ISSN: 1675-7785
eISSN: 2682-8626

Available online at
https://scilett-fsg.uitm.edu.my/

Science Letters 18(1) 2024, 21 – 27.

Science Letters

Effect of Storage Conditions on the Bromelain Activity of Pineapple Peel Variety Josapine

Muhammad Yazid Abd Halim[1], Siti Aimi Sarah Zainal Abidin[1,2], Siti Roha Ab Mutalib[1,2*]

[1]*School of Industrial Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor*
[2]*Malaysian Institute of Transport, Universiti Teknologi MARA, 40450 Shah Alam, Selangor*

ARTICLE INFO

Article history:
Received 25 September 2023
Revised 18 October 2023
Accepted 1 November 2023
Online first
Published 22 January 2024

Keywords:
Bromelain activity
Josapine
Pineapple Peel
Storage Condition

DOI:
10.24191/sl.v18i1.24113

ABSTRACT

Bromelain is a complex mixture of proteolytic enzymes typically derived from pineapple peels, leaves, stems, and fruit. This study aimed to determine the effect of storage conditions on the bromelain activity of the pineapple peel variety Josapine. The production of bromelain powder in this study was done in four steps: the extraction of crude bromelain from pineapple peel extracts, the purification of bromelain using ammonium sulfate precipitation, desalting using the diafiltration process, and finally, freeze drying using a freeze dryer. The casein digestion unit (CDU) analytical method determined the protease activity. The bromelain powder obtained from the pineapple peel extract was stored at three different temperatures: -20 °C, 4°C, and 27°C. Results showed that bromelain powder stored at a freezing temperature had the lowest decrement of bromelain activity when stored for up to one month. Bromelain powder stored at 27°C lost almost 90% of its activity upon one month of storage time. A freezing temperature below -20 °C was the best condition to sustain the enzymatic activity of bromelain.

INTRODUCTION

Pineapple, or its name Ananas comosus, is a perennial plant where from the family of Bromeliaceae [1]. The pineapple fruit is believed to have originated from tropical and subtropical America, and the fruit has been spread elsewhere, where the fruit can now grow throughout the world's tropical and subtropical regions. Pineapple is the most popular tropical fruit and an essential ingredient in fruit and juice products such as juice concentrates, jams, squash, jellies, essence, and pickles [2]. In Malaysia, pineapple is one of the significant fruits, and the fruit is the ninth country in production. Malaysia is one of the world's major pineapple producers, besides Thailand, Philippines, Indonesia, Hawaii, Ivory Coast, Kenya, Brazil, Taiwan, Australia, India, and South Africa. The pineapple variety Josapine is one of the popular pineapple varieties in Malaysia. This pineapple variety combines the 'Johor' variety ('Singapore Spanish' x 'Smooth Cayenne')

* Corresponding author. *E-mail address*: sitiroha7796@uitm.edu.my

with the 'Sarawak' variety ('Smooth Cayenne'). It was found that only about 30% of the whole weight of the pineapple fruit is fresh fruits, whereas the rest, which is 70%, is considered unsuitable components (peel, core, crown, and stem). The extraction of bromelain enzyme from this waste has fortunately been recognized as a value-added product [3].

Bromelain is a mixture of cysteine proteases found in the tissue of plant family Bromeliaceae obtained from pineapple. The proteolytic enzymes obtained from pineapple that are commercially available are stem bromelain and fruit bromelain. Nevertheless, pineapple wastes such as peel, core, and crown were also reported to contain bromelain [3]. Bromelain has many applications in industries, including its uses in food, medical, pharmaceutical, cosmetic, and other industries. Bromelain enzyme is applied for meat tenderization, baking cookies, beer clarification, grain protein solubilization, and protein hydrolysate production. Bromelain is also used as an enzymatic browning inhibitor in fresh apple slices. In cosmetic industries, bromelain is used as an active ingredient to elicit a gentle peeling effect. Also, bromelain can be used to heal acute inflammation and injuries. The bromelain enzyme can also treat many intestinal disorders as it acts as a digestive enzyme and can replace pepsin and trypsin in case of deficiency. In addition, bromelain can also improve the healing of firearm wounds [4]. According to [5], four main factors affecting enzyme activity include the concentration of enzyme, substrate concentration, pH, temperature, and the presence of inhibitors or activators. A study by [6] found that the highest bromelain activity of pineapple variety Phu-Lae of crown, core, and peel bromelain samples for pH and thermal stability was at pH 7 and 35°C temperature. Another research done by [3] found that the bromelain activity extract with crown and without crown decreased with time under refrigerated temperature conditions (5-8°C). Research on the effect of storage conditions on bromelain activity from other pineapple varieties, for example, pineapple variety Phu-Lae by [6] and pineapple variety Morris by [3], has been conducted. However, the research data on the effect of storage conditions on the bromelain enzyme activity of the Josapine pineapple peel variety with maturity indexes 2, 5, and 7 has yet to be reported.

Thus, this study aims to determine the effect of different storage conditions (room temperature, chilled, and frozen) on the bromelain activity of bromelain powder from pineapple peel variety Josapine during a storage period of one month.

EXPERIMENTAL

Preparation of Samples

The harvested pineapple Josapine at three different ripening stages (indices 2, 5, and 7) was purchased from Pasar Moden Seksyen 6, Shah Alam, Selangor, Malaysia. The pineapple peel with maturity index 2, 5, and 7 were cut into small pieces and crushed in a food processor (Model PB-3203L, Brand Pensonic, Country Malaysia) with the addition of water at a ratio of 1:1 to produce pineapple peel extract. Extraction was done at a chilled temperature (4°C). Next, the extract was filtered through a muslin cloth to remove the solid parts. The filtrate was collected and kept in a freezer (-20°C) for further experiments.

Production of Bromelain Powder

Purification of Bromelain Extract

Firstly, the crude bromelain extract was purified using the ammonium sulfate precipitation method. About 10 mL of crude bromelain extract was transferred into a centrifuge tube and placed in an ice bath. Then, about 6 g of ammonium sulfate was added pinch by pinch. Then, the sample is left for incubation for 1 hour until the appearance of precipitation is observed. Then, the incubated sample is centrifuged (Model 5420, Brand Kubota, Country Japan) at 3500 rpm for 30 mins. Then, the pellet was collected by dissolving

https://doi.org/10.24191/sl.v18i1.24113

in 10 mL Tris buffer while the supernatant was removed. The purified bromelain was stored in a chiller for further analysis.

Desalting

The dialysis process was performed using a diafiltrator machine. Firstly, about 1 L of deionized water was allowed to run into the diafiltration machine for washing. Then, before running the sample, the initial salinity of the purified extract was checked using a salinity meter. Then, the purified extract sample was allowed to run into the diafiltrator machine until the salinity percentage reached 0%. The purified extract sample was dialyzed using deionized water. After the salinity reached 0%, the dialyzed sample was stored in a freezer for further.

Freeze Drying

Finally, desalted bromelain was dried using a vacuum freeze dryer (Model Alpha 1-4 LD Plus, Brand Christ, Country Germany), which took four days to dry completely at -60°C. The desalted bromelain was frozen at -20°C before undergoing the freeze-drying process to provide a necessary condition for low-temperature drying.

Storage Study of Bromelain Powder

The freeze-dried bromelain powder with maturity indexes 2,5 and 7 were placed in three glass bottle containers, which were stored at three different temperatures (-20°C, 4°C, and room temperature). For one month, the bromelain activity was determined weekly (0, 7, 14, 21, and 28 days).

Bromelain Activity

Bromelain activity in the sample was determined using the casein digestion unit (CDU) method [7]. Approximately 5 ml of the casein substrate was first transferred into a test tube. The test tube was then immersed in a water bath at 37°C for 10 minutes. Next, about 5 mL of trichloroacetic acid (TCA) stopping reagent and 1 mL of the enzyme solution were added to the blank test tube, while only about 1 mL of the enzyme solution was added to the sample test tube. The test tubes were vortexed and placed back into the water bath at 37°C for 10 minutes. Subsequently, about 5 mL of the TCA stopping reagent was added to both the blank test tube and sample test tube, and they were vortexed again before being placed back into the water bath at 37°C for 30 minutes. Afterward, the blank and sample test tubes were removed from the water bath and left to cool to room temperature. The blank and sample were filtered using filter paper, and the filtrate was collected in a beaker. The absorbance was measured at 280 nm using a UV-visible spectrophotometer. The bromelain activity was calculated using the formula.

$$CDU/mg = [(Et – Eb)/Es] \times 50 \times (11/10) \times DF$$

Where;

DF: Dilution Factor
Et = Absorbance of the enzyme sample tube
Eb = Absorbance of the blank sample tube
Es = Absorbance of standard Tyrosine

Data Analysis

All experiments were performed in triplicate. The data obtained were analyzed using Statistical Package for the Social Sciences (SPSS) software. The analysis of variance (ANOVA) tests was performed.

https://doi.org/10.24191/sl.v18i1.24113

RESULTS AND DISCUSSION

Table 1 shows the storage study of pineapple Josapine. The stability of the bromelain enzyme during storage under different conditions was studied for one month. The following processes produced bromelain powder, including extraction, purification, dialysis, and freeze-drying. The bromelain enzyme activity was determined under storage conditions of room temperature (27°C), chilled temperature (4°C), and frozen temperature (-20°C). The sample used for the pineapple was the pineapple peel with three different maturity indices, including maturity index 2, 5, and 7. The results showed that, at room temperature, the highest bromelain activity at Day 0 was observed for maturity indices 5 (1955.80±1.32 CDU/mg), followed by maturity indices 7 (1636.46±0.93 CDU/mg) and maturity indices 2 (1499.20±17.54 CDU/mg). The same trend was observed for the other two storage conditions, chilled and frozen. For maturity index 2, at week 1, the highest bromelain activity was observed at frozen temperature (1487.57±14.96 CDU/mg), followed by chilled temperature (1369.06±1.47 CDU/mg) and room temperature (1154.70±5.33 CDU/mg) with the percent decrement of 0.78%, 8.68%, and 22.98%, respectively. The same trend was observed for maturity indices 5 and 7 with different bromelain activity and percent decrement values.

To compare, the lowest percent decrement of bromelain activity at week one was observed at maturity index 5 with frozen storage condition (0.55%). In comparison, the highest percent decrement was at maturity index 7 with room temperature storage condition (36.27%). The same trend was observed for other remaining storage periods of week 2, week 3, and week 4. Furthermore, the highest bromelain activity at one month (week 4) storage period was observed at maturity index 5 (1426.67±1.50 CDU/mg) while the lowest at maturity index 2 (123.25±0.17 CDU/mg) with a percent decrement of 27.05% and 91.78%, respectively. The lowest percent decrement was observed at maturity index two at frozen temperature with 10.34%. This value showed that the bromelain activity was mainly maintained for up to one month.

To compare, a previous study by [8] found a similar finding, where the enzyme activity of bromelain was retained about 75% during a storage period of 180 days. According to [3], the bromelain extract should be used as soon as possible, or the fresh extract should be used to obtain a higher specific activity of bromelain extract as the bromelain activity is reduced slightly during storage. Also, according to [2], the increase in protein content and decrease in bromelain activity during low-temperature storage of crude extract obtained from pineapple fruit was reported. So, using the fresh pineapple extract was suggested to obtain higher specific bromelain activity. According to [9], the bromelain enzyme was more stable at frozen temperature (-20°C) with higher enzymatic activity, losing only 5% of its activity at this storage temperature. Also, [9] reported that the bromelain stored in chilled conditions (4°C) was more unstable than those stored in frozen conditions. According to [10], the rate of a chemical reaction is slower at low temperatures, and the enzyme is inactive at the frozen temperature, but it will restore most of its enzyme activity when the temperature increases again. To relate, the enzyme activity is lower at room temperature than frozen during storage, showing that some of the enzyme has lost its activity.

There were several limitations of this study. Firstly, this study only aimed to utilize the pineapple variety from Josapine. Second, the sample was only taken from the peel part other than the crown or flesh of the pineapple variety Josapine. Third, the sample only focused on maturity indices 2, 5, and 7. It was suggested to use samples from other pineapple varieties, such as MD2 and N36, with different parts and maturity indices.

https://doi.org/10.24191/sl.v18i1.24113

Table 1. Storage study of pineapple Josapine.

	Day 0 (CDU/mg)	Week 1 (CDU/mg)	% decrement	Week 2 (CDU/mg)	% decrement	Week 3 (CDU/mg)	% decrement	Week 4 (CDU/mg)	% decrement
Index 2									
RT	1499.20±17.54^{aA}	1154.70±5.33^{bC}	22.98	304.77±0.79^{cC}	79.67	183.45±0.34^{dC}	87.76	123.25±0.17^{eC}	91.78
Chilled	1499.20±17.54^{aA}	1369.06±1.47^{bB}	8.68	1124.39±3.02^{cB}	25.00	989.46±1.04^{dB}	34.00	655.04±0.15^{eB}	56.31
Frozen	1499.20±17.54^{aA}	1487.57±14.96^{aA}	0.78	1463.35±17.57^{aA}	2.39	1370.71±0.99^{bA}	8.60	1344.23±1.62^{bA}	10.34
Index 5									
RT	1955.80±1.32^{aA}	1280.30±1.23^{bC}	34.54	427.56±0.81^{cC}	78.14	239.97±0.37^{dC}	87.73	191.73±0.14^{eC}	90.20
Chilled	1955.80±1.32^{aA}	1724.26±6.47^{bB}	11.84	1496.25±1.76^{cB}	23.50	1198.92±1.16^{dB}	38.70	852.03±0.77^{eB}	56.44
Frozen	1955.80±1.32^{aA}	1945.06±4.43^{bA}	0.55	1790.88±5.71^{cA}	8.43	1529.22±1.51^{dA}	21.81	1426.67±1.50^{eA}	27.05
Index 7									
RT	1636.46±0.93^{aA}	1042.97±0.62^{bC}	36.27	201.23±0.45^{cC}	87.70	186.98±0.21^{dC}	88.57	169.90±0.20^{eC}	89.62
Chilled	1636.46±0.93^{aA}	1491.35±2.53^{bB}	8.87	1239.49±4.31^{cB}	24.26	1059.82±2.05^{dB}	35.24	954.07±2.09^{eB}	41.70
Frozen	1636.46±0.93^{aA}	1618.53±1.09^{bA}	1.10	1400.00±1.47^{cA}	14.45	1132.60±1.07^{dA}	30.79	1046.02±1.75^{eA}	36.08

This means that each row and column with different superscripts are significantly different at $p < 0.05$.
Small letters indicate the effect of storage week on the bromelain activity.
Capital letters indicate the effect of storage condition on the bromelain activity.

* Corresponding author. *E-mail address*: sitiroha7796@uitm.edu.my

https://doi.org/10.24191/sl.v18i1.24113

CONCLUSION

The outcome of this study showed that the storage conditions have influenced the activity of bromelain powder. It is best to store the bromelain powder at frozen temperature because its activity could only lose 10.34% up to one month of storage time for maturity index 2. It has been found that frozen temperatures can prolong the shelf life of bromelain. This finding can be applied to the food and beverages and food processing industry in order to maintain the quality of bromelain enzymes for storage purposes.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Ministry of Higher Education, Malaysia, for funding this project under the Fundamental Research Grant Scheme [RACER/1/2019/WAB01/UITM/3].

AUTHOR'S CONTRIBUTION

Muhammad Yazid carried out the research and wrote and revised the article. Siti Roha and Siti Aimi anchored the review and revisions and approved the article submission.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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https://doi.org/10.24191/sl.v18i1.24113