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การเพิ่มมูลค่าวัสดุเศษเหลือจากสับปะรดเพื่อการผลิตสารสกัดหยาบเอนไซม์โบรมิเลนแบบผง

VALORIZATION OF RESIDUES WASTE FROM PINEAPPLE FOR CRUDE BROMELAIN ENZYME POWDER PRODUCTION

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บทคัดย่อ

วัตถุประสงค์ของงานวิจัยเพื่อศึกษาการสกัดเอนไซม์แบบหยาบจากวัสดุเศษเหลือจากสับปะรด (*Ananas comosus*) และผลิตเอนไซม์โบรมิเลนที่สกัดได้เป็นแบบผง ผลการทดลองพบว่า วัสดุเศษเหลือจากส่วนต่าง ๆ จากสับปะรดมีศักยภาพต่อการผลิตเอนไซม์โบรมิเลน โดยเหง้าสับปะรด (Stem) มีค่ากิจกรรมเอนไซม์และค่ากิจกรรมเอนไซม์จำเพาะสูงสุดเท่ากับ 182.5±1.0 Units/ml และ 16.9±0.5 Units/mg ตามลำดับ มีค่าสูงกว่าส่วนของเปลือก (Peel) แกน (Core) และจุกสับปะรด (Crown) อย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$) นอกจากนี้ได้ศึกษาความคงตัวต่ออุณหภูมิ (25 - 65°C) ต่อประสิทธิภาพการทำงานเอนไซม์ พบว่าเอนไซม์โบรมิเลนแบบหยาบมีค่าความคงตัวต่ออุณหภูมิที่ 35°C ดีที่สุด และลดลงเมื่ออุณภูมิสูงกว่า 45°C และไม่พบค่ากิจกรรมเอนไซม์จำเพาะที่อุณภูมิ 65°C หลังจากนั้นนำส่วนของเหง้าที่ให้ค่ากิจกรรมเอนไซม์จำเพาะสูงสุดมาผลิตเป็นเอนไซม์แบบผง โดยการใช้เทคโนโลยีการทำแห้งเยือกแข็งแบบสุญญากาศ (Freeze-Dry Technology) พบว่าการเก็บรักษาเอนไซม์แบบผงที่อุณหภูมิ 4±1°C จะให้ค่ากิจกรรมเอนไซม์จำเพาะสูงกว่าการเก็บรักษาที่อุณหภูมิ 35±2°C ที่ระยะเวลา 28 วัน ดังนั้นวัสดุเศษเหลือจากส่วนต่าง ๆ ของสับปะรดมีศักยภาพสูงต่อการนำไปใช้ประโยชน์ในการผลิตเอนไซม์และสามารถนำไปต่อยอดผลิตเป็นสารหรือผลิตภัณฑ์ที่มีมูลค่าสูงได้

คำสำคัญ: โบรมิเลนเอนไซม์; สับปะรด; วัสดุเศษเหลือ; กิจกรรมเอนไซม์

Abstract

The objective of this research was to investigate the extraction of crude bromelain enzyme from pineapple residues waste (*Ananas comosus*), including the bromelain enzyme powder production. The results showed that the residues waste had the potential for crude bromelain enzyme production. However, the stem exhibited the highest activity and specific enzyme activity, with values of 182.5 ± 1.0 Units/ml and 16.9 ± 0.5 Units/mg, respectively, which were significantly ($p \leq 0.05$) higher than those of the peel, core, and crown. In addition, the thermal stability of the bromelain enzyme (25-65°C) was studied. It was found that the crude bromelain enzyme had thermal stability at 35°C, but its stability decreased at temperatures above 45°C, with no specific enzyme activity observed at 65°C. Furthermore, the crude bromelain extracted from the stem, which exhibited the highest specific enzyme activity, was processed into powder using freeze-drying technology. The results indicating that the storage of bromelain enzyme powder at 4°C gave higher specific enzyme activity than storage at 35°C for a period of 28 days. Therefore, residual waste materials from pineapples have the potential to be utilized in enzyme production and can be further developed into high-value substances or products.

Keywords: Bromelain Enzyme; Pineapple; Residue; Enzyme Activity

Introduction

Thailand has the potential to become a leader in the export of processed pineapple products, particularly in the categories of canned pineapple and pineapple juice. Statistics indicated that in 2022, Thailand exported pineapple products valued at 23,860 million Baht, marking a 12.14% increase. The United States, the Russian Federation, and Germany stand out as major importers of Thai canned pineapple [1]. In general, pineapple fruit was processed only 30-65% to finished products, resulting in the generation of a substantial quantity of by-products. The periods of April-June and November-January experienced high peaks in by-product generation each year. After fruit harvesting, pineapple residues constitute approximately 40-70% of the process, accounting peel (29-42%), cores (9.4-20.0%), stems (2.4-6.8%) and crowns (2.7-5.9%), thereby contributing to agricultural waste problems [2]. It is estimated that pineapple cultivation generates around 2.7 million tons of waste per year. Despite continuous research aimed at developing new products utilizing pineapple residues, there has been very limited commercial utilization. Currently, scientists and technologists face the challenge of developing innovative products using cost-effective materials that are abundantly available. However, economic instability complicates investment efforts, and the quantity of pineapples is further affected by the drought phenomenon [2].

Pineapple residues waste demonstrates potential as a raw material for the production of high-value-added products such as bromelain, polymer composites, and adsorbents. In agriculture, such waste is

frequently repurposed as fertilizer or animal feed. The peel waste, in particular, serves as an abundant source of substants such as cellulose, hemicelluloses, and carbohydrates. Additionally, various byproducts of pineapple have been repurposed as nutrient sources in culture broth and cellulose production [3]. In addition, these residual wastes can be used for energy generation in the form of methane and ethanol, as well as for the natural compounds production such as citric acid and antioxidants [4-5].

The Bromelain enzyme, a primary protease in *Ananas comosus*, is extracted as a crude aqueous extract from pineapples. It is widely utilized across diverse industries including food, cosmetics, pharmaceuticals, and others. Within the food sector, it serves various purposes such as tenderizing meat, solubilizing grain protein, clarifying beer, baking cookies, and producing protein hydrolysates [5-6]. In biochemical terms, the bromelain enzyme is composed of 212 amino acids, and its molecular weight is 33 kDa [7]. Bromelain, a natural enzyme, possesses therapeutic value as a non-toxic compound. Classified as a protease enzyme, it aids in the digestion of protein compounds. It's noteworthy that bromelain extract includes not only various thiol endopeptidases but also other components such as carbohydrates, cellulases, phosphatases, glucosidase, peroxidases, and glycoproteins. However, bromelain enzyme is inhibited by Hg^{2+}, Ag+, Cu^{2+}, antitrypsin and iodoacetate and organically bound Ca^{2+} [7-8]. The crude bromelain extract consists of 80% stem bromelain, 10% fruit bromelain, 5% ananain, and additional constituents [9-10].

Given the advantages that bromelain offers in diverse industries, extracting and characterizing the bromelain enzyme from various pineapple waste residues becomes a compelling pursuit due to their abundant properties and potential for waste utilization. Consequently, The aim of this study was to determine the extraction of crude bromelain enzyme from pineapple residue waste, analyze its thermal stability, and explore the production and storage of the crude enzyme powder.

Objectives

The objective was to study the extraction and thermal stability of crude bromelain enzyme from pineapple residue waste, as well as to investigate the production and storage of bromelain powder.

Methods

Raw materials

The stem, core, peel, and crown of pineapple (*Ananas comosus* var. Parravia) as residues waste were collected from a farmer's garden that supplies pineapples to processing plants in Pluak Daeng Subdistrict, Pluak Daeng District, Rayong Province, Thailand.

Crude bromelain enzyme extraction from pineapple residues waste

The residues waste from the peel, crown, core and stem were extracted the bromelain enzyme. They were soaked in cold distilled water (DW) at a ratio of 1:2 (w/v) and subsequently blended using a blender. The resulting homogenates underwent filtration before being combined with a cold 0.1 M sodium phosphate buffer (pH 7.0) containing 2 mM EDTA and 5 mM cysteine. The mixture sample was subsequently centrifuged at 4500 rpm at 4°C for 20 min [11]. After that, the supernatant was collected. Subsequently, these filtrates were then subjected to triple-precipitation with 40-60% ethanol to yield the enzyme precipitate. The samples were

recentrifuged and the filtrate (crude enzyme) was used to determine the pH, total soluble solids (TSS), total protein concentration, enzyme activity, and specific activity [11].

Effect of thermal stability for bromelain enzyme activity

The thermal stability of the crude bromelain enzyme, extracted from the peel, core, crown, and stem, was investigated. The samples were incubated sample in water baths at temperatures of 25°C, 35°C, 45°C, 55°C, and 65°C for a duration of 2 hours [12]. Contamination of the sample was prevented by sealing and capping the tubes. After completing the incubation period at each temperature, the specific activity of bromelain was determined [12].

Crude bromelain enzyme powder production

For the production of crude bromelain enzyme powder, the sample with high enzyme and specific activity was prepared for powder production. The pellet sample was freeze-dried using a lyophilizer, a low-temperature dehydration process, at a temperature of -50.0 ± 2.0°C and a pressure of 2.00 mbar until the powder was dry, producing crude bromelain powder. The enzyme activity and specific activity of the crude bromelain powder were then determined [11-12].

Determination of bromelain activity and specific activity

The activity and specific activity of the bromelain extraction sample were determined according to the modified method [13]. The reaction mixture containing the 1.0 ml of 2% (w/v) casein solution in 0.1 M phosphate buffer (pH 7.0) with 5 mM cysteine and 0.5 ml of crude bromelain extract solution. The mixture sample was incubated at 37°C for 20 minutes. To stop the reaction, 2.0 ml of 3% (w/v) trichloroacetic acid (TCA) was added to the sample. Thereafter, the sample was centrifuged at 4°C for 20 minutes. The filtrate was collected, and the enzyme activity was measured at 280 nm using a spectrophotometer. Casein digestive units were used as a standard to express bromelain activity. One unit was defined as the quantity of enzyme capable of releasing the equivalent of 1 µg of tyrosine within a duration of 1 minute at 37°C. Additionally, the total protein content in the crude enzyme extract was determined using the Bradford method (1976), with bovine serum albumin (BSA) serving as the standard [14].

Statistical analysis

All measurements were independently conducted three times, and the results underwent analysis of variance (ANOVA) coupled with Duncan's multiple range test. Differences between means were investigated using the least significant difference test, and statistical significance was defined at $P<0.05$. This analysis was performed using SPSS statistics version 17.0.

Results

Bromelain extraction from pineapple residues waste

The crude bromelain enzyme was extracted from waste residues of pineapple, such as peel, crown, core and stem. Following that, chemical facilitating enzyme extraction, specifically 0.1 M sodium phosphate buffer was used for extraction. Moreover, the enzyme precipitation was performed using a 40-60% ethanol. The results indicated that crude extracts from residues waste had varying pH values within the acidic range of 3.8±0.4 - 4.7±0.2 (Table.1). Moreover, the residues waste from the results exhibited TSS ranged between

2.0±1.0 - 7.0±2.0 °Brix, with the core had the highest TSS of 7.0±2.0 °Brix. Corresponding to Singh [15] reported that pineapple residues waste had an acidic pH of 3.8-5.0 and TSS of 2.0-7.0 °Brix. Additionally, Ketnawa [14] demonstrated that the pH of pineapple crude extracts ranged from 4.0 to 5.0, while the TSS ranged from 2.6 to 6.3. In this work, the results indicated that the stem residue waste demonstrated the highest levels of total protein, enzyme activity, and specific activity, at 215.5 ± 0.5 mg, 182.5 ± 1.0 Units/ml, and 16.9 ± 0.5 Units/mg, respectively, with a 0.1M sodium phosphate buffer at pH 7.0 (Figure 1). This result is in agreement with Al-Saady [16] who reported that pineapple fruit demonstrated the highest bromelain activity when using a 0.1M sodium phosphate buffer at pH 7.0 for enzyme extraction. Sodium phosphate buffer is commonly for enzyme extraction due to its ability to maintain a stable pH level, buffering capacity. Additionally, the controlled ionic strength conferred by sodium phosphate buffer proves advantageous for bromelain extraction [15-16]. Also, Singh [15] reported that pineapple residue waste possessed the potential to serve as a raw material for enzyme production.

Table 1 Characteristics of pineapple residues waste for crude bromelain extraction.

Residues waste from Pineapple	pH	TSS (°Brix)	Total protein (mg)
peel	3.8±0.4[a]	4.0±1.0[b]	91.0±2.0[c]
core	4.0±0.1[b]	7.0±2.0[c]	80.5±1.5[b]
crown	4.7±0.2[d]	2.0±1.0[a]	68.5±1.0[a]
stem	4.2±0.4[c]	2.0±1.0[a]	215.5±0.5[d]

Note: All values are presented as the mean ± S.D. The values in each column are denoted by letters (a, b, c, or d) indicating significant differences ($p \leq 0.05$) based on Duncan's new multiple range test.

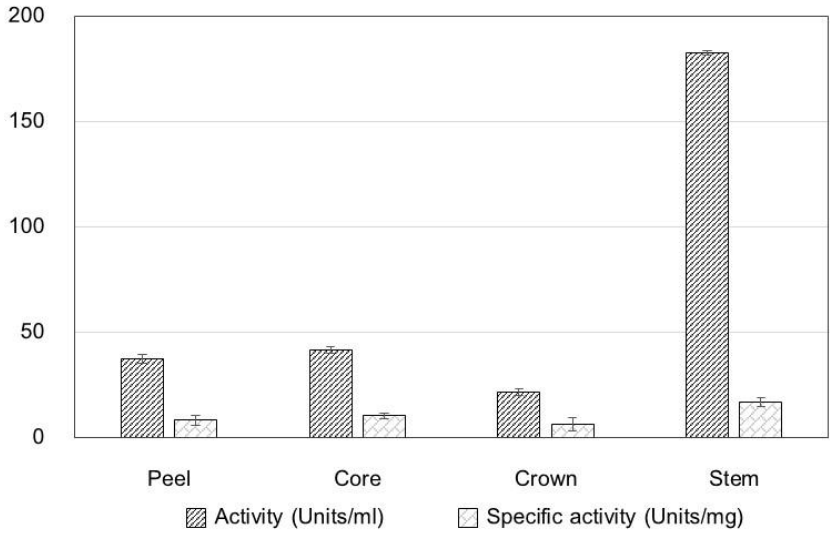

Figure 1 Activity (Units/ml) and Specific activity (Units/mg) from pineapple residues waste. All values are carried out in tripicate as mean ± S.D.

Effect of thermal stability for bromelain enzyme activity

The crude bromelain enzyme, extracted from various pineapple residue wastes, was investigated for its thermal stability across a range of temperatures: 25°C, 35°C, 45°C, 55°C and 65°C. The specific enzyme activity was determined. The results indicated that the residue waste from the stem showed the highest relative specific activity at 35°C (Figure 2). Besides, the relative activity slightly decreased to 76.7% at 45°C. Moreover, at 55°C, the relative enzyme activity continuously decreased to 38.4%. However, at 65°C, no activity was observed. Additionally, the crown residue waste showed high relative activity at 35°C. The efficiency of the relative specific activity enzyme gradually decreases with higher temperatures (45°C), resulting in no activity at 65°C. Furthermore, the results from both peel and core residue wastes exhibited a similar trend. Specifically, the enzyme activity was highest at 35°C, followed by a gradual decline in efficiency as temperatures increased. The bromelain enzyme is a natural enzyme that is sensitive to high temperatures. Denaturation is characterized by a structural change in the enzyme, leading to the loss of its catalytic activity [7-8]. Based on these results, it was observed that the thermal stability for bromelain activity was at 35°C, with a subsequent decrease in enzyme activity observed at temperatures above 45°C, and no activity detected at 65°C.

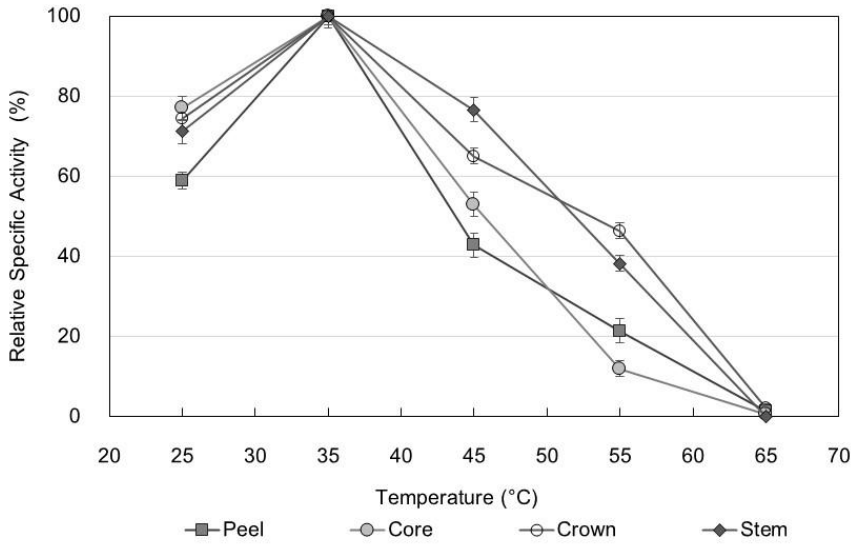

Figure 2 Thermal stability profile of crude bromelain enzyme from residues waste pineapple. All values are carried out in tripicate as mean ± S.D.

Bromelain powder production by using freeze drying technology

The pellet sample from the stem, which extracted and displayed the highest bromelain activity, was processed into powder using freeze-drying technology under the conditions at -50.0±2.0°C, pressure of 2.00 mbar for a period of 15-18 hours until the produced powder was dry. This method can preserve activity by removing water and maintaining the structure of enzyme. After that the crude bromelain powder sample was collected in polypropylene containers with screw caps and stored in a desiccator at room temperature (32±2 °C). The result showed that the crude enzyme powder had a light-yellow color and fine in texture (Figure 3). Furthermore, the effect of storage temperatures at 4°C and 35°C on the enzyme powder was investigated.

The enzyme characteristics and activity were determined every 7 days for a period of 4 weeks. The results indicated that initially, the bromelain powder had a specific activity of 17.9 Units/mg. After 7 days, the specific activity dropped to 15.5 ± 1.5 Units/mg at 35°C and 16.1 ± 0.5 Units/mg at 4°C (Figure 4). Afterward, the specific activity of the bromelain enzyme at 35°C and 4°C continuously decreased. On day 28, the percentage of specific activity at 4°C decreased to 26.8±0.5. In contrast, the percentage of specific activity dropped to 42.1±0.4 at 35°C. The bromelain enzyme is an enzyme that is sensitive with a high temperature. Freeze-drying can preserve the structure and activity stability of bromelain significantly, extending the shelf life of the enzymes. Additionally, the stability of the enzyme depends on various factors, such as extraction methods, humidity, pH, temperature, and storage conditions. All these factors significantly impact the efficiency of the enzyme activity [7-8]. Based on the results, storing bromelain enzymes at 4°C exhibited better specific activity compared to storage at 35°C.

Figure 3 Crude bromelain powder from stem residue waste by using freeze drying technology.

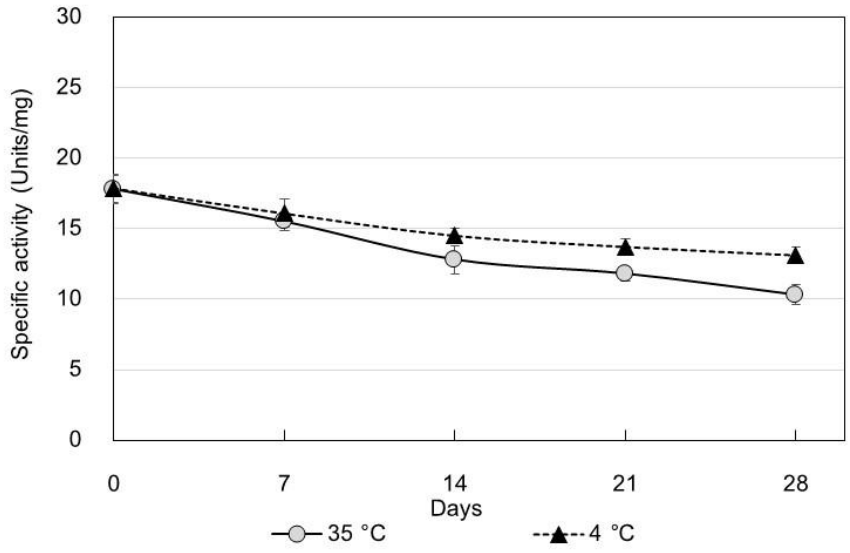

Figure 4 Specific activity (Units/mg) of bromelain enzyme during storage at 4°C and 35°C. All values are carried out in tripicate as mean ± S.D.

Conclusions and Discussion

The crude bromelain enzyme was extracted from pineapple waste residues such as peel, crown, core and stem using 0.1 M sodium phosphate buffer. In the extraction process, the observed high protein content and enzyme activity may be attributed to the influence of salts within the buffer, which facilitates the solubilization of proteins from the compact structure in the sample. The buffering capacity achieved through the use of a sodium phosphate buffer enables the maintenance of the solution's pH by allowing slight adjustments to its value. This property is advantageous for enzyme extraction, thereby promoting maximum activity. The buffer solution proved highly efficient and suitable for specific proteins/enzymes, without inducing excessive denaturation [16]. In enzyme extraction processes, optimal extractants should maintain the system pH close to the target enzyme's optimum pH without altering its activity. Indeed, it is well-established that a sodium phosphate buffer is suitable for bromelain extraction [16-18]. Selecting an extractant buffer capable of maintaining the system pH close to these values is crucial. Furthermore, bromelain extraction containing cysteine and EDTA with a phosphate buffer demonstrated enhanced capability in preserving enzyme activity [19].

Additionally, the temperature had an effect on enzyme stability. Denaturation entails a structural alteration in the enzyme, resulting in the loss of its catalytic activity with high temperatures. Enzyme is highly sensitive to extreme temperature, where excessive heat can disrupt the hydrogen bonds, electrostatic interactions, and hydrophobic forces crucial for maintaining the enzyme's structural integrity [20]. From the result, the thermal stability for bromelain activity was 35°C and the complete loss of bromelain activity occurred when temperatures exceeded 65°C. These results exhibit similarities to studies reported by Gui [20], indicating that the majority of enzymes tend to be unstable at high temperatures. They reported that the thermal stability of residues from peel, crown and core reached its maximum at 35°C. Additionally, Khan [21] reported that at 30°C, the bromelain enzyme exhibits maximum activity and stability. However, the efficiency of the bromelain enzyme dropped to 17% when increased the temperature to 40-60°C. Besides, Omotoyinbo [22] also reported that bromelain activity derived from residues waste pineapple exhibited optimal temperature at 40°C. Additionally, the bromelain enzyme demonstrated stability, maintaining approximately 50% relative activity within the temperature range of 30-60°C, whereas the enzyme's activity decreased under high temperatures (70°C). Also, Febriani [23] identified that at 37°C exhibited high bromelain activity. However, several research have concluded that the temperature range of 40-70°C has an adverse effect on the enzyme activity.

In addition, the stability of the enzyme depends on various factors, such as extraction methods, humidity, temperature, and storage conditions [23]. For the bromelain poeder production method, freeze-drying is advantageous for enzyme preservation maintaining activity by removing water and preserving the enzyme's structure. This method prevents denaturation, ensuring the biological activity of the enzyme. Many enzymes are sensitive to heat and may denature at higher temperatures; however, freeze-drying allows for storage at low temperatures without compromising functionality. Moreover, freeze-drying significantly extends the shelf life of enzymes. Removing water minimizes conditions for microbial growth, reducing the risk of contamination and degradation. Additionally, the post-process storage conditions are crucial for maintaining the activity of bromelain. After freeze-drying, it is essential to store the enzyme in a moisture-free environment to prevent

rehydration and potential loss of activity. Moisture can result in partial rehydration and denaturation over time. This results in a longer storage life for enzyme products and a reduced cost of storage. Enzymes can be stored at lower temperatures without the risk of freezing, making freeze-drying a cost-effective method for long-term storage [24].

Consequently, the pineapple residues wastes (*Ananas comosus* var. Parravia) showed potential for bromelain production. The stem exhibited the highest total protein, enzyme activity, and specific activity at 215.5±0.5 mg, 182.5±1.0 Units/ml, and 16.9±0.5 Units/mg, respectively, significantly ($p \leq 0.05$) surpassing those of the peel, core, and crown. It was observed that the thermal stability for bromelain activity was 35°C, with a decrease in activity at temperatures above 45°C and no enzyme activity at 65°C. Additionally, the crude bromelain from the stem residue waste was processed into powder using freeze-dry technology. Also, storing the enzyme powder at 4°C resulted in higher protein and specific activity compared to 35°C at 28 days. However, the duration of long storage will be studied in the future to assess bromelain powder activity. Therefore, the valorization of by-products from pineapple residues waste holds the potential to yield high-value enzyme products for diverse market applications. Additionally, bromelain offers numerous health benefits, including prophylactic and antibiotic properties [25]. Moreover, achieving sustainable development hinges on policy implementation and the promotion of waste reuse, thereby enhancing opportunities for discovering new products and compounds. Importantly, this approach aids in addressing challenges in agricultural waste management, ensuring efficient and beneficial utilization of remaining materials.

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