**Characteristics of Bromelain Enzyme from Queen Variety Pineapple Crown at Different Drying Temperatures**

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*Received ; Accepted ; Published*

**Novelty statement**

Pineapple crown as post-harvest waste is rarely used as source of bromelain enzyme. These results show that at different drying temperature, the characteristic of bromelain enzyme also different. We found that bromelain enzyme from Queen variety pineapple crown has the best characteristics from drying temperature 40°C and precipitated with 60% ammonium sulfate.

**Abstract**

Pineapple (*Ananas comosus* (L) Merr) is a plant that contains bromelain enzymes in fruit, leaves, crown, and stem with different amounts. Bromelain is included in the protease enzyme group that can break down the molecular structure of proteins into amino acids. Pineapple crown is one of the waste products from pineapple processing that has not been used optimally. This study aims to utilize Queen pineapple crown waste and determine the characteristics of Queen pineapple crown bromelain enzymes which consist of protein content, unit activity, and specific activity at different drying temperatures. Queen pineapple crowns were dried using a cabinet dryer at temperature of 35 °C, 40 °C, 45 °C, and 50 °C before extraction process. The optimum temperature in the Queen pineapple crowns drying process to produce bromelain with a protein content of 4.41 mg/ml, unit activity 1.36 U/ml, and specific activity 0.31 U/ml was 40ºC, then purified by adding 20%, 40%, 60%, and 80% ammonium sulfate. The optimum concentration of ammonium sulfate used to produce pure bromelain (0.33 U/mg specific activity) from Queen pineapple crowns was 60%.

**Keywords**: Bromelain enzymes, drying, pineapple crown, protein.

**Introduction**

Pineapple (*Ananas comosus* (L) Merr) is a tropical fruit that is widely grown and consumed as a source of vitamins and minerals (Hossain, 2015). Pineapple has several varieties, in Indonesia the most commonly cultivated pineapple varieties are Queen and Cayenne pineapples (Lestari *et al*., 2020). Queen pineapple is a population that is sufficiently produced so that easy to find in various places where pineapple is sold at very affordable prices (Boonyaritthongchai and Supapvanich, 2017). In addition, Queen pineapples are always available throughout the year because they are not a seasonal fruit (Sibaly and Jeetah, 2017). The high level of public consumption of Queen pineapples results in large amount of crown as post-harvest wastes (Prado and Spinacé, 2019). In 2011 the harvest of Queen pineapples in Indonesia reached 1.5 million tons, 90% of which are Queen pineapples and the remaining 10% are Smooth Cayenne types (M. Syahirman Yusi, 2016). The weight proportion of pineapple crowns is around 35% of the total weight pineapples when harvested (Roha *et al*., 2013). This means that from the total production of 1.35 million tonnes of pineapples, it is likely that the crown of pineapples will be obtained by weight 472,500 tons. So far, the use of pineapple crowns is still dominated as animal feed and natural fertilizers (Saravanan *et al*., 2013). Pineapple crowns are rich in organic compounds such as cellulose and proteolytic enzymes (Campos *et al*., 2020).

**Matrials and Methods**

**Experimental details and treatments**

**Experimental material**: Experiments were performed in the laboratorium of the Department of Agriculture, Diponegoro University, Semarang, Indonesia during July – December 2020. The Queen pineapple crown used in this study was obtained from Rasamala Traditional Market located at Banyumanik, Semarang, Central Java, Indonesia, ammonium sulfate, aquades, BSA (Bovine Serum Albumin), phosphate buffer pH 6,5, natrium citrate buffer, bromelain enzyme (tablet), folin (Folin-Ciocalteau phenol), casein, Lowry reagent, and TCA (tricarboxylic acid) 10%.

**Treatments**: Fresh pineapple crowns were taken and then selected a good one, cut into small pieces then dried in cabinet dryer with 35 °C, 40 °C, 45 °C, and 50 °C. The dried pineapple crown then crushed using a grinder until smooth then sieved using sieve.

**Extraction and Isolation of Crude Bromelain Enzyme**

Supernatant extract of bromelain enzymes from Queen variety pineapple crown was carried out with 20 g pineapple crown powder was dissolved in 180 ml of cold sodium citrate buffer pH 6.5 and stirred until homogeneous. The solution then filtered with muslin cloth, the filtrate obtained then centrifuged at 4500 rpm for 25 minutes. The supernatant obtained, crude extract of the bromelain enzyme, stored at -20 °C, then be tested for its characteristics.

**Purification of Crude Extract Bromelain Enzyme**

Purification was carried out by the modified method referred to (Dutta and Bhattacharyya, 2013b). Ammonium sulfate added to the enzyme solution with concentrations of 20%, 40%, 60%, and 80% and stirred until homogeneous. The mixed solution was then stored for 24 hours at 4°C. The solution obtained was then centrifuged at 3500 rpm for 25 minutes. The precipitate obtained from the centrifugation process is a protein precipitate (bromelain enzyme) dissolved using sodium citrate buffer pH 6.5 then homogenized and dialyzed overnight. The dialysis process was carried out by putting the solution into a dialysis tube, both ends of the tube tied with twist tie and then immersed in a 10 ml buffer solution.

**Protein Content**

Determination of the protein content was carried out using the modified method (Lowry *et al*., 1951). The test begins with the making of Standard BSA (Bovine Serum Albumin) at several concentration points, 0.5 ml of BSA mixed with 5 ml of Lowry reagent, then vortexed until homogeneous, then incubated for 10 minutes so that the protein binding reaction occurred by Lowry reagent. Then, 0.5 ml of folin was added to the solution and incubated for 30 minutes. The absorbance of the solution was read with UV-Vis spectrophotometer with wavelength 650 nm and curve of the BSA standard then formed. The next step is to read the absorbance of the sample solution, starting with the sample supernatant of the bromelain enzyme extract, diluted 15 times and took 0.5 ml, added 5 ml of Lowry reagent and then vortex until homogeneous and incubated for 10 minutes. The sample solution was given 0.5 ml of folin and incubated for 30 minutes. The absorbance of the solution was read with UV-Vis spectrophotometer with the same wavelength as BSA. The protein content of the sample was determined by linear regression against the obtained BSA standard curve.

**Unit Activity**

Determination of the bromelain enzyme activity unit was carried out by referring to the method (Fathimah and Wardani, 2014) with modifications. The test started with 0.5 ml of extract bromelain enzyme after 15 times of dilution mixed with 0.5 ml of 0.5% casein as substrate, and 0.5 ml of phosphate buffer pH 6.5 then the solution incubated in water bath for 20 minutes at 40 °C. 1 ml of 10% TCA was added to stop the reaction and then incubated for 10 minutes at room temperature. Then sample was centrifuged at 5000 rpm for 10 minutes to obtain the supernatant. The absorbance obtained was measured using UV-Vis spectrophotometer at wavelength of 275 nm. The standard solution was prepared in the same way, but the sample tested was replaced with a bromelain solution from bromelain tablet that had been mashed and dissolved with phosphate buffer pH 6.5 then the absorbance was read with a UV-Vis spectrophotometer with a wavelength of 275 nm to calculate the unit of enzyme activity. The enzyme activity was calculated using the formula described by previous studies (Chaurasiya and Hebbar, 2013).

**Specific Activity**

Specific activity values can be obtained by knowing the protein content (mg/ ml) and unit activity (U/ ml) of the enzyme (Kahiro *et al*., 2017). The enzyme specific activity is then calculated by dividing the enzyme unit activity by total protein content (Nuraini *et al*., 2017).

**Data Analysis**

The data obtained from the test results include data on protein content, enzyme activity unit, and enzyme activity, analyzed descriptively and presented in the form of a bar graph.

**Results**

**Protein Content:** Based on Figure 1(a) it can be seen that the highest protein content in the crude extract of bromelain enzyme was 6.01 mg/ ml found at drying temperature of 50 °C, and the lowest protein content was 4.11 mg/ ml at drying temperature of 35 °C. Based on Figure 2(a), it can be seen that the addition of ammonium sulfate with concentration of 80% produces the highest protein content, 2 mg/ ml. Ammonium sulfate with high concentrations could hydrate protein water so that the protein was precipitated.

**Unit Activity:** Based on Figure 1(b) it can be seen that the optimum temperature of enzyme activity occurs at 40 °C with an activity of 1.36 U/ ml, then at a temperature of 45 to 50 °C the enzyme unit activity decrease. After purification, the highest unit activity was 0.65 U/ ml at the addition of 60% ammonium sulfate (Figure 2(b)).

**Specific Activity:** Based on Figure 1(c), it can be seen that the highest specific activity of the crude extract of bromelain enzymes from Queen pineapple crown varieties was at drying temperature of 40 °C, 0.31 U/ mg. The high specific activity reflects the high amount of pure bromelain enzyme (Setiasih *et al*., 2018). After purification, the highest calculation result of specific activity was 0.33 U/ mg protein obtained through purification with 60% ammonium sulfate (Figure 2(c)).

**Discussion**

**Protein Content:** The protein content of the crude bromelain enzyme increases along with the incretion of the temperature used, because the increases in temperature can accelerate the enzyme reaction, the reaction speed will continue to increase until it reaches the optimal enzyme temperature. This is in accordance with the opinion of (Bell *et al*., 2013) which states that the enzyme reaction will continue to increase until it reaches the optimum temperature, if the temperature exceeds the optimum temperature of the enzyme, protein denaturation will occur. The value of high protein content is not always directly proportional to bromelain activity, this could be because of the presence of other proteins besides bromelain enzyme. This is in accordance with the opinion of (Anjum *et al*., 2011) which states that high protein levels in crude extracts can be caused by the presence of other proteins that participate.

The protein content increased along with the increase in ammonium sulfate concentration, the addition of 80% ammonium sulfate concentration resulted in the highest protein content of 2.00 mg/ml. This is in accordance with the opinion of (Nurhayati *et al*., 2018) which states that the greater concentration of ammonium sulfate added, the greater its ability to coagulate proteins. The protein content of the bromelain enzyme after purification decreased, before purification the protein content obtained was 4.41 mg/ml, after purification the protein content ranged from 1.14 to 2.00 mg/ml. This is due to the reduced of non-enzyme protein contained in the crude extract of the enzyme. This is in accordance with the opinion of (Chen *et al*., 2012) which states that the crude extract of the enzyme still contains many other proteins (non-enzyme) that can interfere. Precipitation using ammonium sulfate produces protein that contains a high salt content, it is necessary to do dialysis to remove salts that can interfere with the calculation of protein content. This is in accordance with the opinion of (Nooralabettu, 2014) which states that the use of ammonium sulfate as a protein precipitant produces protein with a high salt content, the salt content in the protein can be removed by dialysis in a buffer solution.

**Unit Activity:** The highest value of unit activity of enzyme was 1.36 U/ml at 40°C drying temperature, then at a temperature 45 to 50 °C there was a decrease in the unit activity of enzyme. The decrease in enzyme activity can occur because the temperature used exceeds the optimum temperature of the enzyme so that the enzyme undergoes denaturation. This is in accordance with the opinion of (Ren *et al*., 2011) which states that enzyme activity increases with temperature until it reaches the optimum temperature, if the increasing of temperature continue protein enzyme denaturation will occur. Denaturation is a condition which protein enzymes undergo changes in their structure so that they interfere with enzyme activity. This is in accordance with the opinion of (Guzik *et al*., 2014) which states that denaturation is the occurrence of modification or changes of the secondary, tertiary structure and phenomena in proteins without any covalent problems. The unit of enzyme activity is a description of the number of enzymes that work. This is in accordance with the opinion of (Dutta and Bhattacharyya, 2013c) which states that the unit activity is a value to see the transformation or change of one substrate molecule per minute under optimal measurement conditions. The activity of the crude extract of the enzyme can be influenced by several factors, including drying, temperature, pH, and the level of fruit maturity. This is in accordance with the opinion of (Poba *et al*., 2019) which stated that the drying method, temperature, pH, and fruit maturity level could affect the activity of the crude extract of the bromelain enzyme.

The unit activity of enzyme increased with increasing ammonium sulfate concentration up 60%, 0.65 U/ml, then the value of the activity unit decreased. This is in accordance with the statement of (Kumaunang and Kamu, 2011) which stated that the highest bromelain enzyme activity was fractionated with ammonium sulfate at a concentration of 40-60%. The value of the bromelain enzyme activity unit decreased with the use of 80% ammonium sulfate. This is in accordance with the opinion of (Ilyas, 2020) which stated that the enzyme activity unit decreased at a concentration of 80% because at that level of saturation the enzyme activity was not optimal. The decrease in enzyme activity units that occurred with the addition of 80% ammonium sulfate was also supported by (Rozi *et al*., 2020) which states that the decrease in enzyme activity at a concentration of 80% is thought to be due to the influence of salt ions or a concentration of salt that is too high.

**Specific Activity:** The specific activity of the bromelain enzyme extract was highest at a temperature of 40 °C, reaching 0.31 U/mg. The specific activity of the enzyme showed the level of purity of the enzyme, the high value of the specific activity shows the amount of bromelain enzyme in the crude extract of the bromelain. This is in accordance with the opinion of (Pratiwi *et al*., 2014) which states that the higher the specific activity value of the enzyme, the higher the level of purity. The specific activity value was calculated to determine the amount of bromelain enzyme present, with a comparison of protein content with enzyme activity. This is in accordance with the opinion of (Vilanova Neta et al., 2012) which states that the amount of bromelain enzyme present can be determined by calculating the specific activity by dividing the unit value of the enzyme activity by the protein content, which is expressed in units of U/mg.

The highest specific activity was 0.33 U/mg obtained by purification with 60% ammonium sulfate concentration, then decreased at 80% to 0.24 U/mg. This is in accordance with the opinion of (Han *et al*., 2019) which stated that the highest specific activity of the bromelain enzyme occurred in purification with ammonium sulfate in a concentration range of 40-60%. The value of the specific activity of the enzyme after purification process was greater than that of the crude extract of the bromelain enzyme. This is in accordance with the opinion of (Sundarram and Murthy, 2014) which states that after purification there is an increase in the amount of specific enzyme activity because the amount of impurities has decreased. The specific activity of the enzyme is a measure of purity which value will increase after purification process. This is in accordance with the opinion of (Novák and Havlíček, 2016) which stated that the higher the specific activity of the enzyme, the higher the level of purity of the enzyme.

**Conclusion**

The research concludes that the drying stage with cabinet dryer at temperature of 40 °C and purification stage with 60% ammonium sulfate concentration gives the best characteristic of bromelain enzyme isolated from Queen variety pineapple crown.

**Acknowledgements**

This research was funded by the Penelitian Terapan Unggulan Perguruan Tinggi (PTUPT). On this occasion, the author would like to thank the Department Agriculture, for providing lab facilities and constant encouragement to complete this research work successfully.

**Author contributions**

SS, HR, and FA planned the experiments, SS, HR, YP, and SPR interpreted the results, SS, HR, YP, FA, SPR made the write up, analyzed the data, and made illustrations.

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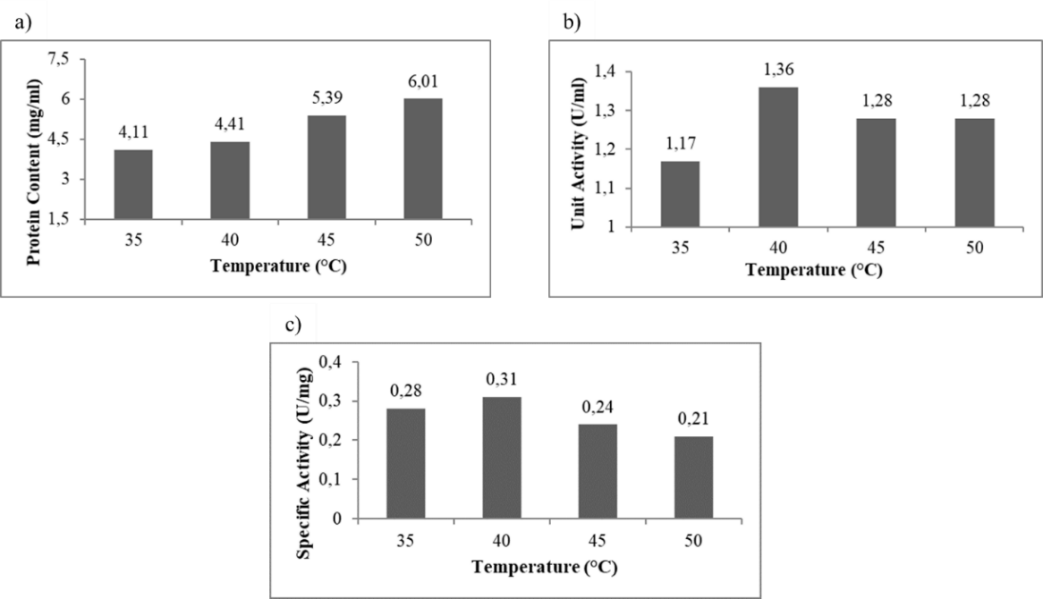
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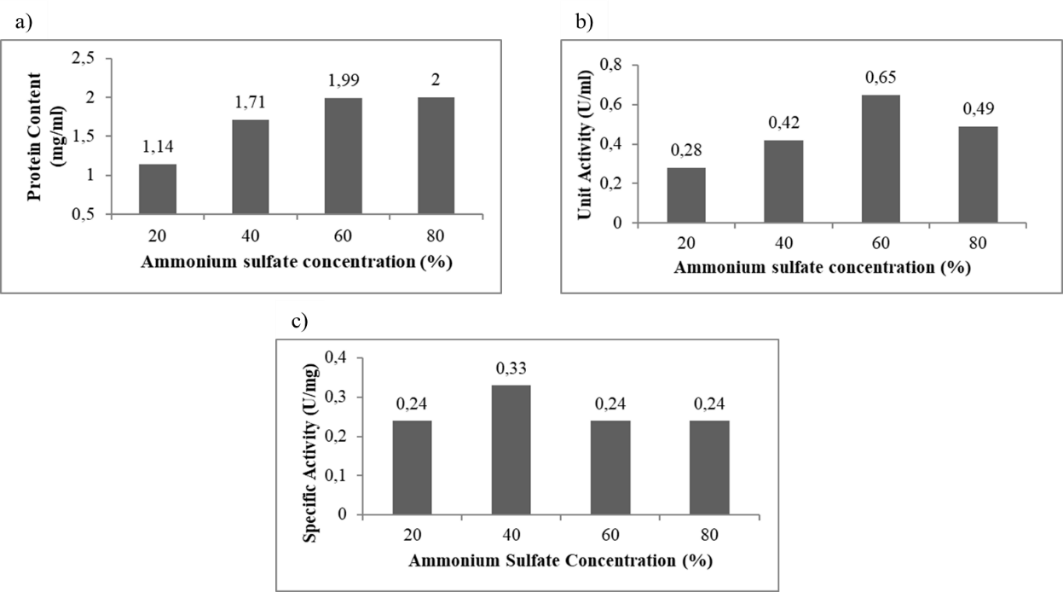
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**Fig. 1**: Protein content (a), unit activity (b), and spacific activity (c) of bromelain crude extract from Queen pineapple crown at various drying temperature



**Fig. 2**:Protein content (a), unit activity (b), and spacific activity (c) of bromelain extract from Queen pineapple crown at various concentration of ammonium sulfate