Characteristics of Bromelain enzyme from Queen variety pineapple crown at different drying temperatures

by Siti Susanti

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Abstract. Pineapple (*Ananas comosus* (L) Merr) is a plant that contains bromelain enzymes in almost all parts of the plant, such as fruit, leaves, crown, and the 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 utilise 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 the temperature of 35°C, 40°C, 45°C, and 50°C before the 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%.

1. Introduction

Pineapple (*Ananas comosus* (L) Merr) is a tropical fruit that is widely grown and consumed as a source of vitamins and minerals [1]. Pineapple has several varieties, in Indonesia the most commonly cultivated pineapple varieties are Queen and Cayenne pineapples [2]. Queen pineapple is a population that is sufficiently produced so that it is easy to find in various places where pineapple is sold at very affordable prices [3]. In addition, Queen pineapples are always available throughout the year because they are not a seasonal fruit [4]. The high level of public consumption of the Queen pineapples results in a large amount of crown as post-harvest wastes [5]. In 2011, the harvest of pineapples in Indonesia reached 1.5 million tons, 90% of which are Queen pineapples and the remaining 10% are Smooth Cayenne types [6]. The weight proportion of pineapple crowns is around 35% of the total weight of pineapples when they are harvested [7]. 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 fertilisers [8]. Pineapple crowns are rich in organic compounds such as cellulose and bromelain enzyme [9].

Pineapple crowns are a source of bromelain enzymes with the highest levels of protein and proteolytic activity among other parts of pineapple waste, such as peel, the core, and leaves [10]. Pineapple crowns with different varieties and growing sites tend to produce bromelain enzymes with varying levels of proteolytic activity [11-13]. On the other hand, the extraction method of proteolytic

enzymes from each part of the plant generally involves a drying process where the high temperature set will affect the characteristics of the enzyme produced [14]. Based on previous research, proteolytic enzyme isolation by involving drying will produce crude extract enzymes with varied proteolytic activity and protein content [10,14,15]. In this study, the isolation and evaluation of the enzyme characterization of bromelain was carried out on pineapple crowns of the Queen's variety by involving different drying temperatures at the extraction stage. The information generated from this study is expected to contribute to increasing the value of the pineapple agro-industry by utilising its waste, namelypineapple crowns as a source of bromelain enzymes in the future.

2. Materials and methods

The materials used in this study were Queen pineapple crown with fruit maturity 4-5 months ware obtained from Rasamala Traditional Market, Banyumanik, Semarang, Indonesia.

2.1. The making of pineapple crown powder

The stage of making pineapple crown powder was carried out based on a modified method [16]. Fresh pineapple crowns were taken and then selected the good one, cut into small pieces then dried in a cabinet dryer with temperatures 35°C, 40°C, 45°C, and 50°C. The dried pineapple crowns then crushed using grinder and sieved through 60 mesh to obtain a fine powder.

2.2. Extraction of crude bromelain enzyme

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

2.3. Purification the crude extract bromelain enzyme

Purification of the crude extract bromelain enzyme from pineapple crown was carried out by the modified method [16]. This process was carried out by adding ammonium sulfate 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 then dissolved using sodium citrate buffer pH 6.5 then homogenised and dialysed overnight. The dialysis process is carried out by putting the solution into a dialysis tube, both ends of the tube are tied with twist tie and then immersed in a 10 ml buffer solution.

2.4. Protein content

Determination of the protein content of the bromelain enzyme from pineapple crown was carried out using the modified method [17] without replication. The test began with the manufacture of Standard BSA (Bovine Serum Albumin) at several concentration points, namely by taking 0.5 ml of BSA mixed with 5 ml of Lowry's reagent, then vortex until homogeneous, then incubated for 10 minutes so that the protein binding reaction occurs. Then, 0.5 ml of folin was added to the solution and allowed to stand for 30 minutes. The absorbance of the solution was read with an UV-Vis spectrophotometer with wavelength 650 nm and curve of the BSA standard was formed. The next step is to read the absorbance of the sample solution, this step is starting with the sample supernatant of the bromelain enzyme extract diluted 15 times and taken 0.5 ml, adding 5 ml of Lowry's 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 an UV-Vis spectrophotometer with the same wavelength as BSA. The protein content of the sample is determined by linear regression against the obtained BSA standard curve.

2.5. *Unit activity*

Determination of the bromelain enzyme activity unit is carried out by referring to the modified method [18] without replication. The test started with 0.5 ml of bromelain enzyme extract from 15x dilution mixed with 0.5 ml of 0.5% casein as a substrate, and 0.5 ml of phosphate buffer pH 6.5 then the solution was incubated with water bath for 20 minutes at 40°C. Next, 1 ml of 10% TCA was added to stop the reaction that occur between the bromelain enzyme extract and casein and then incubated for 10 minutes at room temperature. Then, the sample was centrifuged at 5000 rpm for 10 minutes to obtain the supernatant. The absorbance obtained was measured using an UV-Vis spectrophotometer at a 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 tablets that had been mashed and dissolved with phosphate buffer pH 6.5 then the absorbance was read with an UV-Vis spectrophotometer with a wavelength of 275 nm to calculate the unit of enzyme activity. The enzyme activity is calculated using the formula described by previous studies [19].

2.6. *Specific activity*

Specific activity values can be obtained by knowing the protein content (mg/ ml) and unit activity (U/ ml) of the enzyme [20]. The enzyme specific activity is then calculated by dividing the value of unit activity by the protein content [21].

2.7. *Data analysis*

The data obtained from the test results include data on protein content, enzyme activity unit, and specific activity, analysed descriptively and presented in the form of bar charts.

3. Results and discussion

3.1. *Protein content*

Based on Figure 1a 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 50°C, and the lowest protein content was 4.11 mg/ml at 35 °C. The protein content in the crude extract of bromelain enzyme increases along with the increase 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 temperature. According to [22] which states that the enzyme reaction will continue to increase until it reaches the optimum temperature, if the temperature used exceeds the optimum temperature of the enzyme, denaturation will occur. The high value of the protein content is not always directly proportional to the bromelain activity, this could be because of the presence of other proteins besides bromelain enzyme. In accordance with [23] that high protein levels in crude extracts can be caused by the presence of other proteins.

Based on Figure 2a, it can be seen that 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. According to [24], the greater concentration of ammonium sulfate added, the greater its ability to coagulate protein. 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 reduction of non-enzyme protein contained in the crude extract of the enzyme. In accord with [25], 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. In accordance with [26], 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.

3.2. *Unit activity*

Based on Figure 1b it can be seen that the highest value of unit activity of the bromelain enzyme was

1.36 U/ml at 40°C drying temperature, then at temperature 45 to 50 °C there was a decrease in the unit activity of the bromelain 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. According to [27], enzyme activity increases with the increase of 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. In accordance with [28], denaturation is the occurrence of modification or changes on 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. In accord with [16], 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. In accord with [29], the drying method, temperature, pH, and fruit maturity level could affect the activity of the crude extract of the bromelain enzyme.

Based on Figure 2b, it can be seen that the unit activity of enzyme increases with increasing ammonium sulfate concentration up to 60%, 0.65 U/ml, then the value of the activity unit decreased. According to [30], 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. In accord with [31], 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 is also in accordance with [32] which states that the decrease in enzyme activity at a concentration of 80% is due to the influence of salt ions or a concentration of salt that is too high that can cause denaturation.

3.3. Specific activity

Based on Figure 1c it can be seen that 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 specific activity shows the amount of bromelain enzyme in the crude extract of the bromelain enzyme. In accord with [33], the high the value of specific activity of the enzyme shows that the level of purity of the enzyme is also high. The value of specific activity was calculated to determine the amount of bromelain enzyme present, with a comparison of protein content with enzyme activity. According to [34], the amount of bromelain enzyme present can be determined by calculating the specific activity which is expressed in units of U/mg.

Based on Figure 2c the highest value of specific activity was 0.33 U/ mg protein obtained from purification with 60% ammonium sulfate. In accordance with [35], the highest specific activity of the bromelain enzyme occurred in purification with ammonium sulfate in a concentration range of 40-60%. The value of specific activity of the enzyme after purification was greater than the crude extract of the bromelain enzyme. According to [36] after purification there is an increase in the amount of specific enzyme activity because the number of impurities has decreased. The specific activity of the enzyme is a measure of purity which value will increase after the purification process. In accord with [37], the higher value of the specific activity of the enzyme after purification, the higher the level of purity of the enzyme.

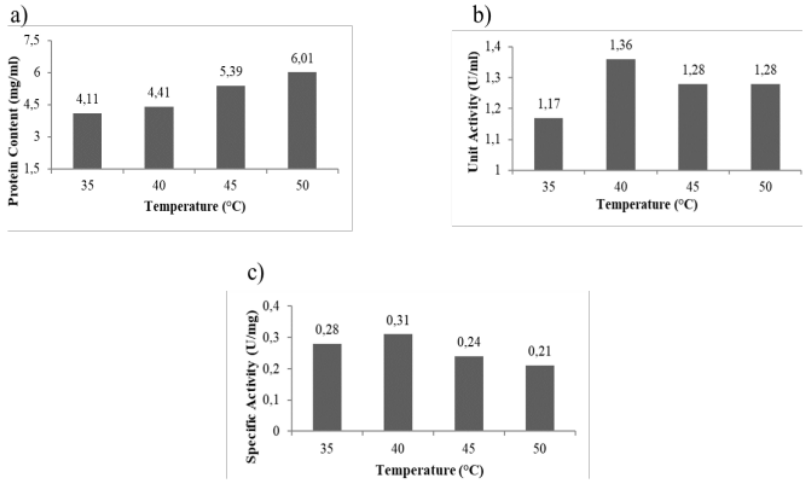

Figure 1. Protein content (a), unit activity (b), and specific activity (c) of bromelain crude extract from Queen pineapple crown at different drying temperatures.

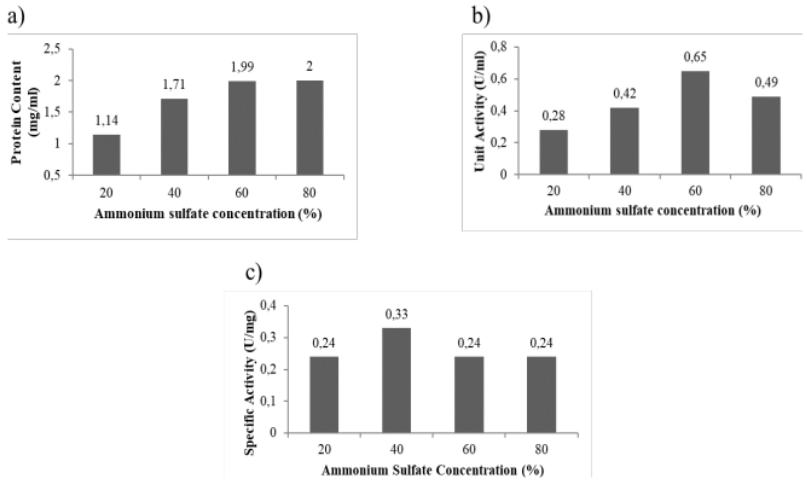

Figure 2. Protein content (a), unit activity (b), and specific activity (c) of bromelain extract from Queen pineapple crown at different concentration of ammonium sulfate.

4. Conclusions

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

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