International Journal of Innovative Research and Scientific Studies, 8(3) 2025, pages: 1312-1321

IJIRSS
International Journal of Innovative Research & Scientific Studies

ISSN: 2617-6548

URL: www.ijirss.com

Performance evaluation of bromelain extraction from pineapple rhizome using in-house freeze dryer

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Abstract

This study introduces an innovative approach for extracting bromelain from pineapple rhizomes using a custom-designed in-house freeze dryer. Equipped with a six-tray layout, the system efficiently processes up to 3 kg of wet bromelain. We employed an air-blast mechanism for rapid initial freezing, followed by a vacuum freeze-drying process, with a key innovation being the deactivation of the cooling fan post-freezing to facilitate efficient ice sublimation. Throughout the entire drying process, the operational temperature and pressure conditions within the freeze dryer's refrigeration system were thoroughly recorded and presented, a crucial step for monitoring and verifying the machine's performance and efficiency. The freeze-drying procedure encompassed three distinct phases: rapid initial freezing, primary drying with pressure reduction and gradual heating, and secondary drying for further moisture removal via increased temperature. Comparative analysis with lab-scale dryers demonstrated that the bromelain extracted using our in-house system retained enzymatic activity levels comparable to conventional methods, validating the efficacy of our machine for consistent, high-quality extraction. This study confirms the effectiveness of our innovative freeze-drying method and provides valuable insights into operational parameters like temperature progression and power consumption, significantly contributing to the broader understanding of freeze-drying techniques in enzyme extraction.

Keywords: Bromelain, desorption, freeze-drying, in-house freeze dryer, pineapple rhizome, sublimation.

DOI: 10.53894/ijirss.v8i3.6792
Funding: This study received no specific financial support.
History: Received: 7 March 2025 / **Revised:** 8 April 2025 / **Accepted:** 14 April 2025 / **Published:** 7 May 2025

Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.
Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.
Acknowledgements: We express our sincere thanks to Rajamangala University of Technology Krungthep and the National Research Council of Thailand for their financial backing. We also thank Weltech Biotechnology Co., Ltd. for supplying experimental materials and results. Additionally, we acknowledge partial support from Burapha University. The authors would like to thank Enago (www.enago.com) for the English language review.
Publisher: Innovative Research Publishing

1. Introduction

Freeze-drying or lyophilization is a vital preservation method, extensively utilized in the food and pharmaceutical industries. Freeze-drying protects the physical and chemical characteristics of products, and it plays a critical role in extending the shelf life of pharmaceutical products and enhancing the quality and stability of food products. The process involves the removal of water from a frozen product under low temperature and vacuum conditions, which helps maintain the product's structural integrity, taste, and biochemical properties [1-5].

The freeze-drying process involves three primary stages, i.e., (1) rapid cooling (freezing), which transforms the water content into ice and determines the ice crystal size essential to minimize cellular structure damage; (2) primary drying (sublimation), where heat applied under reduced pressure removes most of the water content, which requires precise control to preserve product structure; and (3) secondary drying (desorption), where the maximum amount of water is removed without damaging the product by increasing temperature and reducing pressure. This meticulous process reduces the product's weight and volume, and it ensures stability, facilitates easy transportation and storage, and is recognized for its efficacy in preserving the integrity of delicate biological materials [6-9].

Freeze-drying can retain the stability of sensitive substances that are prone to degradation or damage when exposed to conventional drying methods involving heat or pressure. This approach has empowered researchers and industries to tackle the challenges associated with the preservation and transportation of temperature-sensitive materials, ensuring the retention of their original characteristics. Additionally, operational factors, e.g., energy efficiency, processing time, cost-effectiveness, and versatility, are pivotal in optimizing the equipment's performance; thus, freeze-drying is the method of choice in various fields.

However, despite its benefits, the considerable initial investment in freeze-drying equipment is a challenge, especially for small-scale, in-house production environments. In this paper, we investigate the possibility of reducing freeze-drying time by enhancing the heat transfer process to ensure that the temperature of the product remains below its critical collapse temperature. In addition, we compare the cost-effectiveness of in-house freeze dryers with commercial alternatives while considering the relevant expenses in terms of supplies and electricity. Data indicate a cost advantage when using in-house solutions, with commercial freeze dryers exhibiting up to 85% higher markup in comparison [10-13]. With numerous freeze dryers available on the market, making an informed decision about the appropriate equipment for initial, small-scale in-house production becomes crucial. Thus, this study attempts to evaluate the efficiency of in-house freeze dryers systematically using bromelain powder production as a case study to provide clear guidance and insight for potential users.

2. Materials and Methods

To conduct a comprehensive and precise evaluation of the freeze-drying process, particularly when employing an in-house freeze dryer, it is essential to understand the relevant equipment and methodologies. Here, the freeze dryer has been scrutinized in terms of its design, components, and operational parameters, which was done to ensure that all aspects of the freeze-drying process are captured accurately and can be correlated with the observed outcomes. Additionally, understanding the equipment specifications and operational methodology is essential to assess the efficiency and cost-effectiveness of using an in-house freeze dryer rather than commercially available alternatives.

2.1. Freeze Dryer

2.1.1. Equipment

Three-dimensional models of the freeze dryer utilized in this study are shown in Fig. 1 to highlight its design and various components [14-16]. The machine primarily comprises two crucial systems, namely, the vapor-compression refrigeration and water cooling systems. A flow diagram of these systems is shown in Figure 2.

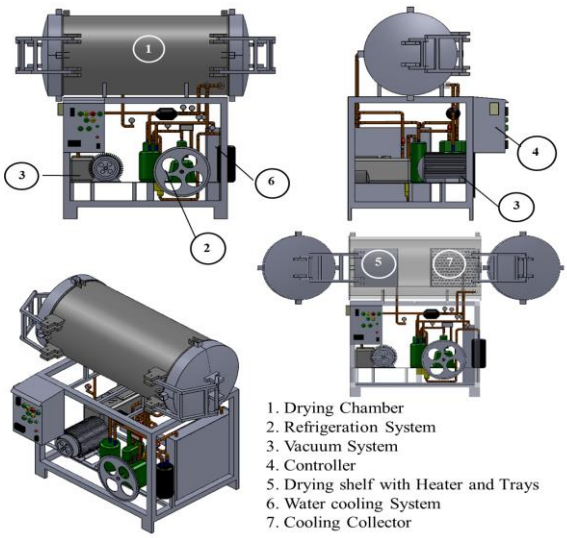

Figure 1.
In-house freeze dryer with the vapor-compression refrigeration and water cooling systems.

Table 1.
Freeze dryer specifications.

Component	Details/specifications
Refrigeration system	Refrigerant: R-404A
Compressor	3.5 kW of cooling capacity on −40°C evaporator unit and 40°C condenser unit, three-phase (open reciprocating)
Condenser	4.82 kW of heat rejection (water cooling)
Expansion valve	3.5 kW (Sporlan, Model CG-032) thermostatic, thermostatic charges available −18°C to −40°C
Evaporator	Cooling capacity 3.75 kW, Ø 15 mm of tubing (50 × 50 mm aligned parallel), 10 mm fin spacing, 10 m^2
Cooling fan	1/3 hp, single phase, 1450 rpm
Tray	250 × 400 × 20 mm^3 (SS-304)
Receiver tank	3.5 kW, Ø 10 mm of tubing
Vacuum pump	2.0 × 10^{-2} mbar (1.5 × 10^{-2} Torr) of ultimate pressure (Total), 3.3 m^3/h of Peak pumping speed (EDWARDS Model RV3 rotary vane pumps)
Water cooling system	Refrigerant: R-134a
Compressor	12000 BTU/h on −20°C evaporator unit, 40°C condenser unit, single phase (rotary type)
Condenser	3.8 kW of heat rejection (air cooling)
Expansion valve	12000 BTU/h (thermostatic expansion valve) −20°C medium temperature
Evaporator	Cooling capacity 12000 BTU/h, Ø 10 mm of tubing (30 × 50 mm^2 Wort chiller)

Table 1 shows the specifications of the various components of the refrigeration and water cooling systems. The refrigeration system comprises a wide range of parts, including a compressor, condenser, expansion valve, evaporator, cooling fan, trays, a receiver tank, and a vacuum pump, each of which is essential to achieve effective and efficient freeze-drying. The water cooling system shares a similar component structure; however, the specifications vary, as shown in Table 1. Collectively, these systems operate harmoniously to ensure a smooth freeze-drying process.

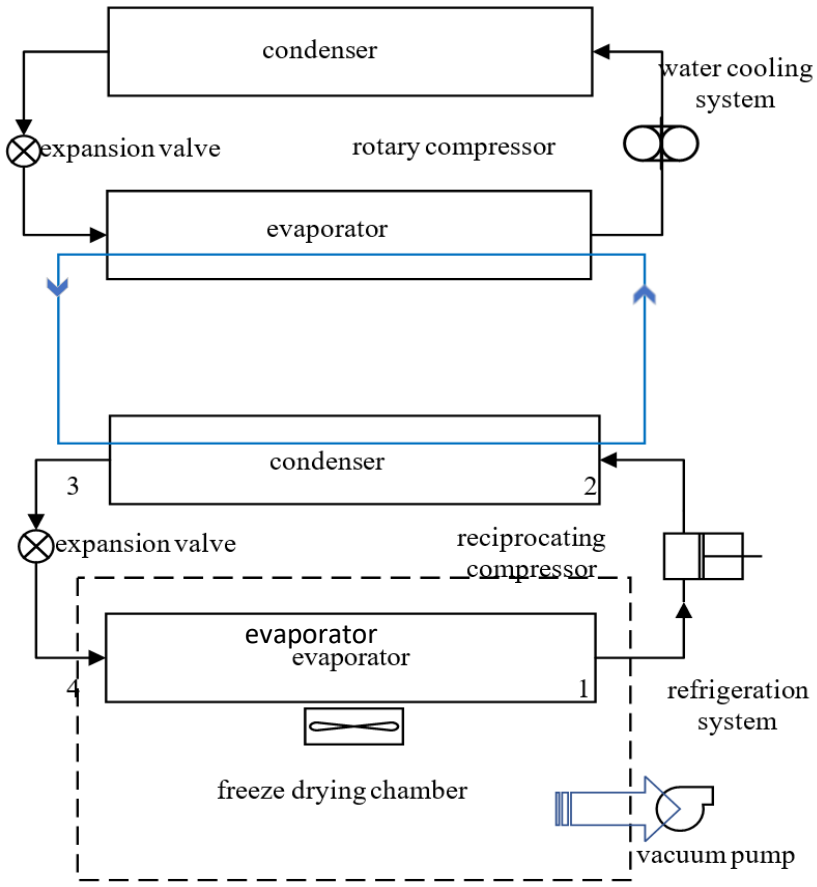

Figure 2.
Flow diagram of the refrigeration and water-cooling systems.

2.1.2. Processes and Procedures

The freeze dryer is designed to work with both freezing (the air blast freezer type) and freeze drying (tray method freeze dryer with heating plates type). The air blast freezing process (Figure 3 (a)): after turning on the cooling fan, air in the drying chamber is circulated, the temperature of the sample solutions is dropped to -20°C and the temperature of the evaporator (cold trap) is dropped to a little lower than -40°C. The freeze drying process (Figure 3 (b)): then shutting off the cooling fan and starting the vacuum pump, the temperature of the cold trap is dropped to about -40°C. The molecules of the water in the sample solutions start to sublimate and condense at the cold trap.

The freeze-drying operation is performed in two primary stages, i.e., initial freezing, air blasting of the product and the subsequent freeze-drying process. The equipment is calibrated to function within a temperature range of −20°C to 40°C, measured via thermocouple (Type T) and data logger (Yokogawa model MV2000), thereby facilitating conversion of the product into powder form. The entire process is completed in approximately 30 hours.

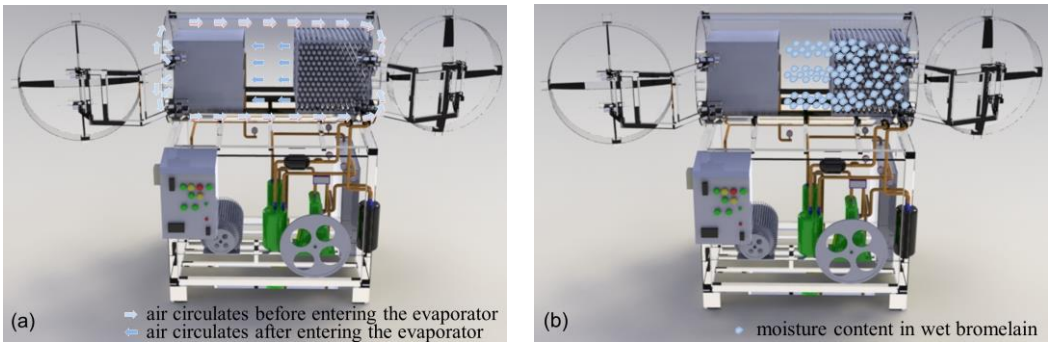

Figure 3.
Freeze dryer consist of two processes: (a) the air blast freezing process and (b) the freeze drying process.

2.2. Bromelain Extraction Process from Pineapple Rhizome

The bromelain purification process was executed using column chromatography. Here, ion-exchange chromatography was used to remove all impurities. Precipitation of wet bromelain was achieved by adding methanol, followed by utilization of the freeze dryer to dry the bromelain, which was then ground into powder form [17-20].

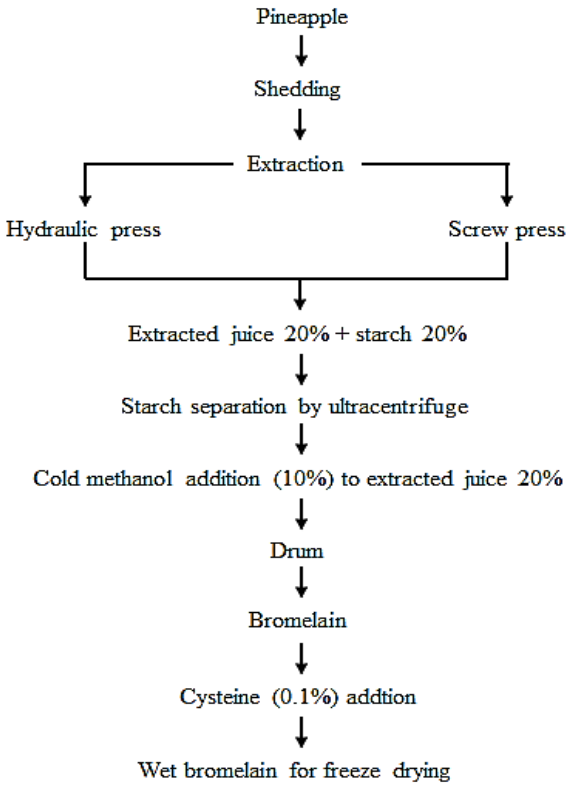

Figure 4.
Crude enzyme process for bromelain extraction.

The bromelain extraction from pineapple rhizome results in a crude enzyme, which is suitable for utilization in the food and cosmetic industries. The crude enzyme production workflow for this extraction process is illustrated in Figure 4. The

procedure begins by shredding the pineapple. Then, two primary methods are employed for extraction, i.e., the hydraulic press and the screw press. Once the juice is extracted using either method, a combination of extracted juice (20%) and starch (20%) is obtained. The starch is then separated from the juice through ultracentrifugation. After separating the starch, cold methanol is added to the extracted juice at a proportion of 10%. The juice is then placed in a drum, resulting in the formation of bromelain. Finally, a 0.1% cysteine addition is added to the wet bromelain for freeze drying. Notably, the properties of wet bromelain can vary depending on the harvest season. These seasonal variations can influence the enzymatic activity and potency of bromelain, affecting its suitability and effectiveness in various applications.

The sample used for the freeze-drying process, i.e., the wet extracted bromelain (Figure 5), was sourced from Weltech Biotechnology Co., Ltd. (WB). The sample was then freeze-dried using our specialized freeze dryer.

Figure 5.
Wet bromelain extracted via crude enzyme process.

2.3. Experimental methods

A total of 3 kg of wet bromelain were distributed across six trays arranged on three shelves in the freeze-drying chamber for three separate batches. Here, the bromelain was frozen in a 5-mm layer. A cooling fan inside the chamber distributed cold air during the freezing process, beginning at an initial temperature of 5°C ± 1°C and ending at −20°C ± 1°C. The freeze-drying phase was initiated with deactivation of the cooling fan, and the fan resumed operation after the freeze-drying phase was complete. The vacuum pump facilitated continuous production of bromelain, and upon reaching a steady state nearly absolute zero pressure, the heating plate was activated. Freeze drying proceeded from an initial temperature of −20°C ± 1°C to a final temperature of 40°C ± 1°C.

2.4. Engineering Analysis

*Coefficient of Performance (COP)***:** The R–404A pressure-enthalpy (p-h) diagram of the refrigeration operational cycle, as presented in Figure 2, is illustrated in Figure 6 via measured pressure and temperature. Points 1 – 4 of the cycle are measured for temperature with the type T thermocouple via data logger and pressure with the bourdon pressure. To evaluate the performance of the process the coefficient of performance (COP) was utilized. The COP quantifies efficiency across both the cooling and heating operations. The COP for the cooling (COP_C) and heating (COP_H) processes are expressed as follows.

$$COP_C = \frac{\dot{Q}_L}{\dot{W}} \quad (1)$$

$$COP_H = \frac{\dot{Q}_H}{\dot{W}} \quad (2)$$

Here, $\dot{Q}_L$ and $\dot{Q}_H$ are the amount of exchanged heat (kW) measured at the evaporator and condenser, respectively. These parameters can be calculated as follows.

$$\dot{Q}_L = \dot{m}\left(h_1 - h_4\right) \quad (3)$$

$$\dot{Q}_H = \dot{m}\left(h_2 - h_3\right) \quad (4)$$

Here, $\dot{m}$ denotes the refrigerant flow rate (kg/s), h_1, h_2, h_3, and h_4 represent the enthalpy at each state on the p-h diagram, and $\dot{W}$, i.e., the required power (kW) at the compressor, is calculated as follows.

$$\dot{W} = \sqrt{3}\, V\, I \cos\phi \quad (5)$$

Here, V is voltage (V), I is the electrical current (A), and $\cos\phi$ is the power factor or energy efficiency [8, 21].

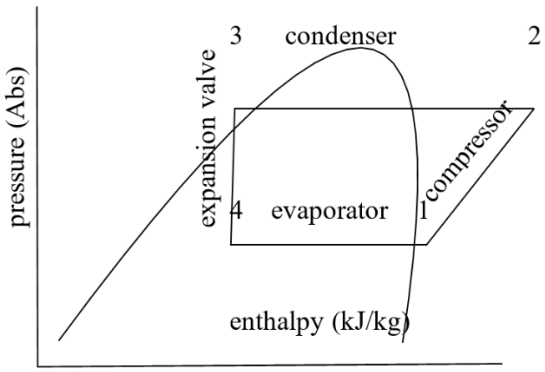

Figure 6.
P-h diagram of the refrigeration cycle in a freeze dryer.

3. Results and Discussion

3.1. Temperature Dynamics and Energy Efficiency in Bromelain Freeze Drying Process

The temperature progression of the bromelain throughout the freeze-drying process is shown in Figure 7, where the x-axis is the processing time (in hours), and the y-axis is the temperature of the bromelain (°C). This progression is categorized into three distinct phases, i.e., freezing, primary drying, and secondary drying. The initial freezing phase exhibits a rapid reduction in the bromelain's temperature, where the substance is chilled rapidly to temperatures significantly less than the freezing point of water (typically in the range of −20°C to −30°C). Here, the vertical bars indicate the variation or uncertainty in the temperature measurements. Using different techniques, the primary and secondary drying processes attempt to eliminate moisture from the material.

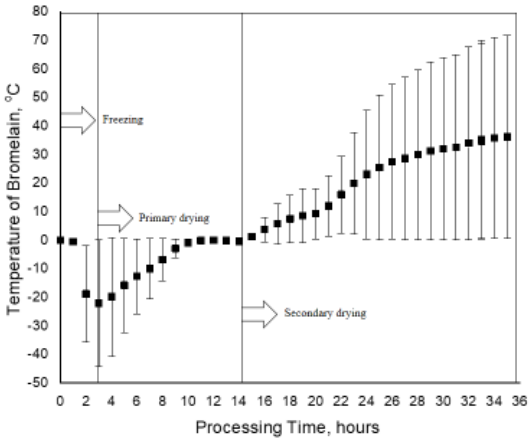

Figure 7.
Correlation between temperature and time for the freeze-drying processes.

The primary drying phase involves a reduction in pressure to almost -1.0×10^3 mbar (vacuum pressure) coupled with a slight introduction of heat, which allows the frozen water content in the bromelain to sublimate (changing from solid to gas).

In this phase, there is a gradual increase in temperature over time. The primary drying phase continues for 11 hours until the temperature stabilizes to slightly above zero.

Note that water molecules may remain attached to the material; thus, the secondary drying phase addresses this issue by increasing the temperature while maintaining this vacuum pressure. This phase involves a more pronounced temperature increase over an extended period. Here, the temperature is increased to between 30°C to 70°C by the end of the processing time. This increase in temperature breaks the molecular attachments binding the water to the bromelain, which facilitates effective extraction. Despite the extended duration of the secondary phase, it is critical to the freeze-drying technique. In addition, research has identified the average rates of freezing, primary drying, and secondary drying as −0.253 °C, 0.137°C, and 0.682°C/min, respectively.

Table 2 shows the temperature, pressure, power consumption, and energy parameters of the refrigeration system during the freeze-drying process. Note that these parameters are interdependent. The parameters, designated by numbers 1, 2, 3, and 4, correspond to positions on the p-h diagram, and E denotes the energy consumption of the freeze dryer and water-cooling systems.

Table 2.
Parameters obtained from refrigeration system during freeze drying.

Description		Freeze-drying stage		
		Freezing	**Primary drying**	**Secondary drying**
$T_{@P-h diagram}$ (°C)	1	21.80 ± 3.19	29.74 ± 1.56	29.43 ± 2.13
	2	78.33 ± 9.38	66.65 ± 4.98	69.35 ± 3.76
	3	41.45 ± 2.91	37.28 ± 3.53	39.88 ± 1.82
	4	−36.58 ± 0.91	−37.42 ± 0.27	−35.67 ± 1.34
$P_{@P-h diagram}$ (mPa)	1	13.75 ± 1.26	13.18 ± 4.21	13.37 ± 2.67
	2	169.75 ± 8.72	157.73 ± 7.62	160.21 ± 7.96
	3	188.00 ± 9.27	173.45 ± 8.68	175.00 ± 6.93
	4	0.28 ± 0.36	3.11 ± 1.47	1.79 ± 1.44
$E_{@freeze dryer}$ (Wh)		4141.05 ± 29.40	3860.45 ± 76.35	3891.00 ± 61.98
$E_{@water cooling}$ (Wh)		1329.35 ± 3.30	1334.60 ± 12.98	1340.61 ± 20.19
$\dot{Q}_L$ (kJ/s)		7.81 ± 2.07	13.80 ± 8.12	10.59 ± 1.86
$\dot{Q}_H$ (kJ/s)		9.79 ± 2.08	15.34 ± 7.13	12.36 ± 1.87

Figure 8 shows the COP profile during the overall freeze-drying process. The average COP_C values for batches 1, 2, and 3 are 4.21 ± 0.48, 4.14 ± 0.55, and 4.12 ± 0.46, and the average COP_H values are 5.18 ± 0.46, 5.15 ± 0.54, and 5.0 ± 0.46, respectively. The COP_C values are less than the COP_H values because the compressor's efforts are primarily applied during the heating phase. The higher efficiency of the 'cooler is attributed to the larger heat quantity, which can be extracted from the refrigerator's interior for a given work amount. Note that the validity of the first law of thermodynamics is upheld in this case ($\dot{Q}_L + \dot{W} = \dot{Q}_H$) [8, 21, 22].

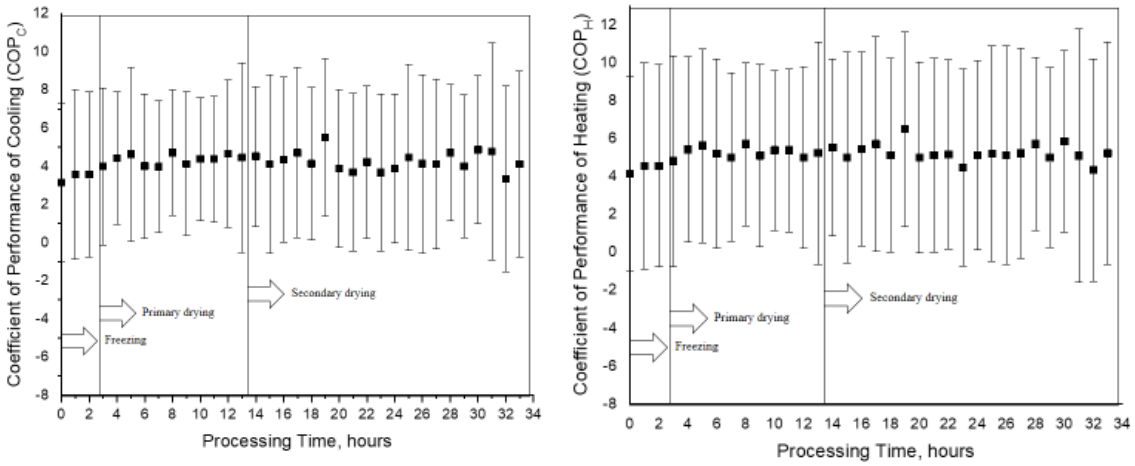

Figure 8.
Evolution of coefficient of performance during cooling and heating operations.

Machine performance is directly linked to its energy efficiency. Continuous power measurements indicate that the energy consumption of the compressor for batches 1, 2, and 3 are 5,224.46 ± 93.62, 5,075.17 ± 142.74, and 5,132.72 ± 166.75 kWh, respectively. This power is primarily sourced from the compressor that operates in both the refrigeration and water cooling systems, with a portion also coming from the heater. The effectiveness of the cooler is determined by multiple elements, e.g., COP_C , the temperatures during evaporation and condensation, and the power consumption. Fig. 8 shows the COP_H values. Note that an in-house research-scale freeze dryer may offer advantages over traditional industrial dryers. The best refrigeration cycle extracts the maximum heat from the refrigerator's interior while using minimal mechanical or electrical energy. The COP_C values provide a measure of the heat extraction capability of the cooler, and it is particularly sensitive to the external temperature and required internal temperature [8, 21, 22].

3.2 Analysis of Bromelain Enzyme Activity

This section presents a comparative analysis of bromelain enzyme activity levels extracted using lab-scale and in-house scale freeze dryers 3. The results indicate a consistent pattern between the two methods. Table 3 compares the enzyme activity levels of bromelain sourced from WB and our in-house results from 3 different harvested batches. As can be seen, the enzyme activity levels of both machines are in good agreement. For example, the April harvest demonstrated lower enzyme activity compared to the January batch. Furthermore, the enzyme activity of batch 1 surpassed that of the other batches. This discrepancy can be attributed to the immediate freeze-drying of batch 2 post-extraction, and the samples from batches 1 and 3 were subjected to extended waiting times prior to drying. Based on these findings, we recommended to either commence the freeze-drying process promptly after bromelain extraction or store the extract at temperatures below −80°C. Conversely, bromelain extracts can be preserved at −20°C for a maximum of two days.

Table 3.
Comparison of enzyme activities.

Batch	Yield of bromelain extracted from pineapple rhizome (%)	Enzyme activity (CDU/mg)		
		WB	In-house	Difference (%)
1	16.03	465.80	476.51	+2.30
2	16.52	575.24	578.68	+0.59
3	14.99	576.37	576.99	+0.10

Note: Batch 1's final weight was not recorded because the sample was immediately sent to WB for enzyme activity analysis.

In this comprehensive analysis, we evaluated the bromelain production process using an in-house freeze dryer, and we compared our results with those obtained from Weltech Biotechnology Co., Ltd. Our findings indicate that the essential properties and enzyme activities of the bromelain extracted via both methodologies exhibited minimal differences. The findings highlight the crucial influence of the crop's harvesting season on enzyme activity levels. In addition, the findings of this study will serve as a valuable reference for evaluating the performance of our in-house freeze dryer, thereby providing a significant contribution to future research and the development of similar technologies.

4. Conclusion

By investigating the freeze-drying process of bromelain, this study thoroughly tracked the temperature changes, emphasizing the critical importance of each phase of the process. The initial rapid drop in temperature during the freezing phase is vital to maintaining the structural integrity of the bromelain. The subsequent primary and secondary drying phases

are essential for moisture removal, ensuring the stability and effectiveness of the acquired product. Our findings also shed light on the complex interactions between the various parameters of the refrigeration system, underscoring the need for precise control to achieve optimal efficiency and product quality. The comparative analysis between different scale freeze dryers revealed similar results in terms of enzyme activity levels, emphasizing the need for either immediate processing or appropriate storage to preserve the enzymatic activity of bromelain. Overall, this study has provided crucial insights, facilitating the refinement of freeze-drying parameters, which are critical for enzyme preservation and biomedical applications.

The designed in-house freeze dryer, via both freezing and drying processes, can be used in continuous production. The main advantage of this machine is that it can be utilized for both freezing and drying processes. It can be used in a cooling step in the freezing process and can also be used in a cold trapping step in the freeze-drying process. Thus, the developed machine can continuously produce bromelain-extracted freeze-dried products. With this machine, the produced bromelain-extracted freeze-dried products also have similar properties to bromelain extracted. The machine could be developed for other types of freeze-drying industries, such as medicine and food.

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