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Optimization of Bromelain Isolation from Honi Pineapple Crown

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Abstract

Bromelain is a proteolytic enzyme that can be found in all parts of pineapple plant varieties. Pineapple crown (PC) has higher bromelain activity than other pineapple wastes such as peels and leaves. This study isolated bromelain from one variety of PC, namely Honi, and determined the optimum drying temperature as well as concentration of ammonium sulphate to obtain the best bromelain characteristics such as protein content, unit activity and specific activity. Honi PC was dried at various drying temperatures (35, 40, 45, 50, and 55 °C), and then extracted and purified using ammonium sulphate in various concentrations (20, 40, 60, and 80%), in order, to get bromelain. Furthermore, the yield of isolated bromelain was calculated and the protein content, unit activity and specific activity of bromelain characterised. The highest yield of crude bromelain (CB) was achieved at 35 °C. However, the highest protein level, unit activity and specific activity of CB were achieved at 55 °C ($p < 0.05$). Purification of CB using concentrations of ammonium sulphate in the range 40 to 80% resulted in a higher protein level ($p < 0.05$). The highest unit activity and specific activity of bromelain were achieved at a 60% concentration of ammonium sulphate ($p < 0.05$). 55 °C and 60% were the optimum drying temperature and concentration of ammonium sulphate respectively to achieve the best characteristics (2.16% protein level, 1.61 U/mL unit activity and 0.75 U/mg specific activity) for bromelain isolated from Honi PC. Honi PC isolated bromelain was shown to inhibit the browning reaction on apple fruits. The agroindustry waste product, Honi PC, has potential as a future alternative bromelain source.

Keywords: Optimum; Drying temperature; Characteristics; Bromelain; Pineapple crown

1 Introduction

There are several varieties of popular pineapple in the Indonesian fruit market such as Smooth Cayenne, Queen, and Honi (Yadi et al., 2020). Honi, which is a new variant of pineapple developed and introduced to the public in 2012 by Sunpride Research and Development located at East Lampung ("Honi, The Featured Pineapple," 2021), is the most popular variety. Lampung is a province in Sumatra, an island of Western Indonesia. People prefer Honi because of the sweeter taste and the fruit is fleshier. Genetically, Honi is a specific strain arising from the crossbreeding of two common strains, namely Smooth Cayenne and Queen ("9 Reasons You

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Should Try Honi Pineapple," 2020).
Nowadays, the total production of Honi pineapple in Indonesia is 1.39 million tons per year ("Top Pineapple Producing Countries," 2018). The high public consumption of pineapple results in abundant waste which is mostly pineapple crown (PC). The crown occupies up to 35% of the total pineapple weight (Campos et al., 2020). So far, the dominant use of PC as a material is for making natural fertilizers and animal feed (Prado & Spinacé, 2019). PC contains cellulose and bioactive components such as antioxidants and proteolytic enzymes, including bromelain (). PC contains lower levels of the bromelain enzyme than pineapple core and flesh. However, compared to other pineapple wastes such as peels and leaves, PC has higher bromelain activity and protein content (Manzoor et al., 2016). In the food Industry, bromelain is used for meat tenderisation, beer clarification, baking cookies, protein hydrolysate production and grain protein solubilisation, and as an anti-browning agent (Mohan et al., 2016). Stability of bromelain is influenced by temperature (40 to 65 ^{o}C) and pH (3.0 to 3.6). The optimum temperature for effectiveness of bromelain is between 50 and 60 ^{o}C (Sarkar et al., 2017).
In general, isolation of a proteolytic enzyme from several parts of pineapple involves a thermal drying process which can affect the yield and characteristics (Kurnia et al., 2018). Isolation and characterisation of bromelain from two PC strains (Morris and N36) has been carried out successfully without a drying stage (). The extraction of proteolytic enzymes from plant material, involving a thermal drying process, resulted in varying values of proteolytic activity and protein content (). In a previous study, 55 ^{o}C was the optimum drying temperature of Cayenne PC for CB production as a meat tenderising agent (Rizqiati et al., 2021).
In this study Honi PC was dried at various temperatures, CB was extracted and the bromelain resulting from a purification stage was characterised for protein content, unit activity and specific activity, and tested as an anti-browning agent on apple fruits. Utilisation of PC as a bromelain source can be expected to increase the added value of the pineapple agroindustry.

2 Materials and Methods

2.1 Preparation and Purification of Honi PC Powder

This research was conducted from July to December 2020 at the Food Chemistry and Nutrition Laboratory and the Food and Agricultural Products Engineering Laboratory, Diponegoro University, Semarang, Indonesia. Honi variety pineapple crown (HVPC) powder was produced by first cutting HVPC into small pieces, placing the crowns on a tray in a cabinet dryer (Maksindo), drying until the water content reached 8 to 10%, at 55 ^{o}C, 50 ^{o}C, 45 ^{o}C, 40 ^{o}C and 35 ^{o}C, and then crushing the dried crowns with a grinder (HR-40B, China) into a fine powder. The supernatant of the bromelain enzyme extract from HVPC was produced by dissolving 20 g of the finely ground crown powder sample in 180 ml of a sodium citrate cold buffer and stirring it until homogeneous. The mixture was filtered and the filtrate was centrifuged (DLab-DM0412 Clinical Centrifuge) at 4500 rpm for 25 minutes to separate the supernatant from the precipitate. The supernatant obtained was frozen at - 20 ^{o}C. Purification of the pineapple crown extract supernatant used a method reported by Hartesi et al. (2020), with modifications. Ammonium sulphate at concentrations of 20%, 40%, 60% and 80% was added to the enzyme solution and stirred until homogeneous. The resulting solution was stored at 4 ^{o}C, overnight or for 24 hours. The solution was centrifuged at a speed of 3500 rpm for 25 minutes. This solution formed a precipitate of the enzyme bromelain. The precipitate was dissolved using sodium citrate buffer, with a pH of 6.5, and homogenized for further dialysis, overnight or for 16 hours.

2.2 Experimental Analysis

Protein content of the crude extract of bromelain enzyme from pineapple crown was determined using the Lowry method (Febriani et al., 2017) with modifications. Bovine Serum Albumin (BSA) standards were prepared at several concentration points by mixing 0.5 ml of BSA with 5 ml of Lowry reagent, vortexing (vortex

mixer, TRSWIX VT02) and incubating for 10 minutes to let the protein binding reactions occur. 0.5 ml of Folin-Ciocalteu reagent was added to the solution and left to stand for 30 minutes. The absorbance of the solution was analysed using a UV-Vis spectrophotometer (Shimadzu UV-1601, Germany), with a wavelength of 650 nm, and a standard value curve of BSA was formed. The supernatant sample of bromelain enzyme extract from HVPC was first diluted 15 times. Then, 0.5 ml of the diluted supernatant was added to 5 ml of Lowry reagent, vortexed and incubated for 10 minutes. The sample solution was added to 0.5 ml of Folin-Ciocalteu reagent and allowed to stand for 30 minutes. The absorbance of the solution was read using a UV-Vis spectrophotometer with a wavelength of 650 nm. The protein content of the sample was determined by linear regression on the obtained BSA standard curve.

Enzyme unit activity testing was carried out using a method reported by Simamora and Sukmawati (2020), with modifications. The sample solution containing 0.5 ml of crude bromelain enzyme extract was diluted 15 times with 0.5 ml casein and 0.5 ml phosphate buffer (pH 6.6). The solution was then incubated using a water bath (Memmert WNB14, Germany) at 40 ^{o}C for 20 minutes. 1 ml of TCA (trichloroacetic acid) 10% was then added to the sample solution and incubated for 10 minutes at room temperature to stop the reaction. Samples were centrifuged at 5000 rpm for 10 minutes to separate the sediment. After obtaining a clear supernatant, the absorbance of the sample was read using a UV-Vis spectrophotometer with a wavelength of 275 nm. The standard solution was prepared in the same way except that the sample was replaced with a bromelain solution from bromelain tablets. Then the absorbance was read using a UV-Vis spectrophotometer with the same wavelength. The enzyme unit activity was calculated according to Sumardi et al. (2019).

The specific activity of the enzyme was calculated by dividing the unit activity (U ml^{-1}) with the protein content (mg ml^{-1}) (Kahiro et al., 2017).

Evaluation of the bromelain activity as an anti-browning agent was performed on apple fruits. Apple slices were soaked (1%, w/v) in Honi PC bromelain and the other natural anti-browning agents, namely onion juice, chili pepper juice, honey, and lemon. As a control, one slice of apple was immersed in distilled water. All treatments were incubated for 12 h at 25 ^{o}C, and the colour change on the surface of each apple slice was observed and recorded in a photograph.

2.3 Statistical Analysis

All data generated were analysed statistically using Analysis of Variance to determine the effect of the given treatment. Further analysis was performed using a Duncan Multiple Range Test to explain the significant difference between each treatment group.

3 Results and Discussion

Drying temperature (35 ^{o}C to 55 ^{o}C) for the Honi PC had a significant effect on the yield (Fig. 1). The highest yield was achieved at a drying temperature of 35 ^{o}C. Above 35 ^{o}C significantly less CB was produced ($p < 0.05$). A decrease in yield with increasing drying temperature was caused by a moisture factor. The higher temperature, the easier it is for water to be released from the materials' surface (Ramos et al., 2016). For dry powdered food products, the maximum water content is 10% so that microbes and fungi can't grow (Zambrano et al., 2019).

3.1 Characteristics of Crude Bromelain (CB)

Drying temperature for the Honi PC significantly influenced the CB characteristics of protein level, unit activity and specific activity (Fig. 2). The highest protein level, unit activity and specific activity of CB were achieved at 55 ^{o}C ($p < 0.05$). The protein content increased in line with increasing drying temperature of PC (Shu et al., 2016). The higher drying temperature caused a lower PC moisture content which resulted in a higher protein content. Extraction of bromelain from PC with high protein content resulted in CB with high protein content as well. Enzyme activity will continue to increase as drying temperature increases until the optimum drying

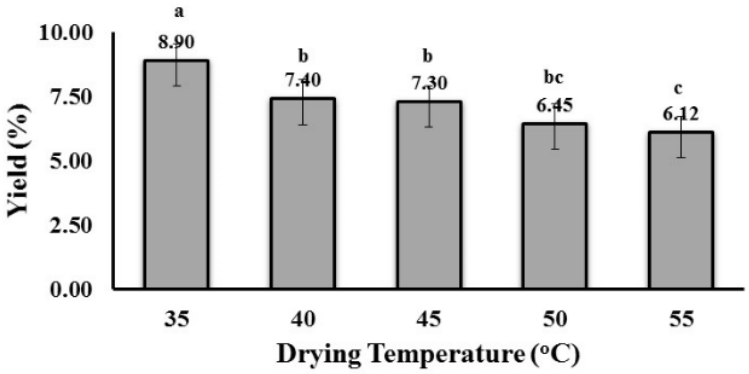

Figure 1: Yield of crude bromelain extract from Honi pineapple crown at various drying temperatures

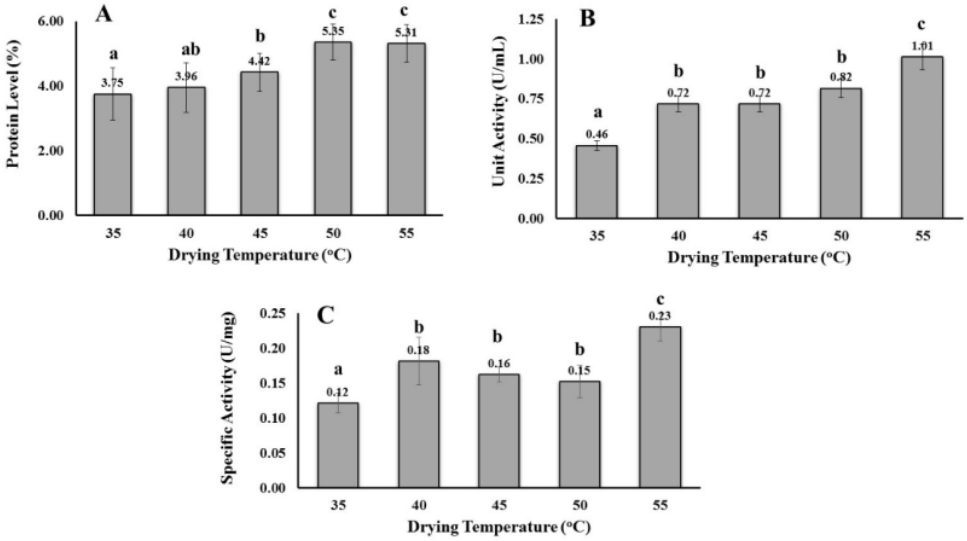

Figure 2: Protein content (A), unit activity (B) and specific activity (C) of crude bromelain extract from Honi pineapple crown at various drying temperatures

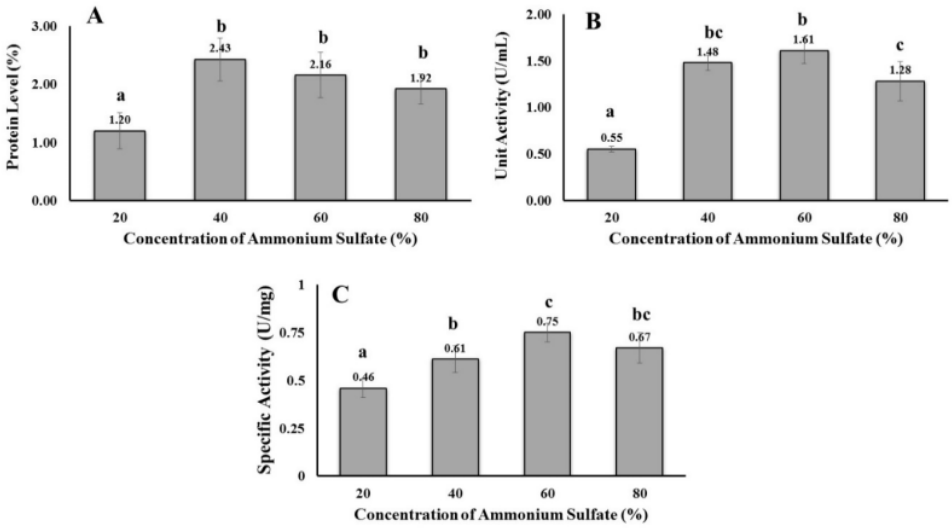

Figure 3: Effect of concentration of ammonium sulphate on the protein content (A), unit activity (B) and specific activity (C) of bromelain from Honi pineapple crown

temperature for isolation of the bromelain enzyme is reached between 50 °C and 60 °C. Above that drying temperature range, the activity of the bromelain enzyme will decrease (Manzoor et al., 2016). High temperatures can accelerate the reaction and activity of the enzyme but if it exceeds the optimum temperature, it will reduce the activity of the enzyme (Poba et al., 2019). The results of the higher unit activity value of the CB show the higher the number of enzymes that can actively catalyse protein breakdown reactions.

This specific activity value aims to determine the amount of bromelain enzyme present and is obtained from a comparison of protein levels with enzyme activity. Ramli et al. (2017) stated that the specific activity of the bromelain enzyme can show the amount of the existing bromelain enzyme expressed in U mg^{-1} which is the unit of enzyme activity per milligram of the total enzyme protein. The samples with a drying temperature of 55 °C showed the highest activity because temperature affects the work of the enzyme. Enzymes at low temperatures have reactions that tend to be slow and if the temperature is increased, the reaction will be faster and will be maximised if it reaches the optimum temperature. As the temperature increases to the optimum, there will be an increase in kinetic energy which will accelerate the motion of the enzymes and the substrate so that there is an increase in the intensity of the collision between the substrate and the enzyme which will facilitate the formation of the enzyme-substrate complex and increase product formation. If the temperature is too high the enzyme will denature, leading to a conformational change in the substrate, and the enzyme activity decreases (Shu et al., 2016). The protein content value will not always be directly proportional to the specific activity because the protein contained in the crude extract of the bromelain enzyme is not specific and there are still other proteins.

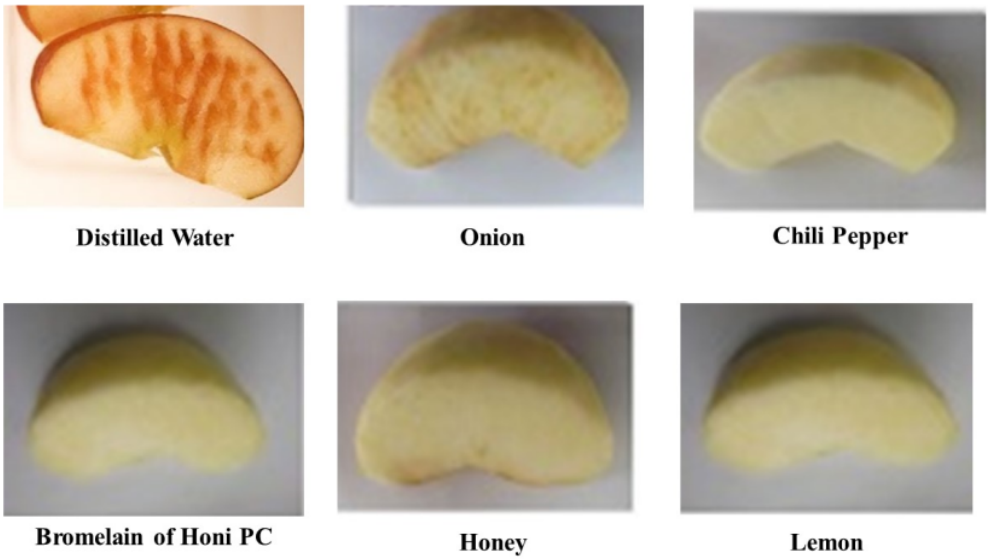

Figure 4: The anti-browning activity of bromelain from Honi PC compared to other natural anti-browning agents

3.2 Characteristics of Bromelain

Bromelain in this study was obtained using ammonium sulphate as a separating agent in the purification of crude bromelain extract. The best CB resulted from PC dried at 55 °C which was then purified using various concentrations of ammonium sulphate (20, 40, 60 and 80 %). The concentration of ammonium sulphate significantly influenced the bromelain characteristic of protein content, unit activity and specific activity (Fig. 3). 60% ammonium sulphate was determined as the optimum concentration to obtain bromelain with higher protein content, unit activity and specific activity ($p < 0.05$). The addition of the ammonium salt will cause the salt ions to compete with the protein to bind water molecules. As the solubility of salt ions is greater than protein, the protein in the enzyme will form clods and settle (Liliany et al., 2018). The 60% concentration of ammonium sulphate produced the highest unit and specific activity ($p < 0.05$). High activity of bromelain was produced by purification with 50 to 80% ammonium sulphate (Setiasih et al., 2018). The water of CB was bound to the ammonium sulphate salt without disturbing the existing protein nor disrupting the enzyme activity. All proteolytic enzyme proteins are optimally soluble and precipitate at a saturation concentration of ammonium sulphate of 60%. At a concentration of 80%, the enzyme proteolytic cannot dissolve and precipitate again because the solution has reached its saturation point (Abd-ElKhalek et al., 2020). The higher the specific activity of the bromelain enzyme, the purer the sample is because at a certain level of salt saturation, the amount of protein in the enzyme bromelain is more than other proteins. The crude extract of the bromelain enzyme has the highest specific activity in fractionation using ammonium sulphate at a concentration of 50 to 80% (Febriani et al., 2017). Bromelain isolated from the purification process using 60% ammonium sulphate showed a reduction in in-

tensity of brown colour formation on the surface of apple fruits compared to the other natural anti-browning agents of honey, chili pepper, lemon, and onion, and to the control of distillated water (Fig. 4). Bromelain, as one of the protease enzymes, can inhibit enzymatic browning by inactivating polyphenol oxidase (PPO) of fruits (Atrooz, 2008).

4 Conclusions

55 °C and 60% were the optimum drying temperature and concentration of ammonium sulphate respectively to achieve the best characteristics (2.16% protein level, 1.61 U/mL unit activity, and 0.75 U/mg specific activity) for bromelain isolated from Honi PC. Honi PC isolated bromelain was shown to inhibit the browning reaction on apple fruits. The agroindustry waste product, Honi PC, has potential as a future alternative bromelain source.

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