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RESEARCH ARTICLE

THE EFFECT OF AMONIUM SULPHATE CONCENTRATION ON THE ISOLATION RESULT OF BROMELIN SKIN AND TUBERS OF PINEAPPLES

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ABSTRACT

Pineapple is widely known by Indonesia people because the plants are widely grown in tropical climates Indonesia. Pineapple is often used as meat diggers because it contains a component of bromelin enzyme which has a sulfhidril (-SH) protease that can hydrolyze peptide bonds in proteins into bundles of amino acid compounds are simpler so that it can make meat softner and help the process of digestion. The aim of this study was to isolate bromelin enzyme from pineapple tubers and pineapple skin which is pineapple waste with ammonium sulphate salt with four treatments (40%, 60%, 80%, and 100%) and three repetitions for having rendemen and activity enzyme. Calculated rendemenanalyzed by one way Anova. The most optimal ammonium sulfate concentration for isolate enzyme is at 60% concentration, with rendemen of 1.67% and enzyme activity of 3.33 U/mL. The concentration of 40% has a rendemen of 0.37% with enzyme activity of 6.67 U/mL, 80% concentration with 0.91% rendemen with 1.67 U / mL enzyme activity, and 100% concentration has 0.94% rendemen with activity enzyme 1.67 U/mL.

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INTRODUCTION

Enzymes are protein compounds that function as biocatalysts in cell metabolism of living things. The use of enzymes has been done in various fields of industry, for food, agriculture, chemical and pharmaceutical products. Protease is one of the enzymes that are widely used for both food and non-food such as research on the addition of papain enzymes in cocoa fermentation which was carried out by Nurhayati (2012), the addition of bromelin enzyme to the manufacture of cassava tape in Wulandari (2008) study, the addition of enzyme is able to enhance protein level, whereas both papain enzyme and bromelin enzyme are protease enzymes that can be isolated from papaya sap papain) and from the pineapple tree (bromelin). Pineapple fruit is widely known by the people of Indonesia because the plants are widely grown in tropical Indonesia. This plant is widely cultivated in the tropics and sub tropics, where originally originated from Brazil (South America). This fruit has a fresh sour taste to sweet and contains nutrients that are high enough to be liked by the wider community either consumed in fresh condition or after processing such as dodol, syrup, jam and others. Pineapplefruit is often used as meat pengempuk because in the pineapple contains a component of bromelin enzyme which is a sulfhidril (-SH) protease enzyme that can hydrolyze peptide bonds on proteins into bundles of amino acid compounds that are simpler so thatit can mengempukan meat. Bromelin can also be utilized other than as for meat busting as well as a drug indigestion, peluruh dead cells and anti-inflammatory. This enzyme is also used for industrial applications in the dissolution of wheat proteins, beersterilization, protein hydrolyzate production, and tannery (Secor *et al.,* 2005 and Fileti *et al.,* 2009). In the pineapple processing industry always leave a lot of waste or waste. Generally, pineapple waste in the form of stems, leaves, skin, bonggol has not been utilized optimally, therefore by isolating the enzyme bromelin from the skin and pineapple, is an alternative in the utilization of pineapple waste so as to provide added value for pineapple fruit in addition to reducing the problem pollution of waste to environment. The process undertaken to obtain pure bromeline enzymes from pineapple fruit is enzyme isolation. The bromeline enzyme can be isolated by separating the cell centrifugation and further purification by precipitation, filtration gel, and ion exchange chromatography (Naiola and Widhyastuti, 2007). How to apply with organic solvents and with salt. The addition of an organic solvent or salt into a solution containing the enzyme causes the solubility of the enzyme in the solution to fall, and the enzyme will precipitate. The results of Kumaunang and Kamu (2011) study entitled "Bromelin Enzyme Activity from Pineapple Skin Extract"using10%, 20%, 30%, 40%, 50%, and 60% ammonium sulphate salt as bromelin enzyme extract and obtained temperature and the optimal pH of bromelin enzyme 65 ^{o}C and pH 5.0-

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6.5 and the highest protein concentration in 60% ammonium sulphate salt extract. In the Masri study (2014), the higher the concentration of ammonium sulphate, the more protein bromelin enzyme from the precipitated pineapple and from the results of this study the concentration of 60% yields the highest protein content. Research on pineapple skin insulation with ethanol 70% by Yulianto *et al.* (2014) obtained 2,580 g of dried bromellin enzyme powder with water content of 5.3259% of 1 kg of fresh pineapple skin derived from 6 kg of fresh pineapple fruit. The result of Salahudin (2011) research that the best settling material in bromelin isolation is ammonium sulphate with 80% concentration which yield 0.61% rendement with 12,5 U/mL enzyme activity compared to ethanol at 80% concentration yield 0.38% with enzyme activity of 0 U/mL. The results of Chaurasiya and Hebbar (2013) study that bromeline isolation with acetone and ammonium salt yielded 4.90and 3.07-fold purification under optimal conditions. Based on the above background the researcher intends to conduct research with the utilization of pineapple waste, such as pineapple skin and pineapple cobs to be taken enzyme. The study to obtain the enzyme bromelin from pineapple waste by isolatingbromelin enzyme using variation of ammonium sulfate salt concentration.

MATERIALS AND METHODS

The study design was experiment with isolation of bromelin enzyme from pineapple fruit by precipitation with ammonium sulphate salt, then calculated the rendemen (yield). Pineapple fruit used is pineapple varieties Subang (Smooth Cayenne). This study is planned to last for 7 months from April to air Okto 201 7. The research location is Integrated Laboratory of Health Polytechnic of Bandung and Chemistry Laboratory UNPAD. The population of this research is pineapple fruit of varieties Subang 7-10 kg and sample of research is leather and pineapple 1-3 kg pine.

a. **Creation of Rough Skin Extracts and Pineapple Fruit Curcuma:** Leather and pineapple fruit culms are used from pineapple fruit that is still mengkal, marked with yellowish-green skin color. Leaf and pineapple washed with aquades, cut into small pieces and weighed as much as 1,500 grams, then homogenized using 200 mL of sodium acetate buffer solution (pH 6.5) then filtered. The crude extract was centrifuged for 25 minutes at 3,500 rpm and stored at 4 ° C (Kumaunang & Kamu, 2011).
b. **Isolation of crude extract of bromelin enzyme:** To the crude extract of bromeline enzyme was added 40%, 60%, 80% and 100%, while stirring using a magnetic dye for 45 minutes, and incubated overnight at 4 ° C. Furthermore, it was centrifuged at 3500 rpm for 25 min. The resulting precipitate was washed with 10 mL of 0.1 M sodium acetate buffer in the pH range 6 - 6.5. The precipitates obtained were dried by frozen drying (Gautam *et al*., 2010).
c. **Rendemen of rough extract of bromeline enzyme:** The yield of bromelin enzyme was obtained from the weight of the enzyme produced by the weight of the skin and the pineapple fruit used.
d. **Test activity of rough extracts of bromeline enzymes:** The activity of bromelain enzyme was determined based on Murachi method using casein substrate. A total of 40 mg/mL casein substrate was treated with 0.4 mg/mL of the enzyme inphosphate buffer solution pH 7,5. Comparison of substrate and enzyme (100: 1). Then incubated at 55 °C for 15 minutes. (Sebayang, 2006). After incubation, into the reaction mixture was added 1 mL of 30% trichloroacetic acid solution. The coagulated protein is separated by centrifugation. The obtained fi eld obtained measured its absorbance at a wavelength of 280nm. This is the wavelength absorbed by the aromatic side-chain amino acids of phenylalanine, tyrosine and tryptophan. As a control used enzyme that has been turned off its activity through heating (Sebayang, 2006).
e. **Preparation of a standard tyrosine solution:** Standard stock solution, weighed as much as 0.1g of tyrosine was dissolved with 7 mL 0.1N HCl in a measuring flask and then added aquadest to 100mL boundary mark. Prepared 5 tubes of reaction then made standard working solution 1-5. Tube 1 (1 mL tyrosine + 9 mL aquadest), tube 2 (2 mL tyrosine + 8 mL aquadest), tube 3 (3 mL tyrosine + 7 mL aquadest), tube 4 (4mL tyrosine + 6 mL aquadest), and tube 5 (5mL tyrosine+5mL aquadest). Each tyrosine concentration measured its absorbance at a spectro photometer λ 280 nm. Created standard curve of tyrosine (Said, 2012).

The data were obtained from isolation of crude extract of enzyme by using ammonium sulfate salt at concentration variationsof 40%, 60%, 80%, and 100%. Calculated the weight and yield of the extract and determined the value of its activity. The result data was processed by one-way ANOVA test

RESULTS

The isolation process for obtaining the crude extracts of bromelin enzymes carried out in this study came from skin samples and pineapple cuttings cut into small pieces to facilitate when mashed with blenders. At the time of added blend ice cubes to avoid the occurrence of damage to the enzyme because there is a mechanical movement that causes the rise in temperature. After blending and then filtered and centrifuged, clear filtrate of crude extract was obtained, then added a 0.1 M sodium acetate buffer in the pH range 6-6.6 to maintain the pH stability of the enzyme.

The result of the crude extract of bromelin enzyme obtained is a clear filtrate. After the ammonium sulphate 40% is added the precipitate is added to the condition of the complete dissolved salt, to which 60% ammonium sulphate is added precipitate with the condition of the complete solute salt, for which 80% ammonium sulfate is added the precipitate is floating on the surface of the solution with a perfect non-soluble salt. For the added ammonium sulfate 100% precipitate formed the same as added ammonium sulfate 80% on the surface of the solution and the salt is not completely dissolved, only at a concentration of 100% more insoluble salt than 80%. The deposition of bromelin enzyme that has been dried is then weighed quantitatively. At 40% ammonium sulfate concentration, the average of 0.25 g of sediment deposition was obtained, at 60% concentration was 0.73 g, at 80% concentration was 0.62 g, and at 100% concentration was 0.63 g. The results of the data obtained were processed by one-way ANOVA test and found that there was a significant difference in the concentration of 40% to 60%, 80%, and 100% because the p-value <0,05. But between the concentrations of 60%, 80%, and 100% there is no significant difference because the p-value> 0,05.

Table 1. The weight of bromelin enzyme obtained in various ammonium sulphate concentration (40%, 60%, 80%, and 100%)

No.	Intervention (%)	Repetition (g)		
		I	II	III
1	40	0,14	0,27	0,34
2	60	0,84	0,56	0,79
3	80	0,84	0,52	0,50
4	100	0,80	0,48	0,63

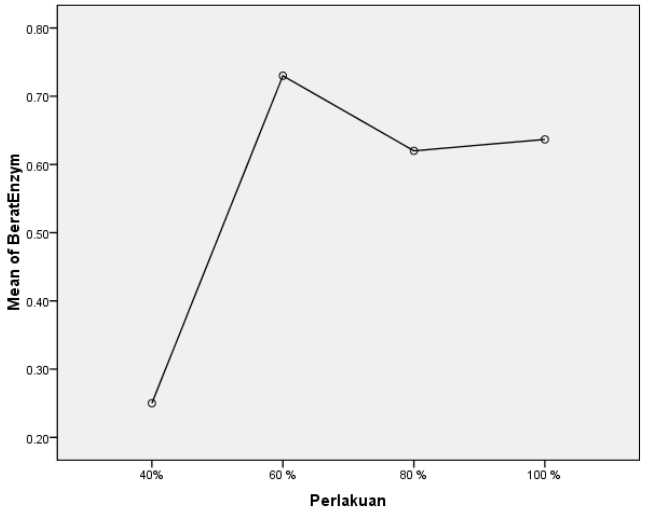

Fig.1. The relationship between treatment of ammonium sulphate concentration (40%, 60%, 80%, and 100%) and bromelin enzyme weight

Table 2. The difference of Bromelin enzyme weight among 40%, 60%, 80%, and 100% groups (One-Way Anova)

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LSD

(I) Perlakuan	(J) Perlakuan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
40%	60 %	-.48000*	.12559	.005	-.7696	-.1904
	80 %	-.37000*	.12559	.019	-.6596	-.0804
	100 %	-.38667*	.12559	.015	-.6763	-.0971
60 %	40%	.48000*	.12559	.005	.1904	.7696
	80 %	.11000	.12559	.407	-.1796	.3996
	100 %	.09333	.12559	.479	-.1963	.3829
80 %	40%	.37000*	.12559	.019	.0804	.6596
	60 %	-.11000	.12559	.407	-.3996	.1796
	100 %	-.01667	.12559	.898	-.3063	.2729
100 %	40%	.38667*	.12559	.015	.0971	.6763
	60 %	-.09333	.12559	.479	-.3829	.1963
	80 %	.01667	.12559	.898	-.2729	.3063

*. The mean difference is significant at the 0.05 level.

Table 3. The yield of bromelin enzyme obtained in ammonium sulphate treatment was 40%, 60%, 80%, and 100%

No	Intervention (%)	Repetition (%)			Average (%)
		I	II	III	
1	40	0,21	0,40	0,50	0,37
2	60	1,24	0,82	1,16	1,07
3	80	0,76	1,23	0,73	0,91
4	100	0,71	0,93	1,18	0,94

Table 4. Activity of Bromelin Fruit Pineapple at concentrations of ammonium sulfate 40%, 60%, 80%, and 100%

No	Intervention (%)	Enzyme activity (U/mL)
1	40	6,67
2	60	3,33
3	80	1,67
4	100	1,67

The yield of bromeline enzyme from concentration 40% - 100% in the average yield at 40% concentration was 0.37%, at 60% concentration was 1.07%, at 80% concentration was 0.91%, and at concentration 100% is 0.94%. The value of bromelin enzyme activity from isolation by ammonium sulphate at concentration of 40% was 6.67 U/mL, at concentration 60% was 3.33 U/mL, at concentration 80% and 100% was 1.67 U/mL (Fig. 1 and Table 1-4).

DISCUSSION

The isolation is done in the study of the skin and tuber pineapple performed to obtain crude extract enzyme bromelain which the skin or hump pineapple is pineapple waste can still be used to produce the enzyme bromelain which is a pro tease enzyme containing sulfhydryl (-SH), which is gluco protein capable of hydrolyzing the proteins into compounds simpler amino acids (Secor *et al.,* 2005). This bromelin enzyme hydrolyzes the peptide bonds in the middle of the peptide chain, thus classified endopeptidase. Side this active bromelin enzyme contains cysteine and histidine which are important for the activity of the enzyme. This enzyme thus specifically intercepts the peptide bonds of the carbonyl group as found in arginine or aromatic amino acids ie phenylalanine or tyrosine (Gautam *et al.,* 2010). The clear filtrate of the pineapple filter is cooled to 4 °C for 3 hours to cool the filtrate before ammonium sulfate salt is added to make it easier to form precipitates when salt is added. Bromelin is easily precipitated by reducing free water in the fruit filtrate. One of the materials capable of binding free water is ammonium sulfate salt. The highly water-soluble ammonium sulfate property and does not react with this enzyme make this salt possible in bromeline isolation (Winarno, 2008).

From the isolation result of ammonium sulphate salt at the most optimal 40%-100% precipitation with the highest weight and yield at 60% concentration, this indicates a 60% concentration reaches the desired isoelectric point of the protein. Precipitation occurs because of the competition between salt and protein to bind water (Masri,2014) because at this concentration is a concentration close to the saturated point of ammonium sulfate salt that is at a concentration of 74.4 g % (Haynes, 2015) . As the result of enzyme isolation research conducted by Nurmala (2012) and Princess *et al.* (2013) at ammonium sulphate concentration of 60% activity test and highest protein content. The enzyme activity test was carried out at 55^{o}C incubation temperature because according to herdyastuti (2006) research the optimum temperature will increase enzyme activity at 55^{o}C with activity value 4.05 U/mL, after passing the temperature its activity decrease, this indicates that at temperature of 55^{o}C enzyme works maximally and at temperature above 55^{o}C enzyme work begin to decrease this because of enzyme is a protein when it is at a high temperature it will experience denaturation so that the enzyme is damaged and activity down.

Considering the enzyme activity calculation results showed that the highest value of enzyme activity of bromelin at concentration 40%, from 60%-100% concentration decrease enzyme activity value, this is probably caused by the influence of salt concentration, where high salt concentration can cause denaturation to protein. saturation of ammonium sulfate salt at room temperature at a concentration of 74.4 g % (Haynes, 2015), so it is possible at concentrations of 60%, 80%, and 100% there are some denatured bromelene enzymes because

high salt concentrations approach and pass through values saturated, so that at the time of activity test the value of activity decreased with increasing concentration of ammonium sulfate salt.

Conclusion

The most optimum concentration of ammonium sulphate to isolate the enzyme bromelin is at a concentration of 60%. The average value of bromelin enzyme yield obtained at 40% concentration was 0.37 %, the concentration of 60% was 1.07%, 80 % was 0.91 %, and the concentration of 100% was 0.94 %. The highest concentration at 60%. The activity of bromelin enzyme at 40 % is 6.67 U/mL, 60 % is 3.33 U/mL, 80% is 1.67U/mL, and 100% is 1.67 U/mL. Performed enzyme isolation using a variation of pH approaching orat its isoelectric pH to obtain a more optimal rendemen of enzyme isolation and to measure the water content of crude extract from bromelin enzyme, to more accurately weight the sample toanalyze other parameters, one for measuring enzyme activity.

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