



Extraction Of Bromelain From Fruit Waste

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Abstract: Bromelain is a group of proteolytic enzymes predominantly found in pineapple (*Ananas comosus*), especially in its waste parts such as the stem, peel, and core. This study focuses on the extraction of bromelain from pineapple waste, promoting a sustainable method of utilizing agricultural by-products. Pineapple processing generates large quantities of waste, which are often discarded, contributing to environmental pollution. By this extraction of bromelain, a valuable enzyme is recovered and waste disposal issue is mitigated. The extraction process uses protein precipitation and the crude extract is then evaluated for enzyme activity. This research highlights the potential of pineapple waste as an abundant and renewable source of bromelain. Factors such as pH, temperature, and solvent concentration were optimized to enhance enzyme yield and activity. This study demonstrates that bromelain can be efficiently extracted from pineapple waste, offering an economical and environmentally friendly alternative to traditional sources.

Keywords: Bromelain, pineapple waste, enzyme extraction, protein quantification, sustainable bioprocess.

I. Introduction

Pineapple (*Ananas comosus*), a tropical fruit cultivated extensively in countries such as India, Thailand, the Philippines, and Brazil, is widely consumed both fresh and in processed forms like juices, jams, and canned products. The processing of pineapples generates a significant amount of waste, approximately 50–60% of the fruit mainly comprising the peel, core, stem, crown, and leaves [13]. These by-products, often discarded or underutilized, pose environmental concerns due to their high organic content. However, they are rich in bioactive compounds, particularly bromelain, a group of proteolytic enzymes with therapeutic and industrial applications.

Bromelain is primarily found in the stem and fruit of the pineapple plant, with the stem being the most concentrated source [9]. It exhibits a wide range of biological properties including anti-inflammatory, anti-edematous, antithrombotic, fibrinolytic, and wound healing effects, making it highly valuable in the pharmaceutical and nutraceutical industries [14]. Additionally, bromelain is employed in the food industry as a meat tenderizer and in the cosmetic industry for exfoliation and skin-care formulations. Despite its economic potential, the commercial production of bromelain remains limited and expensive due to reliance on fresh plant material.

Recent studies have highlighted the potential of extracting bromelain from pineapple waste using environmentally friendly and low-cost techniques. Study shows effective bromelain extraction from pineapple peel and core using aqueous buffer and ethanol precipitation methods, suggesting that waste parts could serve as viable enzyme sources. [13] Also some study shows enzyme recovery from pineapple stem waste, optimizing conditions to increase yield.[11]

This research is aimed at extracting bromelain from pineapple processing waste, specifically the stem, peel, and core using optimized protocols to maximize enzyme activity and yield. By utilizing waste materials, the study supports the principles of sustainable biotechnology and contributes to the circular bioeconomy. The

integration of waste management and enzyme production aligns with global efforts to reduce agricultural waste and promote value added products from renewable resources.

II. Research Methodology

The extraction of bromelain from pineapple waste was carried out using a systematic methodology involving collection, homogenization, and purification steps. Fresh pineapple waste (peels and cores) was thoroughly washed, chopped, and blended with 0.1 M phosphate buffer (pH 7.0) in a 1:2 (w/v) ratio. The homogenate was filtered and centrifuged at 5,000 rpm for 20 minutes at 4°C to obtain the crude extract. Bromelain was then precipitated using ammonium sulfate at 80% saturation, followed by centrifugation and dialysis to remove excess salts. The enzyme activity was quantified using the casein and gelatin digestion method, and protein concentration was determined by the Bradford assay to assess purity and specific activity.

The Pineapple waste was collected from juice centres and the pineapple fruit vendor, and extract was collected from the dried pineapple waste powder (Fig 1.1) using ammonium sulphate precipitation method.

Making of pineapple waste powder:

1. Pineapple waste (Peel, crown, leaves) was dried at 150°C for 72 hours.
2. The dried waste was grinded into fine powder.
3. This powder was then for further extraction of Bromelain.

Extraction of Bromelain from dried powder:

1. Mix the pineapple waste powder with PBS in the proportion of 1:10. (Fig1.2)
 2. Keep the mixture for continuous stirring on a magnetic stirrer for 30 minutes.
 3. Filter the mixture using a muslin cloth.
 4. Crude bromelain extracts were saturated with 30%, 40%, 50%, and 60% (NH₄)₂SO₄ (w/v).
 5. The required amount of ammonium sulphate was calculated to attain the desired concentrations.
 6. Crude extracts from pineapple crown and peel were transferred to a beaker and was placed on a magnetic stirrer at 4°C.
 7. While stirring, ammonium sulphate was slowly added to the extracts.
 8. The extracts were left overnight at 4°C for precipitation.
 9. The precipitated extracts were subjected to centrifugation at 5,000 rpm for 20 minutes at 4°C.
 10. The supernatant was carefully discarded, and the pellet was retained.
 11. The pellet was resuspended in 0.05M phosphate buffer (pH 7).
- The extracted bromelain was stored in a freezer at approximately 4°C to prevent protein degradation.

III. Characteristic Testing Of Bromelain

Various tests were performed to confirm the presence of bromelain in the extracted sample.

Casein digestion test- This test assesses the proteolytic activity of bromelain by measuring its ability to hydrolyse casein, producing smaller peptides that were detected spectrophotometrically

Gelatin digestion test - This test evaluates bromelain's ability to hydrolyze gelatin. The digestion of gelatin was observed visually and quantified spectrophotometrically.

Bradford Assay - The Bradford assay is a quick and reliable spectroscopic method used to determine the protein concentration in a sample. This technique is based on the interaction between Coomassie Brilliant Blue G-250 dye and proteins. When the dye binds to proteins, it undergoes a shift in its absorption spectrum, leading to a visible colour change. The intensity of this colour change is directly related to the amount of protein present, making it suitable for accurate quantification.

IV. Result And Discussion

The Bromelain extract was proved to be satisfactory.



fig 1.1 dried pineapple waste powder



fig1.2 bromelain extracted from dried pineapple waste powder

pH test- The pH of the Bromelain extract was measured and found to be 5.6, indicating a mildly acidic nature. This aligns well with reported pH values of standard Bromelain, which typically range between 5.5 and 6.5, suggesting the extract retained its native characteristics.

Tube no.	Sample	Enzyme used	Incubation time (min)	Absorbance at 280nm	Observation
1	Control(blank)	None	30	0.07	No digestion
2	Positive control	Trypsin (1mg/ml)	30	0.90	Strong digestion
3	Test sample	Bromelain (1mg/ml)	30	0.70	Moderate to high digestion
4	Test sample (low concentration)	Bromelain (0.5mg/ml)	30	0.50	Partial digestion

Table no. 1 Observation table for casein digestion test.

Casein test- The casein digestion test results, as shown in the provided table (Table 1), demonstrate the proteolytic activity of bromelain in the tested samples. The absorbance values indicate the extent of casein breakdown, with higher readings reflecting greater enzymatic activity. The results show whether the Bromelain extract effectively digested the casein substrate (indicating a positive proteolytic reaction) or showed minimal activity (indicating partial or no digestion).

Gelatin test- The gelatin digestion test results, as shown in the provided table (see Table 2), reflect the proteolytic activity of Bromelain when tested with a gelatin substrate. The absorbance values at 280 nm correlate with the extent of gelatin degradation. Samples that showed higher absorbance indicate stronger

enzymatic activity, confirming bromelain's ability to hydrolyze gelatin. Lower absorbance values represent partial digestion or limited activity, depending on the enzyme concentration used.

Tube no.	Sample	Enzyme used	Incubation time (min)	Absorbance at 280nm	Observation
1	Control(blank)	None	30	0.06	No digestion
2	Positive control	Trypsin (1mg/ml)	30	0.80	Strong gelatin digestion
3	Test sample	Bromelain (1mg/ml)	30	0.60	Moderate to high digestion
4	Test sample (low concentration)	Bromelain (0.5mg/ml)	30	0.50	Partial digestion

Table no. 2 Observation table for gelatin digestion test.

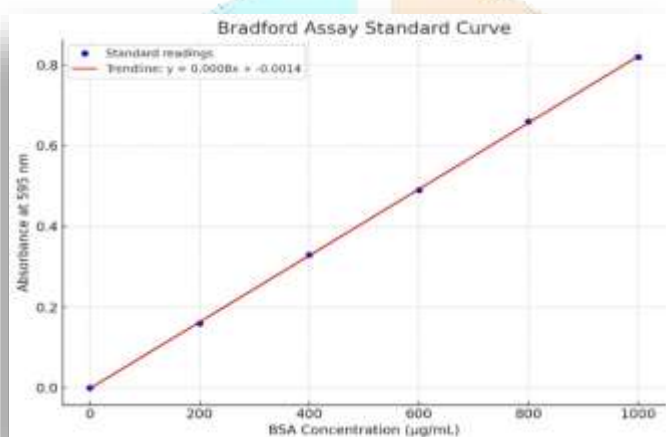


Fig 2.1 Standard curve of BSA for Bradford assay

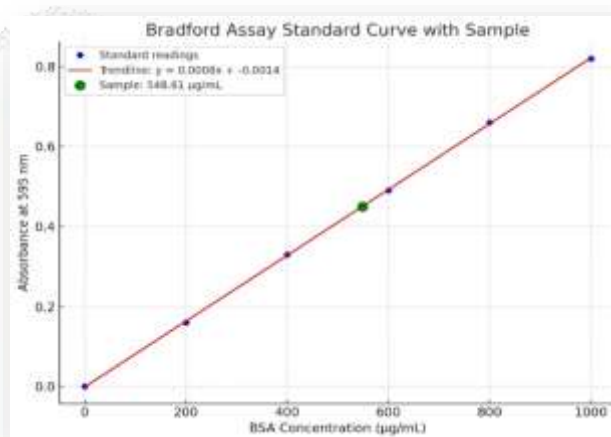


Fig 2.2 Standard curve with sample

The Bradford Assay- It confirms the presence and concentration of protein in the extracted bromelain sample. (Figure 2.1 and Figure 2.2)

Figure 2.1 shows the standard curve generated using known concentrations of Bovine Serum Albumin (BSA), establishing a direct correlation between protein concentration and absorbance at 595 nm. Figure 2.2 displays the absorbance of the unknown sample, which was interpolated using the standard curve. The resulting concentration of the bromelain extract was approximately 561.25 µg/mL, indicating a significant protein content and supporting the successful extraction of bromelain from pineapple waste.

Stability test- It shows the stability of the extracted bromelain under different conditions and varying temperatures. The extract was kept at room temperature, refrigeration (4°C), and slightly elevated temperatures over 15 days to observe changes in clarity, color, and enzymatic activity.

As we see the Lowry method, the Bradford assay is easy and accessible, its speed and high sensitivity. Certain substances like detergents, reducing agents, or high salt levels can interfere with the results and affect accuracy (Lu et al., 2010). Additionally, since different proteins bind to the dye with varying strengths, the presence of mixed proteins in a sample can lead to inconsistencies in measurement.

Because of this, it's important to use proper controls and be aware of the specific characteristics of the proteins being analyzed when using the Bradford.

This study successfully demonstrated a simple and sustainable method for extracting bromelain from pineapple fruit waste such as peels and crowns materials that are typically discarded. The extracted enzyme was confirmed to have proteolytic activity through casein and gelatin digestion tests, showing significant protein breakdown at higher concentrations.

The protein content of the extract was further validated using the Bradford assay, which indicated a concentration of approximately 561 µg/mL.

On testing the stability of extracted Bromelain, the stored Bromelain at 4°C maintained its stability the best, showing minimal change in appearance and no significant loss of activity. In contrast, samples exposed to higher temperatures showed slight cloudiness and reduced activity over time. These results suggest that refrigeration is ideal for preserving the quality and effectiveness of the extracted bromelain, especially if it is to be used in formulations that require long-term storage.

These findings not only confirm the presence and functionality of bromelain in fruit waste but also highlight the potential of using agricultural by-products as valuable raw materials.

By converting waste into a useful bioactive compound, this research supports eco-friendly and cost-effective approaches in enzyme sourcing, especially for use in industries like cosmetics, food, and pharmaceuticals. This study demonstrates that pineapple fruit waste, particularly peels and crowns, can serve as an effective and sustainable source of bromelain. The extracted enzyme displayed notable proteolytic activity in both casein and gelatin digestion tests, confirming its functional integrity. The Bradford assay further validated the presence of protein, estimating a concentration of approximately 561 µg/mL, while the pH of the extract remained within the ideal range for bromelain activity.

V. Conclusion

Bromelain was effectively extracted from pineapple peels and crowns using a simple and sustainable method. The enzyme demonstrated effective proteolytic activity in both casein and gelatin assay, and protein quantification confirmed a substantial yield. The extract showed maximum stability when stored at 4°C, supporting its suitability for further applications. These findings highlight the potential of pineapple waste as an economical and eco-friendly source of bromelain for industrial use.

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