Impact of pineapple core storage duration on the properties of the developed bromelain

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Abstract. This study examines the impact of pineapple core storage duration on the properties of the bromelain enzyme produced. Bromelain is a protease enzyme used in many applications in the agro-food, pharmaceutical and cosmetic industries. This study aimed to determine changes in bromelain enzyme activity during storage of pineapple core at various durations (0, 12, 24, and 48 hours). Bromelain was extracted by mixing pineapple core juice with 90% ethanol, followed by a separation process with filtration. The results showed that bromelain enzyme activity peaked at 12 hours of storage and then loss significantly after 24 to 48 hours. This loss was caused by changes in pH, increased water content, and protein degradation that affect enzyme stability. This study highlights the importance of optimizing storage duration to maintain bromelain enzyme activity and supporting sustainable pineapple waste management by using pineapple core as a source of high-value enzymes.

1 Introduction

Pineapple (*Ananas comosus* L.) is a well-known southern fruit that is not only popular for its fresh taste but also for its widely recognized health benefits [1]. As one of the largest pineapple producers in Asia, Indonesia produces up to 1,805,506 tons of pineapple per year. However, the short shelf life of pineapple, only about 7-9 days at room temperature, often leads to unsold fruit becoming waste. Processable products

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include hemicellulose and cellulose extracted from pineapple core and used as animal feed [2], [3]. Ironically, this pineapple waste actually has great potential as a resource for high quality proteolytic enzymes, namely bromelain [4].

One part of the pineapple plant that has not been optimally utilized is the core. Pineapple core are a major waste that contains high concentrations of bromelain with significant commercial value. Bromelain, a protease enzyme, has the ability to hydrolyze proteins into peptides or free amino acids, making it very useful in various industries. This is an enzyme that has been used extensively in the food industry to tenderize meat, in the pharmaceutical field as a therapeutic agent, in cosmetics to improve skin health, and in the fishery products processing sector [5], [6]. Using pineapple core as a source of bromelain offers a solution to reduce waste and opens up great opportunities for diversifying value-added products.

Despite its great potential, the stability and activity of bromelain are greatly influenced by the storage conditions of the raw materials, such as storage duration after harvest. Bromelain activity is affected by various factors, such as temperature, metal ions, and pH value [7]. Stability during storage is also an important challenge, as storage duration and conditions can affect enzyme activity and structure. Information regarding the effect of storage duration on bromelain activity in pineapple core is still limited.

This study was implemented to observe the effect of pineapple core storage duration (0, 24, 48, and 72 hours) on the properties of the bromelain enzyme. The results of this study are intended to provide data for storage optimization of bromelain raw materials, support sustainable pineapple waste management, and increase the application of this enzyme in various industries.

2 Methodology

2.1 Study area

This research was implemented in Instrumentation and Biochemical Chemistry Laboratory, Faculty of Agricultural Technology, Universitas Andalas, Padang.

2.2 Extraction of Crude Bromelain Extraction

The pineapple used in this experiment was a queen type that was still intact from the core, fruit, and leaves, which was three months and ripe, and it was treated according to the length of storage, namely hours 0, 12, 24, 48, 72.

One kilogram of well-washed pineapple flesh was cut into small pieces and blended until smooth. Pineapple juice was obtained by squeezing the blender through a strainer. The result was pineapple juice. To extract the crude bromelain enzyme, they were then mixed with 90% ethanol in a specific ratio. The mixture was incubated at 4°C for 24 hours. The formed precipitate was separated by filtration through a 0.45 µm filter paper. The resulting precipitate is the paste of crude bromelain enzyme. This paste was then flattened on a glass panel, and the excess ethanol evaporated naturally in an open space. If not used immediately, the crude bromelain enzyme paste was stored at 4°C.

2.3 Crude Bromelain Analysis

2.3.1 Yield

The calculation of the percentage of the yield of the crude bromelain can be seen in the following formula:

$$\text{Yield (\%)} = \frac{final\ sample\ weight}{initial\ sample\ weight} \text{ x } 100$$

2.3.2 pH Value of Pineapple Core Juice

The pH of pineapple juice was measured before the crude bromelain enzyme was extracted using a pH meter by taking 100 ml of pineapple juice. The measurement results were recorded

2.3.3 Measurement of Color of Crude Bromelain

The crude extract of bromelain enzyme was poured into a glass container until it covered the entire bottom. The color was accomplished using Hunterlab ColorFlex EZ spectrophotometer with Hunter L*, a*, b* score. Before analysis, the chromameter was calibrated using the white and black color standards contained in the device. The measurement results produced L* (brightness/brightness), a* (redness/redness), and b* (yellowish/yellowness) values. The white and black color standards are used as a reference in measuring the degree of color. The L*, a*, and b* values are used to calculate ^{o}Hue and white degree, where ^{o}Hue is calculated using the formula:

$$^{o}Hue = \text{-Tan b/a}$$

2.3.4 Scanning Of Bromelain Content Using Spectrophotometry.

A sample bromelain enzyme solution was prepared with the same concentration using 96% ethanol solvent. Samples that had been weighed as required were dissolved until homogeneous, then vortexed for 30 seconds to ensure complete mixing. Afterwards, the solution was ultrasonicated for 15 min at room temperature (25-30°C) to enhance bromelain extraction. Prior to analysis, the UV-Vis spectrophotometer was calibrated using 90% ethanol as a blank. The sample solution was then put into a quartz cuvette with a capacity of 1 mL and an optical path length of 1 cm Scanning was performed in the 200 to 800 nm spectrum to detect the specific absorption peak of bromelain. The scanning results determined the bromelain content based on the maximum wavelength (λ max) and the resulting absorbance value.

2.3.5 Determination of Proteolytic Activity

2.3.5.1 Preparation of Tyrosine Standard Curve

0.1 g of tyrosine accurately and dissolve it in a 100 mL volumetric flask with distilled water to prepare a tyrosine parent standard solution with a 1000 mg/L concentration. Then, prepare a series of tyrosine standard solutions with concentrations ranging from 1 to 10 ppm. Calibrate the spectrophotometer using distilled water as a blank to set the absorbance to zero. Measure the absorbance of each standard solution at 275 nm.

Plot absorbance against concentration and determine the linear regression equation in the form of y = ax + b.

2.3.5.2 Determination of Proteolytic Activity

Add 1 mL of 1% casein into three test tubes. Then, 1 mL of 0.1% crude bromelain was added to each test tube, followed by 2 mL of phosphate buffer at pH 7. Incubate the test tubes at 37°C for 30 minutes. After incubation, add 1 mL of 30% trichloroacetic acid and continue the incubation for another 30 minutes. Next, centrifuge the mixture at 3500 rpm and 4°C for 15 minutes. Then the absorbance of the supernatant is measured at 275 nm. The proteolytic activity is determined using the standard curve linear regression equation, where y = sample absorbance and x = tyrosine concentration.

The enzyme activity is determined based on this formula:

$$\text{Enzyme activity (U/mL)} = \frac{\text{AA supernatant x}\frac{total\ volume\ of\ solution}{1000}}{1000\ x\ BM\ tyrosine}$$

3 Result and discussion

3.1 Yield of crude bromelain

The results showed that the yield of crude bromelain enzyme from pineapple core changed during the storage process at varying durations, as shown in **Fig** 1. The greatest yield was recorded at a storage duration of 12 hours before finally decreasing at storage for 24 and 48 hours. This phenomenon is consistent with research conducted by Poba et al [8], who found that the ripeness of pineapple affected the crude bromelain content. The higher the maturity level, the content of crude bromelain tends to rise, although the enzyme activity losss. This is because, after harvesting, the pineapple fruit still undergoes metabolic processes that can affect 1enzyme stability and activity.

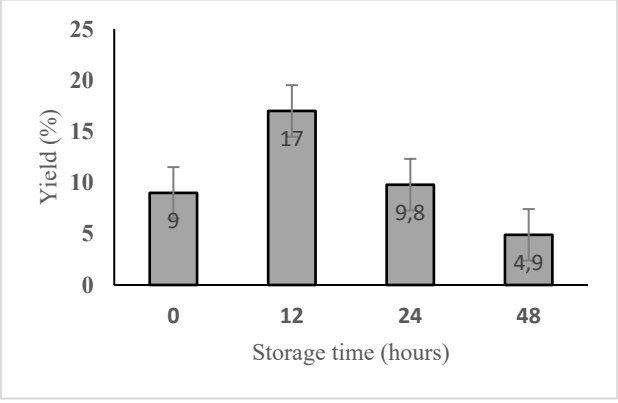

Fig 1. Yield Percentage of Crude Bromelain.

However, the loss in yield after 12 hours can also be attributed to the reduced metabolic activity with storage duration. A loss in bromelain enzyme activity after a

certain duration indicates enzyme degradation due to metabolic processes or environmental influences during storage. Fresh pineapples or those in optimal conditions at harvest have a higher bromelain content, which is about 2.31%. The results of this study indicate that to maximize crude bromelain yield, the ideal storage duration is about 12 hours, before the enzyme activity starts to loss significantly. Thus, it is important to consider storage duration as a key factor in the processing of crude bromelain from pineapple [9].

3.2 pH Value of pineapple core juice

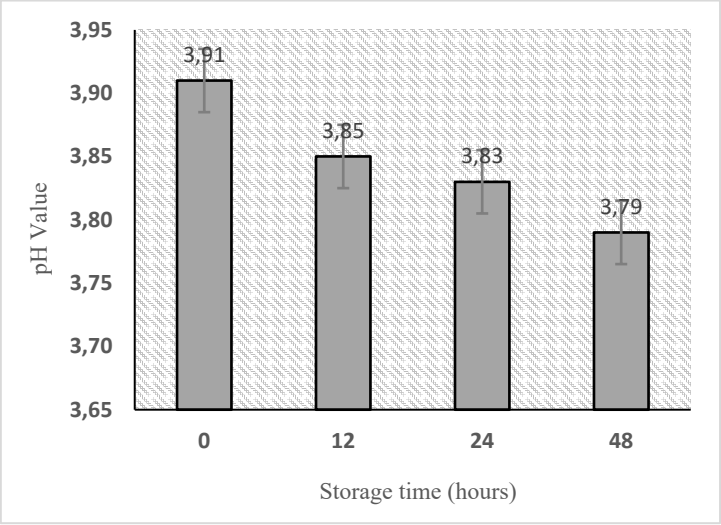

Fig 2. pH Value of Pineapple Core Juice.

Based on **Fig 2**, the pH value of crude bromelain from the pineapple core loss during storage. At the beginning of storage (0 hours), the pH value was recorded to be the highest, around 3.90. However, as the storage duration increased to 48 hours, the pH value loss gradually, reaching the lowest value of about 3.70. This loss in pH may be due to the natural metabolic and fermentation processes that still take place in the material during storage. After harvesting, pineapple core likely undergo degradation of organic compounds, which produce organic acids as by-products [10]. This process may also affect the stability of the bromelain enzyme, as enzymes are sensitive to changes in environmental conditions, including pH [11], [12].

A loss pH value can affect the activity of bromelain, as this enzyme has an optimum pH for its maximum activity. A loss in pH outside the optimal range can lead to a reduction in the catalytic efficiency of the enzyme. Therefore, controlling storage duration and environmental conditions is important to maintain pH stability and activity of crude bromelain enzyme from pineapple core. A loss in pH occurs due to the conversion of citric acid and malic acid to lactic acid during storage. A low pH is also caused by the bacteria contained in the product which break down organic by bacteria present in the product [2], [13].

3.3 Color measurement of crude bromelain

The table 1. shows the color changes of crude bromelain during storage at various durations (0, 12, 24, and 48 hours) based on the parameters of hue (°Hue), brightness (L*), and dominant color. The highest hue value was recorded at 0 hours storage (96.61 ± 1.23) and lossd to 91.15 ± 0.67 at 48 hours. This loss in value indicates a change in color hue, although it is still in the yellow color category. Meanwhile, the L* value, which indicates the brightness of the color, was initially 63.00 ± 0.44 at 0 hour and slightly lossd to 56.05 ± 0.32 at 12 hours, before increasing again to 59.145 ± 1.48 at 48 hours.

Table 1. Color Measurement of Crude Bromelain

Storage duration (Hours)	°Hue (average ± SD)	L* (average ± SD)	Color
0	96.61 ± 1.23	63.00 ± 0.44	Yellow
12	93.24 ± 0.29	56.05 ± 0.32	Yellow
24	94.97 ± 1.01	58.94 ± 1,05	Yellow
48	91.15 ± 0.67	59.15 ± 1.48	Yellow

These variations in brightness values may be due to chemical reactions, pigment degradation, or environmental influences during storage [14]. Despite the changes in hue and brightness parameters, the predominant color of crude bromelain remained yellow throughout the storage period. Such changes are likely influenced by oxidation processes, pigment degradation, or changes in chemical compounds within the crude bromelain due to environmental conditions such as temperature and oxygen exposure

3.4 Scanning of crude bromelain content using Spectrophotometry UV-Vis

Fig 3. shows the scanning results of bromelain levels using spectrophotometry in the wavelength range of 200-800 nm during different storage durations, namely 0 h, 12 h, 24 h, and 48 h.

The results of the scanning UV-Vis spectrum of bromelain enzyme from pineapple core at shelf life of 0, 12, 24, and 48 hours are shown in **Fig** 1. The spectrum shows absorbance values in the wavelength range of 200-800 nm, focusing on the 200-300 nm region which is relevant for protein and enzyme absorption. At a wavelength of about 280 nm, which is a typical peak for proteins (including bromelain).

At the beginning of the observation (0 h), the spectrum showed a sharp absorbance peak in the wavelength range of 250-300 nm. This indicates that bromelain in fresh condition has high activity, with a stable active structure, and has not undergone significant degradation. However, after 12 hours of storage, there was an increase in absorbance at the same wavelength. This increase is thought to be due to temporary molecular restructuring that may activate some parts of the enzyme molecule before finally degrading.

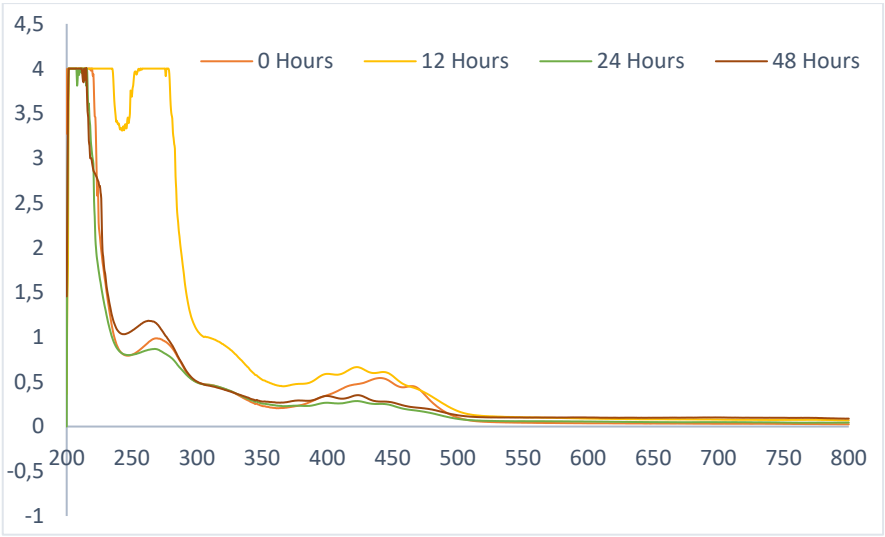

Fig 3. Scanning of Crude Bromelain Content Using Spectrophotometry UV-Vis

At a shelf life of 24 hours, there was a loss in the absorbance peak compared to 12 hours. This loss indicates that the enzyme structure starts to undergo partial denaturation, which reduces the catalytic activity of bromelain. Environmental factors such as storage temperature and air exposure most likely affect the stability of the enzyme structure [14].

As storage duration increased, there was a loss in absorption intensity, indicating a degradation or loss in bromelain levels. A significant loss began after 12 hours of storage and continued at 24 to 48 hours, where bromelain levels were less and less detectable. This suggests that bromelain has a limited shelf life, and environmental factors such as temperature, pH, or the presence of other enzymes may affect its stability [14].

3.5 Determination of proteolytic activity of crude bromelain

Several factors, such as changes in pH, moisture content, and loss protein content can explain the loss in proteolytic activity of bromelain during pineapple storage. In addition, the increase in water content and loss in protein content in raw bromelain during storage also contributed to the loss in enzyme activity. Pineapple spoilage is also an important factor that causes protein degradation and the appearance of compounds that inhibit enzyme activity, following the findings of [8], who mentioned that high levels of pineapple maturity reduce bromelain activity. The use of technology can cause differences in data findings. This study used low-cost. technology without a purification process.

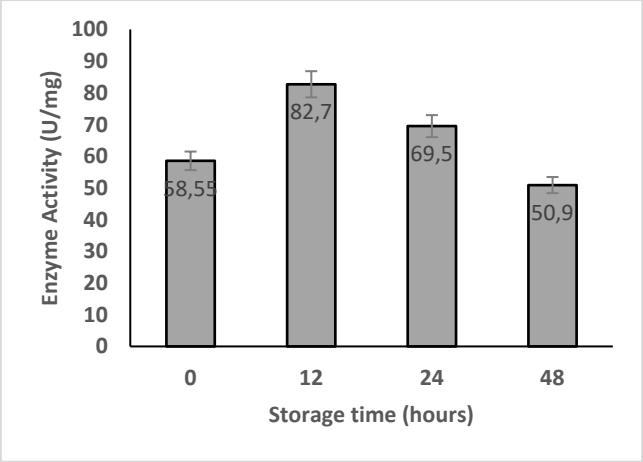

Fig 4. Proteolytic Activity of Crude Bromelain

In the data shown in **Fig 4**, bromelain activity peaked at 12-24 hours of storage and lossd significantly after 48 hours. The combination of these factors suggests that the loss in bromelain proteolytic activity is due to changes in chemical and physical conditions during pineapple storage, with optimal activity at shorter storage durations. To obtain maximum results, it is important to consider proper storage conditions and the use of improved purification technologies.

4 Conclusion

This study shows that the storage duration of pineapple stems significantly affects the characteristics of the bromelain enzyme produced. Bromelain activity peaked at 12 hours of storage, which is the best duration to maintain enzyme activity before a significant loss occurred after 24 to 48 hours. This loss in activity was caused by changes in pH, increased water content, and protein degradation during storage. The results of this study confirm the importance of proper storage duration management to maximize the potential of bromelain from pineapple stems. In addition, using pineapple stems as a source of bromelain enzyme supports the sustainable management of pineapple waste. For more optimal results, future research is recommended to explore storage conditions that can maintain enzyme stability for a longer period of duration.

Acknowledgements

This study and publication have been supported by the grant for the Faculty of Agricultural Technology Universitas Andalas with the scheme of DIPA 2024 with number 01D/PL/PN-UNAND/FATETA2024

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