

Characteristic of isolated crude bromelain extract from cayenne pineapple crown in various drying temperature and its effect on meat texture

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Article history:

Received: 30 December 2020

Received in revised form: 25

January 2021

Accepted: 13 April 2021

Available Online: 10

September 2021

Keywords:

Bromelain enzyme,

Pineapple crown,

Temperature

DOI:

[https://doi.org/10.26656/fr.2017.5\(5\).692](https://doi.org/10.26656/fr.2017.5(5).692)

Abstract

Bromelain enzyme is a protease enzyme that is abundantly found in pineapples. Pineapple crown is a part of pineapple that contains bromelain enzyme which its content remains unknown. This study was aimed to discover the effect of drying on the moisture content, yield, and characteristics of the enzyme of cayenne pineapple crown, namely protein content, activity units and specific activities. This study also aimed to discover its effect on the beef texture with a difference in duration in the immersion process. The method used was different drying temperatures of 55°C, 50°C, 45°C, 40°C, 35°C, and 30°C which was analysed descriptively and the difference of immersion duration in beef using the Completely Randomized Design (CRD) method with four treatments, namely immersion duration of 0-hour, 2-hrs, 4-hrs, 6-hrs with 5 repetitions. The results obtained was the optimal drying temperature was 55°C due to its best moisture content, protein content, and best enzyme characteristics and it was known that there was a significant effect ($p < 0.05$) of immersion duration on the meat texture with the optimal immersion duration of 4 hrs with the best texture.

1. Introduction

Pineapple cultivation is quite big in Indonesia because as tropical fruit, it becomes one of the promising export commodities. Pineapple production takes place in the fourth rank of the most produced commodity in Indonesia after banana, mango and orange with a total production of 1.8 million tons in 2018 (BPS, 2018). The varieties grown in Indonesia are very diverse, one of them is the cayenne pineapple variety because it is easy to cultivate. Along with an increase in pineapple production and export, it will be directly proportional to the increase of the waste produced. Fruit waste is an inedible part of the pineapple which are usually the skin, hump and pineapple crown (Wahyuni *et al.*, 2016). Generally, pineapple wastes are still not optimally utilized, whereas it is well known that pineapple is a fruit that has rich bromelain enzyme content.

Bromelain enzyme is a protease enzyme functions to accelerate the simplification of protein compound elements using the hydrolysis process. Bromelain

enzyme comes from a combination of protease enzyme which is mostly obtained from pineapples (Banerjee *et al.*, 2018). The source of the bromelain enzyme comes from several utilized parts of pineapple such as the fruit (Kusuma *et al.*, 2015), skin (Kumaunang and Kamu, 2011) and stem (Masri, 2013). Meanwhile, for the pineapple crown itself, there has not been much research conducted about its utilization. The enzyme is usually produced by extracting directly from fresh ingredients which makes it difficult to store in its raw form. The innovation conducted by drying the pineapple crowns into powder before taking the crude extract of the bromelain enzyme. In the drying process, it is necessary to pay attention to its optimal temperature so that it will not cause damage to the active substance in non-heat resistant raw materials that is possible to reduce the quality of the products produced (Widhyastini, 2013).

In the drying process to obtain powdered products, there are several things to consider such as the yield content produced and the moisture content of the product. Moisture content is a parameter of the product

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durability during the storage period and the durability of microorganism attacks (Zambrano *et al.*, 2019). The yield resulted from the drying process of the pineapple crown is related to the level of effectiveness and economic value that it becomes one of the important parameters (Lekahena *et al.*, 2014). The specific characteristics of crude enzymes can be discovered by conducting several tests, namely protein content, activity unit and enzyme-specific activity. The protein content is an illustration of enzyme content, activity unit as the amount of enzyme needed to hydrolyse protein in time units, and enzyme-specific activity shows the purity level of an enzyme (Atmaja, 2013). The application is conducted by testing the beef texture with the treatment of different immersion duration.

A previous study has been conducted by Nadzirah *et al.* (2016) about the application of bromelain powder produced from pineapple crowns in tenderizing the round cut of beef, but in this study, there is no further analysis regarding the characteristics of the bromelain enzyme, especially in the activity unit, enzyme-specific activity, and the raw material used, namely cayenne pineapple crown which is an Indonesian local fruit. This study is aimed to discover the drying effect on moisture content, yield, and characteristics of cayenne pineapple crown enzyme, namely protein content, activity unit and specific activities also to discover its effect on the texture of beef. The advantages of knowing the optimal drying temperature for the drying process of the cayenne pineapple crown based on parameters of moisture content, yield, and enzyme characteristics are protein content, activity unit and specific activity also to discover the optimal immersion duration of beef with texture parameters.

2. Materials and methods

2.1 Materials

The materials were used cayenne pineapple crown, distilled water, tissue, aluminium foil, muslin cloth, solution of sodium citrate buffer pH 6 (Merck, Germany), BSA (Sigma-Aldrich, USA), Lowry reagent (Thermo, USA), foline solution (Merck, Germany), TCA 10% (Merck, Germany), casein (Sigma-Aldrich, USA), standard bromelain enzyme (Life Extension, USA), phosphate buffer (Merck, Germany) and beef.

2.2 Method

The study was conducted with raw pineapple crowns dried at the temperatures used at 55°C, 50°C, 45°C, 40°C, 35°C, and 30°C. The optimal temperature obtained was applied to beef with different in immersed duration of crude pineapple crown. Immersion was carried out to determine its effect on the texture of beef with CRD

method in 4 treatment 5 replications obtained 20 experimental units. Immersion treatments were varied at 0 hr (T1), 2 hrs (T2), 4 hrs (T3) and 6 hrs (T4).

2.2.1 Preparation of pineapple crown powder

The preparation of pineapple crown powders was carried adapted the method by Hanifah *et al.* (2017) with modifications. The pineapple crown of each variety was sorted to obtain the best result. Pineapple crowns were reduced in size by the cutting and grinding method. It was then arranged on a tray for drying in a cabinet dryer with a determined drying temperature difference of 55°C, 50°C, 45°C, 40°C, 35°C and 30°C for 5 hrs. The dried pineapple crown was then ground with a grinder for 4 mins and sifted with 40 mesh.

2.2.2 Process of bromelain enzyme extract isolation

The process of isolated the crude bromelain enzyme extract of the pineapple crown was conducted by referred to Kumaunang and Kamu (2011) with modifications. Pineapple crown powder was weighed 20 g and dissolved with sodium citrate buffer solution (pH 7) then homogenized. The solution was then filtered with a muslin cloth and then centrifuged at 5,000 rpm for 15 mins, the supernatant from the centrifugation process was separated and stored at a temperature of -25°C.

2.2.3 Moisture content

The moisture content of pineapple crown powder adapted from the method of (AOAC, 2005) with modifications. The porcelain dish was dried at 105°C for 1 hr. Then, it was placed in the desiccator and weighed. The sample was weighed 3-5 g and placed in a dish. The sample was dried at 105°C for 3 hrs. The sample was then placed in a desiccator until it was cold and weighed again. Then, after weighing the dish, it was dried again in the oven until a constant weight was obtained. Determination of moisture content can be calculated according to Aguirre-Loredo *et al.* (2016).

2.2.4 Yield

The yield calculated for pineapple crown powder product was based on the ratio of the weight of the powder produced (final weight) to the initial weight expressed in per cent (%). The yield was calculated with the according formula showed by Bhattacharjee *et al.* (2020).

2.2.5 Protein content

The procedure was tested for protein content according to Özel *et al.* (2010) with modifications. The protein content was determined by the Lowry method starting with a standard curve from the BSA standard

solution of 1.00 mg/mL and reacted with the Lowry reagent then incubated for 10 mins. The combined solution was added with Folin-Ciocalteu reagent and incubated again for 30 mins. Next, the absorption was measured using a UV-Vis spectrophotometer at a wavelength of 650 nm. The standard curve was made based on the determination of the wavelength from a solution with various concentrations with a range of 0.1 mg/mL. Furthermore, the protein content of the sample was tested by reacting it with 0.5 mL of the enzyme supernatant with Lowry-Folin reagent and measured at a wavelength of 650 nm.

2.2.6 Enzyme activity unit

The bromelain enzyme activity unit was tested in crude extract adapted from Soares *et al.* (2012) with modifications. 0.5% casein as a substrate was added as much as 0.5 mL into the pineapple crown bromelain enzyme crude extract of 0.5 mL which was diluted 15 times. The phosphate buffer solution was added and incubated in a 37°C water bath for 20 mins. Then, 10% TCA was added and incubated for 10 mins at room temperature. Centrifuge the solution for 4000 rpm for 20 mins. The absorbance of the supernatant was read at a wavelength of 280 nm using UV-vis spectrophotometry.

2.2.7 Enzyme specific activity

Enzyme specific activity was calculated adapted the method from Kusuma *et al.* (2015). The specific activity was obtained from the result of the unit of enzyme activity divided by the protein content of the enzyme.

2.2.8 Meat texture

The texture of the meat was tested used a texture analyzer adapted Dalvi-Isfahan *et al.* (2016). The meat sample was placed right under the cylindrical meat probe. The center of the sample was then pressed and a number is printed on the equipment, beef tenderness was an observed parameter.

2.3 Data analysis

The data analysis for the parameters of moisture content, yield, enzyme characteristics including protein content, activity units and enzyme-specific activity were analysed descriptively. The data of beef texture was analysed using the analysis of variance (ANOVA) test. Parametric testing was conducted using the SPSS 26.0 application at a significance level of <0.05 and the test was continued with Duncan Multiple Range Test (DMRT) to find the mean value of the differences.

3. Results and discussion

3.1 Moisture content

The moisture content of pineapple crown powder ranged from 6.954% to 7.285% (Table 1). The moisture content difference contained in pineapple crown powder is caused by the differences in the drying temperature which is conducted at 55°C that has the moisture content of 6.954% while at the temperature of 30°C, it has a moisture content of 7.285%. The increasing temperature will cause faster evaporation and transfer of moisture content that moisture content is reduced. The increase of temperature and drying time will cause various water molecules to evaporate which causes a decrease in the moisture content of pineapple crown powder (Ikhsan *et al.*, 2016). The higher the drying air temperature produced, the faster the heat transfer and the faster the evaporation process of water from the material (Dewi and Satibi, 2015). A pineapple crown powder which has a low moisture content will make it easier in its usage, which is more practical and effective due to its ability to shorten the enzyme production process that is usually directly from fresh material and has a long shelf life because it is free from microbes and fungi. Low moisture content causes longer microbial growth because a moisture content that is less than 14% can prevent mould growth and will extend the shelf life of food (Lisa *et al.*, 2015).

Table 1. Moisture content and yield of pineapple crown powder

Drying Temperature	Moisture (%)	Yield (%)
55°C	6.95	10.00
50°C	6.98	9.80
45°C	6.95	8.50
40°C	6.95	9.00
35°C	7.00	13.06
30°C	7.28	13.43

3.2 Yield

In this study, the yield is the ratio of the pineapple crown powder to the wet pineapple crown weight. The highest yield resulted from the drying temperature of 30°C (13.43%) while the range temperature of 40-55°C resulted in a lower yield (9.32%). Based on the yield resulting from the drying process with different levels of temperature, the best temperature for the drying process to produce pineapple crown powder was 55°C. This temperature is ideal for the drying process because obtain a high yield and is low in moisture (Table 1). The quality of the dried product is determined by yield and moisture content (Martinus, 2012). In this case, moisture content was affected by drying temperature also play a role in the final yield. The moisture content of the

material contributed to its weight (Hatfield and Prueger, 2015).

3.3 Protein content

The protein content in the crude pineapple crown extract can represent the amount of enzyme content contained in the crude pineapple crown. Based on the data in Figure 1. It is known that the drying temperature changes used may not affect the protein content in the crude extract of pineapple crown because there is no significant decrease in the protein content. The range of protein produced from the pineapple crown ranging from 6.845-6.148 mg/mL, which the highest protein content obtained was the cayenne pineapple variety with a drying temperature at 55°C. The activity of the bromelain enzyme is generally the most optimum at 55°C (Ketnawa et al., 2010). At this temperature, each pineapple variety has a high protein content value that indicates a high enzyme activity value. The reaction that occurs in the enzyme will increase with the speed of the reaction but has certain limits until it reaches the denaturation temperature (Wardhani et al., 2016). Protein denaturation will cause a decrease in enzyme activity due to conformation changes. In terms of, unsuitable heating will also obtain transformation in the structure and original properties of the protein (Zhang and Romero, 2020). The drying temperature used for each enzyme production is different, however, the bromelain enzyme can work optimally at a temperature of 55°C. When the temperature is very high the enzymes can be damaged.

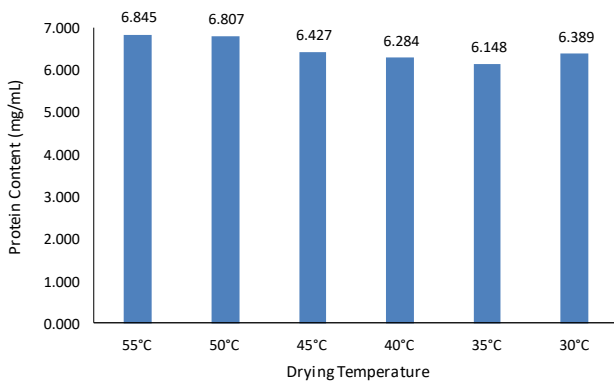


Figure 1. Protein content of crude extract pineapple crown

3.4 Enzyme activity unit

The enzyme activity unit is the number of enzymes that can be obtained in the transformation or changes of one substrate molecule per min in optimal measurement conditions (Djarkasi et al., 2017). At temperature 55°C, the enzyme activity unit value was 1.964 U/mL, it did not decrease at 50°C drying temperature, but at 45°C it decreases to 1.63 U/mL (Figure 2). The changes in drying temperature in the 10°C range will cause a change in the value of the bromelain enzyme activity unit, while

the 5°C temperature range does not give a significant change in the value of the enzyme activity unit. Enzyme activity will increase until the optimal temperature is reached, but will extremely decrease if the temperature used exceeds the optimal value due to the enzyme damage (enzyme inactivation) or modification of the protein (Han et al., 2016).

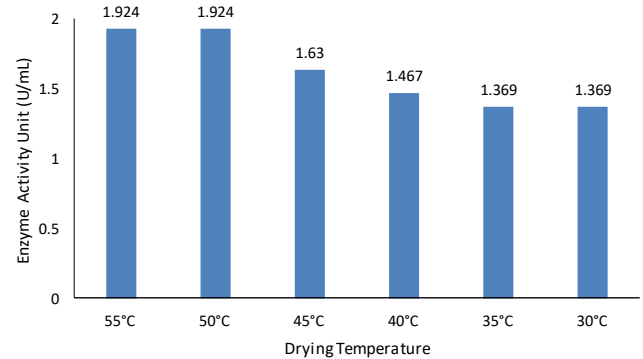


Figure 2. Enzyme activity unit of crude extract pineapple crown

3.5 Enzyme specific activity

The specific activity can be interpreted as a unit of enzyme activity in every milligram of protein contained inside the enzyme crude extract (Putri et al., 2013). Enzyme specific activity is one of the indicators to measure the purity of an enzyme (Feng et al., 2017). The value of specific enzyme activity in crude pineapple crown extract ranges between 0.283 – 0.214 U/mg (Figure 3), shows a decrease that is in line with the value in protein content and the unit activity of the enzyme. The specific enzyme activity also showed a decrease with increasing the drying temperature. The specific activity of the enzyme is related to the formation of lipids in the presence of temperature during the process (Zhang et al., 2020). In general, every enzyme has a different optimum temperature to carry out its function. Bromelain enzyme will be active in rank temperature from 50 to 55°C. The rate of activity of the bromelain enzyme will increase along with temperature rise until its optimal limit. If the temperature exceeds the

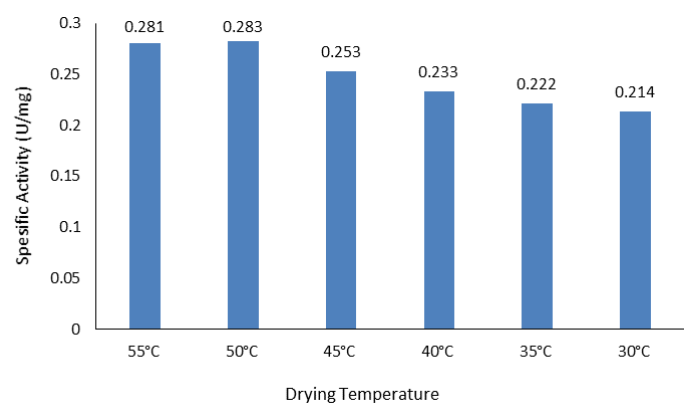


Figure 3. Bromelain enzyme specific activity of crude extract pineapple crown

optimal limit, it will cause enzyme denaturation and disrupt the active side of enzyme-substrate interaction (Nurhidayat *et al.*, 2018).

Table 2. Beef texture immersed in the solution of bromelain crude extract isolated from pineapple crown solution

Immersion Duration (hour)	Tenderness
0	5,540.17±767.48 ^a
2	4,067.15±510.46 ^{ab}
4	1,766.81±402.22 ^c
6	3,090.72±348.12 ^{bc}

Values are presented as mean±SE. Values with different superscript within the same column are significantly different ($p < 0.05$).

3.6 Texture

A texture test on the beef sample was carried out by using the crude extract that had been dried at 55°C because it has the most optimal bromelain enzyme characteristic. Based on Table 2 treatment of immersion beef in crude pineapple extract with different immersion duration used gives a significant effect on the quality of the meat texture ($p < 0.05$). Immersing the meat inside the solution of crude pineapple crown extract is one of the techniques to tenderize meat, where the longer the optimal immersion duration used will cause the meat to become more tender. The duration of treatment used for immersion of the meat is 2 to 6 hrs with T1 (control or fresh meat). Bromelain enzyme is a potential proteolytic enzyme that can tenderize meat because it has a softening effect on the myosin and myofibrillar protein in the meat (Arshad *et al.*, 2014). The best meat texture is the immersion duration for 4 hrs because the immersion duration for 6 hrs shows an increase in texture due to the clumping of meat during the storage process in the refrigerator. The texture of meat that becomes tender is also influenced by the protein denaturation that is hydrolysed by the bromelain enzyme, which is a proteolytic enzyme that the meat will loosen and becomes more tender (Paramartha *et al.*, 2019).

4. Conclusion

Based on the result obtained, drying the pineapple crown does not damage the bromelain enzyme content if it is carried out at the optimal temperature. Drying the pineapple crown powder will also give the advantage of being able to store it longer. The application of bromelain enzyme from the crude pineapple crown cayenne variety can be used as a meat softener. The 55°C drying temperature has the best enzyme characteristics as well as low moisture content and high yield produced, meanwhile the best tenderness texture occurs when the immersion duration of the beef is 4 hrs.

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