other biological processes. Mainly at the C-terminal side (carboxyl side) of the amino acids lysine or arginine, residues, Trypsin crackles peptide chain [7].

Protein which has high solubility has the potential to have good functional properties to be applied in food processing [8]. Hydrolytic enzymes enhance the functional properties of protein foods without deteriorating their nutritional value. In our previous study [9], time hydrolysis and enzyme concentration have significantly influence on soluble protein content. Increasing of the hydrolysis time, soluble protein content increases ranged from 0,5%-2,9%. On the other hand, an increase of the enzyme concentration, soluble protein content increases ranged from 0,02%-2,6%. Until now, no research has been conducted on the hydrolysis of apple snail using trypsin enzyme. So, the aim of the present study was to determine the soluble protein content of apple snail by trypsin enzyme as affected by enzyme/substrate ratio and time of hydrolysis during the enzymatic process using.

## 2. Material and Method

### Sample preparation

Apple snail (*Pila ampullacea*) (AS) was a purchased 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). Trypsin was purchased from Merck Millipore (Burlington, Massachusetts, USA).

### Enzymatic hydrolysis

Protein hydrolysates preparation was determined according to the method that described by Andre *et al* 2020 [9]. *Pila ampullacea* meat was cut into 1x1cm and then blanched at 93-94$^0$C for three minutes. *Pila ampullacea* meat was minced using a grinder with suspended in distilled water with 1:1 (w/v) ratio at temperature room. Enzymatic hydrolysis was performed in a water batch at room temperature (30$^0$C). The hydrolysis condition was treated using different enzyme concentrations from 0.01 to 0.1 E/S, hydrolysis times between 3 and 18 h. The treatments used were E1H1: 0.01 E/S, 3 h; E1H2: 0.01 E/S, 6 h; E1H3: 0.01 E/S, 9 h; E1H4: 0.01 E/S, 12 h; E1H5: 0.01 E/S, 15 h; E1H6; 0.01 E/S, 18 h; E2H1: 0.05 E/S, 3 h; E2H2: 0.05 E/S, 6 h; E2H3: 0.05 E/S, 9 h; E2H4: 0.05 E/S, 12 h; E2H5: 0.05 E/S, 15 h; E2H6; 0.05 E/S, 18 h; E3H1: 0.1 E/S, 3 h; E3H2: 0.1 E/S, 6 h; E3H3: 0.1 E/S, 9 h; E3H4: 0.1 E/S, 12 h; E3H5: 0.1 E/S, 15 h; E3H6; 0.1 E/S, 18 h. The resulting hydrolysates were heated in boiling water for 3 minutes to inactivate the enzyme. After cooling to room temperature, the hydrolysates were centrifuged at 3,000 rpm for 30 minutes to remove impurities. The supernatant was transferred in a refrigerator until it was ready to be analyzed.

Table 1. Hydrolysis condition of apple snail by trypsin enzyme in relation to E/S ratio and hydrolysis time

| | Enzyme concentration (E) | | |
|---|---|---|---|
| Hydrolysis time (H) | 0.01 E/S (E1) | 0.05 E/S (E2) | 0.1 E/S (E3) |
| 3 h (H1) | E1H1 | E2H1 | E3H1 |
| 6 h (H2) | E1H2 | E2H2 | E3H2 |
| 9 h (H3) | E1H3 | E2H3 | E3H3 |
| 12 h (H4) | E1H3 | E2H4 | E3H4 |
| 15 h (H5) | E1H4 | E2H5 | E3H5 |
| 18 h (H5) | E1H5 | E2H6 | E3H6 |

### Soluble protein content

Soluble protein content of the sample, was measured by Silvestre [10] with modifications. Aliquots of Aliquots of 1000µL of the Apple Snail 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 x 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.

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## 3. Results and Discussion

### Soluble Protein Content

The effect of E/S ratio and hydrolysis time of apple snail hydrolysate is presented in Fig. 1. For 0.01 E/S ratio the highest value, 3.72% was obtained at 12 h (E1H4); the lowest value, 2.3% was obtained at 3 h (E1H1). For 0.05 E/S ratio the highest value, 4.14% was obtained at 12 h (E2H4); the lowest value, 2.4% was obtained at 3 h (E2H1). For 0.1 E/S ratio the highest value, 4.52% was obtained also at 12 h (E3H4); the lowest value, 2.4% was obtained at 3 h (E3H4). The increase in the E/S ratio affected to soluble protein content value. But, at low hydrolysis time, 3h, higher E/S ratio was not affected to soluble protein content. The highest soluble protein occurred at 0.1 E/S ratio and 12 h (E3H4), whereas The lowest soluble protein occurred at 0.1 E/S ratio and 13 h (E1H1).

In the case of hydrolysis time, an increase soluble protein occurred at 3 h to 12 h, but there was a decrease at 15 h and 18 h. The soluble protein content of apple snail hydrolysate treated 0.01 E/S ratio increased from 2.3% at 3 h to 3.72% at 12 h, but decreased at 15 h dan 18 h. The same trend showed treated by 0.05 E/S ratio and 0.1 E/S ratio; increased from 2.33% to 4.14%, but decreased after that at 15 h and 18 h. Also, the same trend showed treated by E/S 0.1 ratio, an increase from 2.4% to 4.52%, but decreased after that at 15 h and 18 h.

Hydrolysis time have significantly influence on soluble protein content. This trend similar with our previous study Andre et al [9] (2020) that increase hydrolysis time, soluble protein content was increased. According to Saidi et al [11](2013) said that the amount of the soluble fraction increased sharply during the first few minutes of hydrolysis and then stabilized at a value dependent on the enzyme concentration. Similar results have been reported [12] in Cowpea (*Vigna unguiculata*) protein hydrolysates produced with alcalase and flavourenzyme that 60 min was the best results of protein solubility. Segura-Campos [12] stated that the increase hydrolysis time, the higher solubility and degree of hydrolysis. The analysis of the soluble protein content shows that the rate of formation soluble protein increased with increasing time during the first 12 h min. From this behavior, we concluded that it is not advisory to continue hydrolysis over 12 h, because there was not a significant increase in rates of soluble proteins.

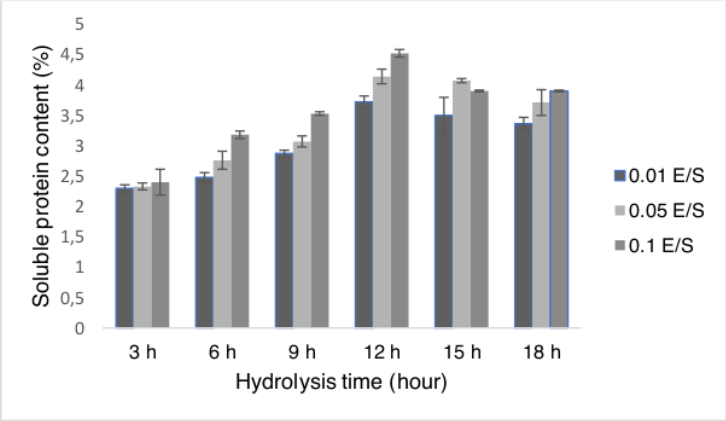


**Fig. 1** Soluble protein content of Apple Snail hydrolysate with trypsin enzyme on different E/S ratio and hydrolysis time

In this study, enzyme:substrate ratio (E/S) showed a positive effect on soluble protein content, that is, the increase on this parameter resulted in higher soluble protein content.. The highest value of soluble protein content in E3H4 was 4.52%. But, at low hydrolysis time, i.e. 3 h, the soluble protein content does not affected by E/S ratio. The value of soluble protein content treated E1H1, E2H1, and E3H1 at 3 h was 2.3%, 2.33%, 2,4%, respectively. At higher hydrolysis time, E/S ratio significantly affected to soluble protein content. At E/S 0.1 ratio, significantly affected to value of soluble protein content. According to Saidi et al [11] the concentration

enzyme was the variable that has the greatest effect on enzymatic hydrolysis, followed by the hydrolysis time and and temperature. Solubility changes in the apple snail hydrolysates may have been caused by a reduction in molecular weight of the enzymatically modified proteins. The increased solubility observed in this study makes the apple snail hydrolysates potentially useful in applications requiring high solubility profiles to impart certain characteristics to a food application. According to Montilha et al [13], investigated the hydrolysis with alcalase enzymes of protein isolate from okara. The authors verified that degree of hydrolysis increased with enzyme concentration. 8% enzyme:substrat showd the highest of degree of hydrolysis.

## Conclusion

Soluble protein content increase with increasing of enzyme concentration and hydrolysis time, but decreased after hydrolysis time at 12 hours. Optimum treatment to hydrolyzing apple snail using trypsin enzyme was E3H4 treated (E/S 0.1 ratio and 3 h).

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