ISOLATION OF PROTEASE ENZYME FROM CHAYOTE FRUIT (*Sechium edule* (Jacq.) Sw.) WITH AMMONIUM SULFATE FRACTINATION METHOD

Ketut Ratnayani* and Lia Kusumaningrum
Chemistry Department, Faculty of Mathematics and Natural Science, Udayana University
*Corresponding author : ratnayaninew@gmail.com

ABSTRACT

Protease is an enzyme that is capable to hydrolyze (breakdown) protein molecules into simpler compounds such as small peptides and amino acids. The aim of the research was to isolate protease enzyme from chayote (*Sechium edule* (Jacq.) Sw.) using fractination ammonium sulfate method and to find out the optimum saturation level of the ammonium sulfate. P rotease activity examination of each fraction of ammonium sulfate was performed using Anson method. Protein content assay was determined using Biuret method. The results showed that crude extract protease of chayote had specific activity of 3,7338 x 10^{-3} U/mg. The optimal saturation levels of ammonium sulfate for protease chayote precipitation was 40-50%. At this saturation level, the highest enzyme spesific activity were 16,00 x 10^{-3} U/mg, with four times purifying of protease enzyme from the crude extract protease.

Keyword : protease, protease activity, ammonium sulfate, specific activity

INTRODUCTION

Protease is an enzyme that is capable to hydrolyze (breakdown) protein molecules into simpler compounds such as small peptides and amino acids. The source of protease enzymes comes from microorganism, animal and plant. Plant is the biggest source (43.85%) followed by bacteria (18.09%), fungi (15.08%), animal (11.15%), algae (7.42%) and viruses (4.41%) (Mahajan and Shamkant, 2010). Plant protease has higher activity and stability on various temperature, pH, inhibitor and metal ion (Mehrnoush *et al.*,, 2011).

One of the subtropical plant in Indonesia is *labu siam* or chayote (*Sechium edule* (Jacq.) Sw) which is commonly served as cooked vegetable. Plant latex that causes itching may due to its protease component (Lavinka and Dong, 2013). It is predicted that chayote fruit has protease activity because its latex causes itching as well as papaya's (Joseph, 2010). Juwarni *et al* (2014) found that the chayote latex contained protease with protease activity as much as 0.0264 U/mL. However, there are some problems in latex collection from chayote fruit. The latex is rapidly agglomerated, few in number and easily sticking to the blade when tapped from the fruit. So it is necessary to find another alternative method for obtaining proteases from chayote fruit, in this case by directly squeezing the flesh without collecting the sap.

For characterization and next application purposes, protease need to be isolated from chayote fruit with fractionation using ammonium sulfate salt. Ammonium sulfate salt often used to fractionate enzyme and protein due to its high solubility, giving stabilizing effect on the enzyme and do not disturb protein structure (GE Healthcare Life Sciences, 2011). Protease enzyme isolation using ammonium sulfate have been reported by some researchers. Noda, Koyanagi and

Kamiya (1994) isolated protease from melon fruit using ammonium sulfate with 50% saturation level. Asakura *et al.,* (1997) extracted and purified *oryzasin* from rice seed using ammonium sulfate 30-60% saturation level.

The aim of the research was to isolate protease enzyme from chayote fruit (*Sechium edule* (Jacq.) Sw.) using fractination ammonium sulfate method and to find out the optimum saturation level of the ammonium sulfate.

MATERIAL AND METHODS

Material

Chayote fruit, aquades, ammonium sulfate ($(NH_4)_2SO_4$), bovine serum albumin (BSA), tyrosine, natrium metabisulfite 1%, casein 0,65% (b/v), K_2HPO_4 0,05 M, KH_2PO_4 0,05 M, Na_2CO_3 0,5 M, TCA (Trichloroacetic Acid) 0,11 M, Biuret reagent and Folin-Ciocalteau reagent.

Crude Extract Preparation

The crude protease extract was obtained by separating the enzyme from chayote fruit. Two hundreds grams of chayote fruit was added with 90 mL of cold buffer fosfat 0.05 M (pH of 7) which contained 1 % of natrium metabisulfite. Then it homogenized and filtered using a filter paper. The filtrate then was centrifuged at 5000 rpm for 15 minutes, at a temperature of 4^{o}C. The supernatant was collected and separated from the sediment. Supernatant obtained from this step referred as supernatant I, which will be used for the fractionation process.

Protease Isolation Method Using Ammonium Sulfate Fractionation

A hundred mL of the supernatant I (crude extract protease) was fractionated with ammonium sulfate. Determination of the amount of ammonium sulfate that is used to achieve the expected saturation level refers to a table compiled by the Scopes (1982). The percentage of saturation of ammonium sulfate used was 0-20%, 20-50% and 50-70%. The addition of ammonium sulfate salts was gently done using a magnetic stirrer until a certain saturation level was achieved. The precipitation obtained by centrifugation at 5000 rpm and a temperature of 4 ° C for 15 minutes. Then it dissolved in 12 mL of 0.05 M of cold phosphate buffer (pH 7) containing 1% sodium metabisulfite. Ammonium sulfate fraction with the highest specific activity occurred prior to fractionation protease.

Assay of Protease Activity

Determination of enzyme activity using the method of Anson (Anson, 1938) with a colorimetric technique that utilizes absorption of the blue complex formed by reaction of tyrosine with Folin Ciocalteu reagent (Folin and Ciocalteu, 1927). Casein solution 0.65% (w/v) of 2.5 mL in the pre-incubation at 37°C for 4 minutes added 1.0 mL sample of precipitant. This mixture incubated at 37°C for 30 minutes. Hydrolysis reaction was stopped by addition of 2.5 mL of 0.11 M TCA and allowed to stand for 5 minutes. This mixture was centrifuged for 15 minutes at a speed of 5000 rpm. Protease activity was determined by measuring the levels of tyrosine generated using colorimetric reagent Folin-Ciocalteau. As many as 2.0 mL of the supernatant was reacted with 5.0 mL of 0.5 M Na_2CO_3 and 1.0 mL reagent Folin-Ciocalteau then the mixture was left for 30 minutes. Read of the absorbance of this solution was done using a UV-Vis spectrophotometer at 755.4 nm. As a blank, the addition of 0.11 M TCA conducted

before the addition of a protease extract sample. Protease activity (U / mL) is expressed in units of activity. one unit (U) is expressed as the amount of protease that can hydrolyze a substrate (casein) and produce a color equivalent to 1 µmol tyrosine product (181 µg) every minute incubation period in experimental conditions. Protease enzyme activity can be determined by the formula (Sigma, 1999):

$$\text{Protease Activity}\,(\text{U/mL}) = \frac{\mu\text{mol tirosin} \times V_1}{V_2 \times t \times V_3}$$

V_1 = total volume used on sample protease activity assay; V_2 = volume of crude protease extract sample ; V_3 = volume of supernatan used on tyrosin concentration analysis; t= incubation time.

Determination of Total Protein Content (AOAC, 1995)

Precipitate of 1.0 mL sample was reacted with 5.0 mL of Biuret reagent and then allowed to stand for 20 minutes at room temperature. The absorbance of mixture was measured using a UV-Vis spectrophotometer at 547 nm. Total protein content is calculated by converting the absorbance to the standard linear regression equation of BSA.

RESULT AND DISCUSSION

In the preparation phase of the crude extract of the fruit flesh of squash obtained filtrate (filtering results juice) is solid green. The filtrate was centrifuged resulting in crude protease extract (supernatant I) light green, 200 grams of chayote fruit obtained crude extract as much as 200 mL.

The determination of the specific activities carried out to determine the level of purity of the enzyme, the higher the value of specific activity, the higher the purity of enzymes that have been isolated (Lehninger, 1990). The specific activity of the protease enzyme is an enzyme activity for each milligram of total protein extracts of chayote. The specific activity of chayote protease in the crude extract, fractions of 0-20%, 20-50% and 50-70 can be seen in Table 1.

The highest specific activity found in fractions of 20-50%, so it was conducted the next fractionation using ammonium sulfate saturation level with a more narrow range that is 20-30%, 30-40% and 40-50%. The results of the specific activity value can be seen in Table 2.

Table 1. Specific activity of chayote protease on crude extract, 0-20% fraction, 20-50% and 50-70% fraction

Description	Protease Activity (U/mL)	Total Protein Level (mg/mL)	Specific Activity (U/mg)
Crude extract	5,13 x 10^{-3} ± 0,0775	1,3782 ± 0,0822	3,7338 x 10^{-3} ± 0,0018
0-20% fraction	1,09 x 10^{-3} ± 0,0000	0,8431 ± 0,0125	1,2931 x 10^{-3} ± 0,00002
20-50%fraction	25,30 x 10^{-3} ± ,0002	1,4938 ± 0,0125	16,9000 x 10^{-3} ± 0,0003
50-70%fraction	4,05 x 10^{-3} ± 0,0000	0,6551 ± 0,0000	6,1823 x 10^{-3} ± 0,0000

Table 1 shows that there was an increasing of protease activity in fractions of 20-50% i.e. 25.30 x 10^{-3} U/mL, whereas in Table 2 it appears that protease activity as a whole has increased from crude extract (5.13 x 10-3 U/mL). From the crude extract to the addition of salt $(NH_4)_2SO_4$ 30-40% rise and drop upon addition of salt $(NH_4)_2SO_4$ 40-50%. The decrease and increase in the activity of this protease can be caused by variations in the amount of protease enzyme protein and non-protein enzyme that settles in each fraction as protease results fractionation was still consists of protein enzymes and non-enzyme proteins (Wang, 2004), as well as due to the respective each type of protein has a different solubility (Scopes, 1982).

The total protein content shows a decrease in the fraction of 0-20% and 50-70% and an increase at the fraction of 20-50% compared to the protein content of the crude extract (Table 1). The total protein content in decreased in each additional level of salt saturation $(NH4)_2SO_4$ (Table 2). This is caused by each protein has a different solubility (Scopes, 1982) and in this case a protein thought to have much precipitate in the fractions of 20-30%, so that the protein is not too much precipitate in the fraction of 30-40% and 40-50% , The addition of ammonium sulfate salt resulted in water molecules bind to the salt ions, so that the amount of water that bind to protein is reduced and resulting precipitation of proteins (Seidman and Mowery, 2006).

Although the value of the highest protease activity was found in fractions 20-30% and the highest protein content was found in fractions 30-40%, but the fractions has a lower specific activity. The specific activity showed a purity level of protease enzyme. The more pure protease enzyme obtained, would have a value of specific activity increasing (Lehninger, 1990). During the process of purification, enzyme concentration increased relative to the total protein content to a certain extent (Lehninger, 1990). The purity of protease obtained in fractions of 40-50% (16.00x10^{-3} U/mg) reach 4 times than the specific activity of the crude extract (3.7338 x 10^{-3} U/mg)

CONCLUSION

It can be concluded that the value of the specific activity of the crude extract protease (before the stage of salting out) was 3.7338 x 10^{-3} U/mg. The most optimal of ammonium sulfate saturation level to precipitate chayote protease was 40-50%.

Tabel 2. Specific activity of chayote protease

Description	Protease Activity (U/mL)	Total Protein Content (mg/mL)	Specific activity(U/mg)
20-30% fraction	9,6933 x 10^{-3} ± 0,0074	0,7563 ± 0,0821	12,30 x 10^{-3} ± 0,0119
30-40% fraction	13,7700 x 10^{-3} ± 0,0124	0,4671 ± 0,0102	11,05 x 10^{-3}± 0,0303
40-50% fraction	5,4933 x 10^{-3} ± 0,0039	0,3588 ± 0,2622	16,00 x 10^{-3} ± 0,0049

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