RESEARCH ARTICLE | OCTOBER 10 2018

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AIP Conf. Proc. 2019, 020014 (2018)
https://doi.org/10.1063/1.5061850

Bromelain Content of Extract from Stem of Pineapple (*Ananas comosus* (L.) Merr)

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Abstract. Bromelain is a proteolytic enzyme from Bromeliaceae plants that can be found in pineapple (*Ananas comosus* (L.) Merryl). Bromelain has activity as a pharmaceutical and food. The aim of this study was to determine the bromelain content from stem bromelain extract (SBM). The stem of pineapple was extracted using a grater and then concentrated by a freeze dryer to obtain a pure concentrate. SDS-PAGE, Bradford assay and protease specific activity was used to analyze bromelain activity. The results of this study showed that bromelain from pineapple stem has a molecular weight between 19-22 kDa. Bradford assay showed 0.476 µg/mL bromelain concentration. Bromelain activity of Non freeze dried (NF) extract showed 0.317 U/mL, while freeze dried (F) extract showed 0.212 U/mL. This study concluded that the pineapple stem has bromelain activity.

Keywords: Bromelain, pineapple, proteolytic enzyme

INTRODUCTION

In industry, pineapple is a fruit with considerable waste. Waste from pineapple fruit such as leaves, stems and skin are usually only used as animal feed. However, pineapple fruit waste can be optimized for reuse because it contains complete and quite high nutrition, such as protein, minerals, fats, carbohydrates, vitamins and bromelain.[1]

Bromelain is a major sulfhydryl proteolytic enzyme found in pineapple plants (*Ananas comosus* (L.) Merryl). Bromelain is usually found in the fruit and stem, but also easy to find in small amounts in leaf, skin, core, etc.[2] Bromelain from stem is known as stem bromelain (SBM). SBM has a uniqueness content compared with bromelain from fruit (FBM) because SBM contains many mixtures of various types of thiol-endopeptidases.[3] Based on the research of Heinecke and Gortner, SBM concentrations are better than FBM. [4]

Bromelain has been reported to have medical efficacy. Bromelain has been used traditionally to relieve tense muscles, inflammation and connective tissue. In recent years, bromelain has been used as an anti-inflammatory, antiedematous and antithrombotic agent (preventing blood clotting). In addition, bromelain showed activity to prevent lung metastases and potentially as an anticancer agent.[5]

The development methods for proteinase assay and enzyme activity are important to standardize bromelain to be a therapeutic product in the future.[6] The objective of this study was to determine the SBM activity from *Ananas comosus* (L.) Merryl.

The 9th International Conference on Global Resource Conservation (ICGRC) and AJI from Ritsumeikan University
AIP Conf. Proc. 2019, 020014-1–020014-5; https://doi.org/10.1063/1.5061850
Published by AIP Publishing. 978-0-7354-1737-3/$30.00

MATERIALS AND METHODS

Preparation and Extraction

Pineapples were originated from Desa Sugihwaras, Ngancar, Kediri, East Java. Pineapples were identified at the Plant Taxonomy Laboratory, Biology Department, Mathematic and Life Science Faculty, Brawijaya University.

The pineapple's stem (SBM) was separated from the fruit, shredded, filtered using a thin cloth, filtered with standard filter paper and filtered again using Whitman filter paper (2.5 μm). The last step was to put the SBM solution into the freeze dryer to get a pure dry extract.[7]

SDS-PAGE

The bromelain stem molecular weight was determined under denaturing conditions [1.5 M Tris, pH 8.8] by subjecting the sample to 12% SDS-PAGE (SDS Promega, H5113; Agarose, Promega V3121). The protein sample was run along with a standard molecular weight marker and bands were visualized using the acrylamide/bis-acrylamide staining technique. [8]

Bradford Assay

Bovine serum albumin (BSA) (Intron Biotechnology, J642) standard (800 μL) or the test sample solution was pipetted into a dry test tube. Bradford reagent (200 μL) (Pro-Measure Protein Measurement Solution, Qiagen, 21011) was added to each tube and mixed thoroughly. The samples were incubated at room temperature for 10 minutes. The absorbance of the samples at a setting of 595 nm were measured using a UV-visible spectrophotometer. The unknown concentrations were determined using the standard curve developed using the BSA protein standards.[9]

Specific Activity

Tyrosine (Merck, 1.08371.0100) was used as a standard for determining the protease activity of bromelain. Casein (Merck, 1.02245.0500) (200 μL SPB, pH 7.0) was used as a substrate in the presence of phosphate buffer, di-sodium-hydrogenphospate-12-hydrate (Pro-analysi Art.6579) and potassium dihydrogen phosphate (Pro-analysi CAS-No. 7778-77-0) (300 μL, pH 7) and 0.1 mM EDTA (Promega, H5032). The assay test was performed at 37 °C for 10 min. Adding 10 mL of 4% (w/v) TCA (trichloroacetic acid) (Merck, 1.10801.0250) stopped the reaction. The solution was centrifuged at 10,000 rpm for 10 min to remove the precipitated protein. The clear supernatant was analyzed after setting the absorbance to 275 nm. One unit of protease activity was defined as the amount of enzyme releasing product equivalent to 1 μmol of tyrosine/min/mL under the assay conditions. [10]

RESULTS AND DISCUSSION

SDS-PAGE Analysis

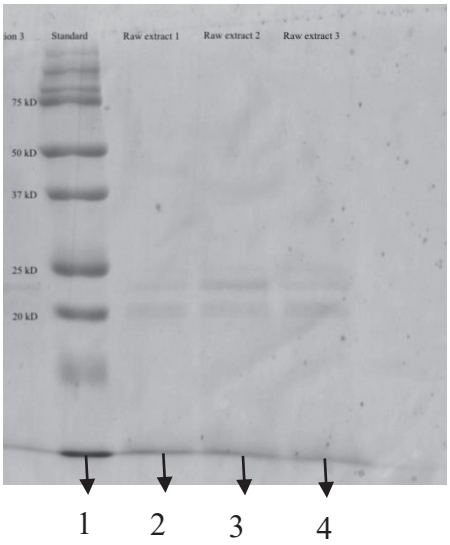

FIGURE 1.SDS PAGE showing the protease bands. Lane 1: Molecular weight marker, Lane 2: Extrcat SBM 1, Lane 3: Extrcat SBM 2, Lane 4: Extrcat SBM 3.

The molecular weight of SBM proteinase purified bromelain was determined by 12% SDS-PAGE. The fractions collected from SBM 1 to 3 were observed between 19 kDa and 22 kDa, which might correspond to bromelain bands, Fig. 1. This study had the same result as Ramalingam, et al. and Hale et al., which showed bromelain molecular weight to be between 20 to 30 kDa and 22 to 28 kDa.[6,8]

SDS-PAGE results showed thin bands, probably due to very low concentrations of bromelain proteins. It also showed that the content, or amount of protein, in similar sized bands had the same molecular weight. Charged molecules move freely under the influence of electric fields where molecules of equal charge size will accumulate in the same band or zone.[11-12] In addition, thin bands are also thought to be due to enzymes denatured by chemicals found in SDS and EDTA.[13]

Bradford Assay

The Bradford is a method to measure the total protein concentration in a solution using a spectrophotometer. The absorbance value is used to create a standard curve as below.

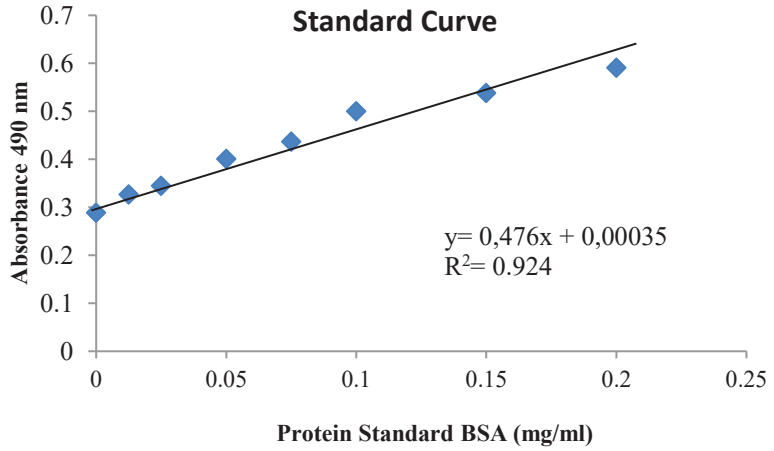

FIGURE 2. Spectrophotometer values (λ 490 nm) then obtained by gelatin standard curve with regression equation y = 0.476x + 0.00035.

The Bradford method is a method of measuring the total protein concentration in a solution by calorimetry. Bradford's test uses Coomassie Brilliant Blue (CBB), resulting in a bluish tint on targeted proteins.[14] In this study, the protein concentration obtained by the regression value reached 0.924 with a significance value of t smaller than 5%, which means that the protein concentration obtained was close to the standard curve value. This equation then used to determine the sample protein content (x) by substituting the absorbance value of the sample into the regression equation as Y value. The following are the results of measurements of protein levels in A4 and A8 samples.

TABLE 1. Results of Sample Spectrophotometry and Protein Levels

Protein Sample	Absorbance 490 nm	Protein Level (mg/ml)
A4	0,401	0,842
A8	0,591	1,24

The linear curve showed a directly proportional relation between the value of absorbance and protein content. The relationship is determined by the value of R^2 and the result is close to 1 which is indicates a strong relationship between this two parameter. Using the linear regression equation on the standard curve of Y = 0.476x + 0.00035. This equation is then used to determine the protein content of the sample. The protein content of sample obtained for A4 sample is 0.842 mg / ml and A8 sample is 1.24 mg / ml.

Protein Specific Activity

This test objective was to determine protease activity of stem bromelain (SBM) extract based on a spectrophotometry method. The results below show a different treatment of SBM extract:

TABLE 2. Bromelain Activity in Different Treatment. NF = Non freeze dried, F = Freeze dried.

Sample	Enzyme Activity (units/mg)
NF	0.032
F	0.212

Table 1 shows that bromelain activity in freeze dried extract (0.212 units/mg) was higher than non-freeze dried extract (0.032 units/mg). This is due to the effect of freeze drying limiting the oxidative changes of metabolites due to

the very low concentration of oxygen in the vacuum. With this working principle, the freeze dry method is well suited to determine the natural content of an extract. The results obtained have similarities to the study by Herdyastuti showing that the activity in the pineapple rods was 0.521 U/mg[15]. In addition, the activity of enzyme bromelain obtained in pineapple stalks was 0.1623-0.2887 U/mg[16]. Another study showed that unpurified recombinant bromelain had an activity of 0.03 U/mg[17].

In this study, it was found that bromelain activity was lower than the previous study, which was thought to be caused by the protein molecule in the cob sections being transported to the flesh along with the fruit ripening process[18]. Crude extract of bromeline enzyme is a mixed protein that is still dilute. The reproduction of bromelin in the crude extract of this enzyme is easily denatured so less profitable for the use of industrial enzymes. therefore it is necessary to purify and immobilize the enzyme. Enzyme mobilization is an enzyme trapping technique in a polymer matrix or enzyme binding on a carrier material by maintaining its catalytic properties. The immobilized enzyme has several advantages than pure immobilized enzymes, such as more easily separated from the reaction mixture, can be used repeatedly, and directly produce enzyme-free products[19].

Bromelain is sensitive to pH changes and this enzyme acts well within the range 3-8. The optimum pH of this enzyme is 7.0. At low pH, bromelain activity decreases and at high pH the adsorption decreases[20]. The activity decrease is due to the active side of the enzyme environment experiencing proton deficiency and increase in pKa[21]. Various approaches have been used to optimize the activity and increase the purity of bromelain enzyme preparation for application in commercial, food and therapeutic industries[17].

SUMMARY

Based on the research, the bromelain was detected by SDS-PAGE in pineapple stem.. When the pineapple stem was in non freeze dried the enzyme activity higher than in freeze dried.

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