

Cost-effective drying methods for edible bird's nest hydrolysate: Impact on nitrite levels and sialic acid content

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Abstract. To produce powdered edible bird's nest hydrolysate (EBNH), a significant amount of water must be removed after hydrolysis to improve the product's stability, extend shelf life, and reduce transportation and storage costs. This study investigates the application of cool-air and hot-air drying methods, with a particular focus on strategies suited for small and medium-sized enterprises (SMEs). Cool-air drying was performed at 17 °C using a fan and air conditioner, while hot-air drying was conducted at 55 °C and 65 °C using a dehydrator. The study evaluated drying time, as well as the quality, moisture content, nitrite levels, total sialic acid content, and antioxidant activity of EBNH produced by each method. The quality, cost, and feasibility of these methods were compared with freeze-drying and spray-drying techniques from previous studies. Results showed that higher temperatures and lower relative humidity improved drying efficiency, with hot-air drying at 65 °C requiring the shortest drying time. Chemical analysis revealed significant differences in nitrite and total sialic acid content: cold-air-dried samples had higher nitrite levels, while hot-air drying at 65 °C produced the highest total sialic acid content. Cost and feasibility assessments demonstrated that hot air drying at 65 °C was the most efficient and cost-effective method. This method is especially advantageous for SMEs due to its balance of efficiency, affordability, and ease of implementation, making it ideal for smaller-scale operations.

Keywords: edible bird's nest, hydrolysate, drying, total sialic acid, feasibility analysis

INTRODUCTION

Edible bird's nest (EBN) is a traditional Chinese delicacy that has received worldwide attention in recent years (Jamalluddin *et al.*, 2019). It is the solidified saliva of the Swiftlets, usually produced and consumed as food and as traditional medicine in South Asian countries (Acharya and Satheesh, 2023). In the past few years, the investigations have revealed that birds' nests possess the antibacterial, antiviral, and immunomodulation effects that could be applied for cancer, regeneration of tissues, cardiometabolic disorders, and neuroprotection (Chok *et al.*, 2021; Permatasari *et al.*, 2023). Bird's nests are harvested by the farmer and sold as fresh, uncleaned raw bird's nests commonly referred to as raw unclean

(RUC) bird's nests or as raw clean bird's nests known as RC bird's nests where they undergo through a cleaning process. The method used in cleaning the bird's nests is through rinsing the bird's nests in water until they get wet, soft, and the tightly bound bird nest threads get partly relaxed. Finer and smaller feathers are picked out by using the tweezer and the cleaned feathers are again arranged and shaped in different form, air-dried then packed and marketed across the globe (Babji *et al.*, 2015).

EBN's principal target market consists of Chinese communities worldwide, particularly in China, Taiwan, Singapore, and North America. However, new emerging markets have also been identified in Japan and South Korea, where there is growing interest in EBN hydrolysates (EBNH).

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These emerging markets place greater emphasis on EBN hydrolysates compared to the traditional forms of EBN. EBN hydrolysates are prepared through an enzymatic process, where RC EBN undergoes an initial water treatment followed by enzymatic hydrolysis (Yeo, Wong, Tan, Rukayadi, *et al.*, 2023). This process breaks down proteins into smaller peptides and amino acids, enhancing the product's bioactivity, solubility, and nutritional value (Unal *et al.*, 2024). EBN, a traditional Chinese delicacy, has historically been consumed for its flavour, texture, and medicinal properties, especially in soups and desserts. It has also been recognized for its role in traditional medicine across South Asia, notably for improving respiratory problems, strengthening the immune system, moisturizing the lungs, resolving phlegm, and alleviating coughing (Chua and Zukefli, 2016; Ramachandran *et al.*, 2018). However, recent advancements have expanded its applications beyond traditional uses. Modern enzymatic hydrolysis processes have enhanced the bioactivity, solubility, and nutritional value of EBN, leading to the development of EBNH. These innovations have paved the way for EBNH to be incorporated into functional foods, dietary supplements, and pharmaceutical formulations. Despite this progress, small and medium-sized enterprises (SMEs) face significant challenges in adapting to these modern processes, particularly in drying EBNH to a powder form, which is critical for extending shelf life and reducing transportation costs. The transition from traditional to modern production methods highlights the need for scalable, cost-effective solutions tailored to the unique constraints of SMEs.

There are many challenges in producing EBNH that affect the efficiency, quality, and economic feasibility of the process. An important challenge is the drying technique used after hydrolysis. The hydrolysate must be dried from liquid to powder to extend its shelf life and facilitate storage and transportation. Freeze-drying (also known as lyophilization), and spray-drying are two common drying techniques, each with distinct advantages and limitations. Freeze-drying is a process in which water is sublimated by the direct transition of water from solid (ice) to vapor, thus omitting the liquid state, and then desorbing water from the “dry” layer (Nowak and

Jakubczyk, 2020). This method involves freezing the product and then reducing the surrounding pressure to allow the frozen water to sublime directly from ice to vapor. The freeze-drying process is known for its ability to preserve the structure and nutritional content of the product. However, it is often cost-prohibitive due to high energy requirements and equipment costs. Spray-drying, on the other hand, is the process of converting a solution into dried powder in a single step by passing an atomized spray through a high-temperature gaseous medium (Ziaee *et al.*, 2019). This method atomizes the liquid into fine droplets and rapidly dries them with hot air, producing a powder. While spray-drying is faster and less expensive than freeze-drying, it can compromise the quality of heat-sensitive compounds.

In a study conducted by Du *et al.* (2022), salted duck egg white was used to investigate the effects of three drying methods—freeze-drying, vacuum drying, and spray drying—on the physicochemical and nutritional properties of protein powder. The results showed that freeze-dried protein peptide samples had significantly higher solubility than those that were vacuum- or spray-dried. Freeze-drying also had the least effect on the microstructure of protein and peptide powder, with the freeze-dried samples exhibiting greater antioxidant capacity than unhydrolyzed salted egg white (Du *et al.*, 2022). Similarly, Chen *et al.* (2022) investigated differences in tissue structure and protein physicochemical properties of golden pompano fish under various drying conditions, including freeze-drying, hot air-drying, and heat pump-drying. The study found that freeze-drying caused the least harm to fish tissue compared to other drying methods, preserving the fibrous structure and smooth surface, and maintaining more concentrated protein bands (Chen *et al.*, 2022). Thus, drying methods affected the physicochemical and nutritional properties of food.

Both freeze-drying and spray-drying are expensive and energy-intensive processes, posing challenges for producers, particularly small and medium-sized enterprises (SMEs) that may lack access to advanced technology (Gan *et al.*, 2020). Given these limitations, it is essential to explore alternative drying methods that can maintain the quality of EBNH while being cost-effective and accessible to a broader range of producers. In this study, various drying techniques for EBNH were

compared highlighting their effects on the nutritional and functional properties of the final product. In contrast, air drying methods, including cool air and hot airdrying, present more economical alternatives for SMEs involved in EBN processing.

The efficacy of cost-effective drying methods (cool air and hot airdrying) was obtained by comparing their drying efficiency and the quality of the resulting EBNH powder. Key parameters such as moisture content, nitrite levels, total sialic acid content, and antioxidant activity will be analysed to determine effective drying times and assess the overall feasibility of these methods for SMEs. Additionally, these parameters will be compared with those obtained from previous studies on spray drying and freeze-drying methods. A cost analysis and feasibility study of these different drying methods will also be conducted, highlighting the potential benefits and limitations SMEs. The findings are expected to provide insights into alternative drying techniques that enhance the quality and commercial viability of EBNH products, thereby supporting the sustainability of SMEs in the industry. By addressing the need for cost-effective solutions, this research contributes to the ongoing optimization of EBN processing and its market potential.

MATERIALS AND METHODS

Materials

All laboratory analyses were conducted using analytical grade chemicals. Phenol, N-acetylneuraminic acid, resorcinol, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were sourced from Sigma-Aldrich (USA), while 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, and methanol were obtained from Thermo Fisher Scientific (USA). All other reagents and solvents used were of analytical grade.

Preparation of EBN hydrolysate samples

Enzymatic hydrolysis process

The raw material used in this study was the EBN by-product (Figure 1A) provided by Think Birdnest Sdn. Bhd (Segamat, Malaysia). These by-

products are fragments of EBN containing tiny feathers. During the primary processing quality control step, EBN fragments with attached feathers were separated from the RC EBN to enhance cleanliness before sterilization and packaging. These fragments called as EBN by-product used in this study.

The crushed EBN by-product was thoroughly washed and soaked for five minutes. The soaked EBN was then placed in a clean pot and subjected to double boiling until the temperature reached approximately 95 °C. This process continued for one hour. Afterward, the pot was removed from the heat and allowed to cool to 50 °C. Bromelain, constituting 0.8% of the dry weight of EBN, was added to the pot. The mixture was intermittently heated, maintaining the temperature between 48-55 °C for two hours. Subsequently, the sample underwent another round of double boiling for thirty minutes to deactivate the bromelain, maintaining a temperature range of 85-90 °C. The pot was then removed and cooled for ten minutes. The resulting liquid hydrolysate sample was filtered through an 80-mesh sieve to remove impurities (EBN hydrolysate as shown in Figure 1B and transferred into several clean containers for the subsequent drying process. The steps were adapted from a study by (Yeo, Wong, Tan, Rukayadi, *et al.*, 2023).

Drying process

This study employed an innovative approach to drying the liquid EBN hydrolysate samples obtained through enzymatic hydrolysis. Two different drying methods were utilized: hot air and cool air-drying. For the cool air-drying method, a stand fan and an air conditioner set to 17 °C were used, following the methodology described by Yeo *et al.* (2023). For the hot air-drying method, samples were placed in a hot air dehydrator (Excalibur 9 tray dehydrator, USA) with temperatures set at 55 °C and 65 °C, representing new drying techniques for EBN hydrolysate. Each method was applied to two distinct initial sample volumes: one derived from 200 mL and the other from 70 mL of liquid hydrolysate. This differentiation in sample volume was strategically chosen to investigate whether the initial volume impacts the drying efficiency and the final quality of the hydrolysate. By comparing these volumes, we aimed to determine not only the optimal

drying technique but also to assess how volume variations might affect product quality. This approach allows us to provide more nuanced recommendations for EBN processing, tailored to different production scales and requirements. Throughout the drying process, the temperature (°C) and relative humidity (RH, %) were monitored and recorded every hour. The dried EBN hydrolysate products are shown in Figure 1C.

Moisture content analysis

The moisture content (MC) analysis of the EBN sample was conducted using the oven drying method. EBNH sample was accurately weighed and placed in a drying oven (Binder FD 115, Tuttlingen, Germany). The sample was dried at 105 °C for a minimum duration of 24 hours or until no further changes in weight were observed, following the procedure outlined by (Tan *et al.*, 2020). The weight of the sample was recorded before (*w*) and after drying (*d*), with meticulous documentation of any changes.

The moisture content was determined using the following formula:

$$MC(\%) = \frac{w - d}{w} * 100$$

where *w* is the initial wet weight of the sample, and *d* is the final dry weight of the sample. This calculation provided the moisture content as a percentage of the initial wet weight, enabling the assessment of water content in the EBN sample.

Nitrite content analysis

The nitrite content of the EBNH samples was determined using the Aquaquant® 1.14424 NO₂-method. For pre-treatment, 0.5 g of EBN was combined with 25 mL of ultrapure water in a 1:50 ratio and soaked for 1 hour at room temperature. The sample was then incubated at 80 °C for 30 minutes (Quek *et al.*, 2018). After cooling to room temperature, the EBN mixture was centrifuged at 8000 rpm for 5 minutes. The resulting supernatant was collected for analysis.

To analyse the nitrite content, 5 mL of the supernatant was mixed with one level blue micro spoon of ready-to-use nitrite reagent (Cat. No. 1.14776.0001, Merck Millipore). The mixture was vigorously shaken to dissolve the reagent and then allowed to sit at room temperature for 10 minutes. Following this incubation, the mixture was centrifuged again at 8000 rpm for 10 minutes. The absorbance of the sample mixture was measured at 543 nm using a visible spectrophotometer (SCILOGEX SCI-V1000, USA). Nitrite standard solution was used as the analytical standard for this study.

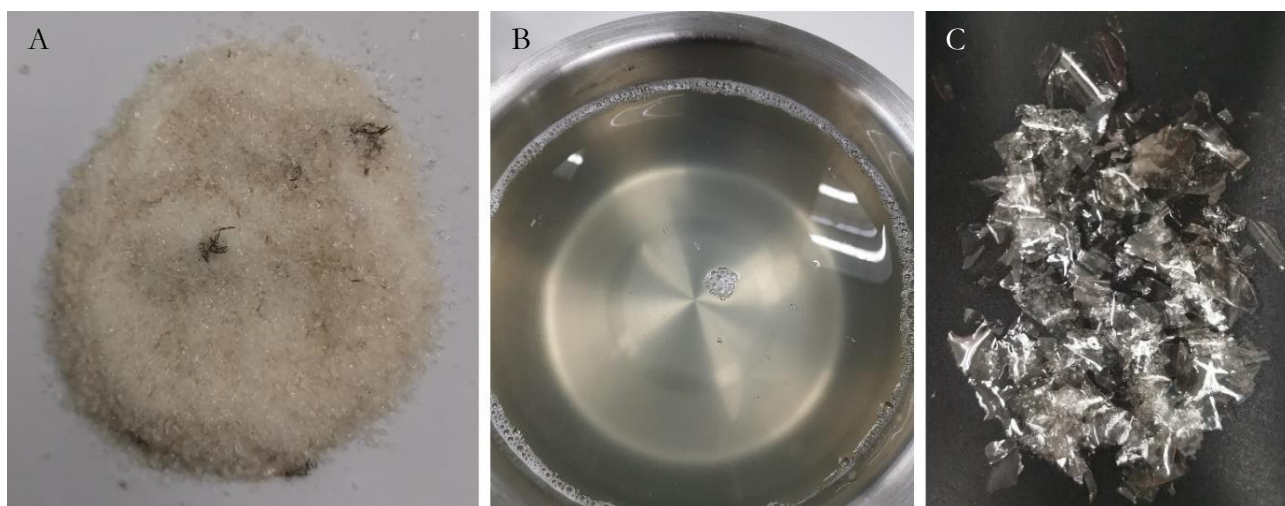


Figure 1. A: Raw material for hydrolysate- by product of EBN; B: liquid EBN hydrolysate; and C: EBN hydrolysate after drying.

Total sialic acid (SA) content analysis

The total sialic acid (SA) content of the sample was determined using the periodate-resorcinol assay, as described by Hui Yan *et al.* (2022) and Yeo *et al.* (2023). A 0.5 mL sample (2.0 mg/mL) was mixed with 0.5 mL of resorcinol reagent in a test tube. The test tube was covered with a chilled marble and placed in boiling water for 15 minutes. After incubation, the samples were cooled to room temperature for 10 minutes. Subsequently, 2.0 mL of 1-butanol (Merck Millipore, USA) was added to the sample, and the mixture was vortexed vigorously for at least 10 seconds to form a single-phase solution. The samples were then incubated in a 37 °C water bath for 3 minutes to stabilize the colour. After cooling to room temperature, the absorbance of the sample was measured at 580 nm using a visible spectrophotometer (SCIOGEX SCI-V1000, USA). To prepare 100 mL of resorcinol reagent, 0.22 g of resorcinol (Sigma-Aldrich, USA) was dissolved in 10 mL of distilled water. This solution was then mixed with 80 mL of concentrated hydrochloric acid (HCl) (Merck Millipore, USA) and 0.25 mL of 0.1 M copper sulfate (CuSO₄) (Merck Millipore, USA), and the final volume was made up to 100 mL with ultrapure water. N-acetylneuraminic acid (Sigma-Aldrich, USA) was used as the analytical standard for this assay.

Antioxidant activity analysis**DPPH assay**

The antioxidant activity of the EBNH samples was assessed using the DPPH assay as described by Yeo *et al.* (2023). A 1 mL aliquot of the sample, at a concentration of 2 mg/mL, was mixed with 14 mL of a 0.036 mM DPPH solution. The DPPH reagent was prepared by dissolving 14.07 mg of DPPH powder (Sigma-Aldrich, USA) in 1 L of methanol (Thermo Fisher Scientific, USA). For the control sample, ultrapure water was used instead of the EBNH sample. The mixture was incubated in the dark for 30 minutes. After incubation, the sample or control was filtered through a 0.45 µm PTFE syringe filter, and the absorbance was measured at 517 nm using a visible spectrophotometer (SCIOGEX SCI-V1000, USA). Ultrapure water was used as the blank. Free radical scavenging activity was calculated using the following equation:

$$\text{Free radical scavenging (\%)} = \frac{a - b}{a} * 100$$

where a represents the DPPH control absorbance; and b represents the DPPH sample absorbance.

ABTS assay

The ABTS assay was conducted following the procedure outlined by Yeo *et al.* (2023). A 0.2 mL sample, at a concentration of 2 mg/mL, was mixed with 1.8 mL of ABTS reagent. The ABTS reagent was prepared by combining 7 mM ABTS (Thermo Fisher Scientific, USA) and 2.45 mM potassium persulfate (Thermo Fisher Scientific, USA) in a 1:1 ratio. The resulting ABTS reagent was incubated for 14 to 16 hours at room temperature in the dark. The reagent was then diluted with methanol (Thermo Fisher Scientific, USA) to achieve an absorbance of 0.7 ± 0.2 . For the control sample, ultrapure water was used instead of EBNH. The mixture was incubated in the dark for 10 minutes, then filtered through a 0.45 µm PTFE syringe filter, and the absorbance was measured at 734 nm using a visible spectrophotometer (SCIOGEX SCI-V1000, USA). Ultrapure water was used as the blank. Free radical scavenging activity was calculated using the following equation:

$$\text{Free radical scavenging (\%)} = \frac{a - b}{a} * 100$$

where a represents the ABTS control absorbance; and b represents the ABTS sample absorbance.

Statistical analysis

All experiments and analyses were performed in triplicate to ensure reliability and reproducibility of the results. Microsoft Excel (Microsoft 365, version 2395) was employed for statistical analysis. Significant differences among the samples were determined using a T-test, with a p-value of less than 0.05 ($p < 0.05$) considered statistically significant. The data are presented as the mean of three replicates along with the standard error (SE).

Drying cost analysis

To conduct a comprehensive drying cost analysis for 1 L of liquid EBNH, four drying technologies: freeze-drying, spray-drying, cold air-drying, and

hot air- drying were evaluated. The analysis is based on equipment costs, preparation and drying times, and operational costs, referencing the equipment models used in Yeo, *et al.*, 2023 for the freeze and spray drying methods and this study for the cold and hot air-drying methods. To ensure accuracy and relevance, updated equipment costs were sourced from publicly available prices listed on the internet. If prices were not readily available, suppliers were contacted directly to obtain the most current prices, providing a realistic and current market perspective.

Preparation and drying times were measured for each method, focusing on the duration required to dry 1 L of liquid EBNH. Preparation time includes setting up the drying equipment and any necessary preprocessing of the hydrolysate. Operational costs were estimated by calculating the energy consumption of each drying method based on the specifications of the equipment. The energy costs were then calculated using the bill calculator provided by Tenaga Nasional (<https://www.mytnb.com.my/residential/unders tand-your-bill/bill-calculator>), inputting the estimated kWh usage for each method. This tool allows for precise calculation of energy consumption costs, and reflective of real-world conditions. By using a standardized tool for calculating energy costs, the study ensures consistency and reliability in the operational cost data. This method ensures that the time-related costs and efficiencies of each drying method are accurately analysed.

Feasibility analysis

To assess the feasibility of four drying methods—freeze drying, spray drying, hot-air drying, and cool-air drying—for EBNH, a set of comprehensive criteria was established. The criteria included space requirement, initial capital cost, operation cost, installation complexity, cleaning and maintenance needs, equipment/part replacement ease of access, required operational skills, and scalability potential.

Each criterion was evaluated using a standardized scoring system, with scores ranging from 1 to 4, where: 1 represents the least favourable outcome, 4 represents the most favourable outcome. For example, a score of 4 for the operational skills criterion would indicate that

minimal expertise is required to operate the equipment, while a score of 1 would suggest that highly specialized skills are necessary.

The installation complexity criterion was evaluated based on the time and ease required to set up the equipment, with higher scores assigned to methods that required less time and effort. Cleaning and maintenance scores reflected the frequency and complexity of maintenance tasks, with simpler and less frequent tasks receiving higher scores. Equipment/part replacement was scored based on the cost and accessibility of replacement components, with more accessible and affordable parts receiving higher scores. The required operational skills criterion assessed the level of training needed, with lower requirements leading to higher scores. Finally, scalability was evaluated based on the potential to increase production capacity with minimal additional costs and disruptions.

The total score for each drying method was calculated by summing the scores across all criteria. The method with the highest total score was deemed the most feasible for SMEs. This systematic scoring approach ensures a balanced evaluation of both technical and economic factors, identifying the most practical and cost-effective drying method for EBN hydrolysate.

RESULTS AND DISCUSSION

Drying efficiency

Liquid EBNH obtained after enzymatic hydrolysis was dried using two methods: hot-air and cool air drying. The cool airdrying method involved a stand fan, and an air conditioner set to 17 °C. Meanwhile, samples were dried using a hot air dehydrator at temperatures of 55 °C and 65 °C. The temperature (°C) and relative humidity (%) were monitored and recorded every one hour throughout the drying process. The purpose of this study was to compare drying methods using two different types of samples with varying initial liquid hydrolysate volumes: sample (1) was 200 mL and sample (2) was 70 mL. Furthermore, samples that were dried using the cool air-drying method were named as (A), samples that were dried using hot air- drying at 65 °C were named as (B), and samples that were dried using hot air

drying at 55 °C were named as (C). The results of the drying process are shown in Table 1 and Table 2. Cool air drying had a drying period of 9.10 hours for 200 mL and 7.00 hours for 70 mL. Hot airdrying at 55 °C reduced the drying time to 4.10 hours for 200 mL and 3.00 hours for 70 mL, increasing the drying efficiency by more than 50% compared with cool airdrying. Hot airdrying at 65 °C was the fastest, drying 200 mL in 3.35 hours and 70 mL in only 2.30 hours, which increased the drying efficiency by more than 20% when compared to hot airdrying at 55 °C. These results suggested that the efficiency of higher drying temperatures for faster moisture removal and improved product stability.

The drying process is profoundly affected by relative humidity and temperature. As the temperature of the air rises, so does its ability to hold water, resulting in more efficient moisture removal. Drying at higher temperatures reduces overall drying time and energy consumption because faster-moving water molecules evaporate faster (Patel *et al.*, 2023). Relative humidity (RH), which measures the amount of moisture in the air relative to its maximum capacity, influences the drying air's ability to remove water from the product. Previous studies have reported that decreasing of RH improved the drying rate of papaya slices (Ju *et al.*, 2023); reduced humidity could effectively improve drying kinetics and

preserve nutritional quality in onion (Sasongko *et al.*, 2020); and a study suggested that maintaining lower RH is crucial for effective moisture removal during drying operations, where this study found that drying medium exceeded 60% RH, it significantly hindered the drying process (Xing *et al.*, 2023). Therefore, hot air-drying in this study, with higher temperatures and lower RH, demonstrated higher efficiency in the drying process.

Moisture content

The moisture content of the EBNH samples was determined after they had been dried using cool air and hot air-drying methods. The dried samples were then crushed into powder and oven-dried for moisture content analysis. The moisture content was determined using the formulae stated in methodology and the data presented in Table 3. The data indicates the moisture content of EBN hydrolysate samples that were dried for 24 hours. The data show that the moisture content of the samples decreases as drying time increases. According to Malaysia Standard (MS 2334: 2011, Edible-Birdnest (EBN)- Specification), and China Standard (CAIQRZ- 2015002, Bird's Nest Product Certification Implementation Rules), the acceptable moisture content limit for RC EBN product is less than 15%. All samples in this study exhibited moisture content within this limit.

Table 1: Samples dried using the cool air method (Mean $\pm$ SE)

Sample ID	Initial liquid hydrolysate volume (mL)	Effective drying period (in hours)	Temperature (°C)	Relative humidity, RH (%)
A1	200.00	9.10	21.19 $\pm$ 0.47	58.22 $\pm$ 1.68
A2	70.00	7.00	21.66 $\pm$ 0.48	59.29 $\pm$ 2.00

Table 2: Samples dried using the hot air dehydrator with temperatures set to 55 °C and 65 °C (Mean $\pm$ SE)

Sample ID	Temperature that was set to the hot air dehydrator	Initial liquid hydrolysate volume (mL)	Effective drying period (in hours)	Temperature (°C)	Relative humidity, RH (%)
B1	65 °C	200.0	3.35	66.10 $\pm$ 0.67 ^a	39.67 $\pm$ 1.45 ^b
B2	65 °C	70.00	2.30	65.65 $\pm$ 0.85 ^a	40.50 $\pm$ 1.50 ^b
C1	55 °C	200.00	4.10	57.18 $\pm$ 0.61 ^b	46.88 $\pm$ 0.77 ^a
C2	55 °C	70.00	3.00	56.90 $\pm$ 0.78 ^b	47.33 $\pm$ 0.88 ^a

Different superscript letters *a-b* in the same column represent significant differences at $p < 0.05$.

Table 3: Moisture content (%) of the EBN hydrolysate samples (Mean $\pm$ SE)

Drying method	Sample ID	Effective drying period (in hours)	Initial liquid hydrolysate volume (mL)	Moisture content (%)
Cool air drying at 17°C	A1	9.10	200.00	8.33 $\pm$ 4.41
	A2	7.00	70.00	15.00 $\pm$ 5.00
Hot air drying at 65°C	B1	3.35	200.00	11.67 $\pm$ 4.41
	B2	2.30	70.00	10.00 $\pm$ 2.89
Hot air drying at 55°C	C1	4.10	200.00	5.00 $\pm$ 2.89
	C2	3.00	70.00	3.33 $\pm$ 1.67

Nitrite content

This study investigated the impact of different drying methods on the chemical properties of EBN hydrolysates, with a particular focus on nitrite content. Table 4 shows that there is a significant difference in nitrite content among EBN hydrolysates dried using various methods and starting with different initial sample volumes. In August 2011, the Chinese government imposed a ban on EBN products imported from Malaysia due to elevated nitrite levels (NO_2) (Yeo *et al.*, 2021). High nitrite levels in EBN have been a public concern, questioning their safety for consumption. Nitrite is often used as a preservative and anti-botulin agent in food processing, necessitating strict control to prevent toxicity. One significant concern is its role in inducing methemoglobinemia, a condition that reduces the oxygen-carrying capacity of blood. Symptoms of methemoglobinemia include cyanosis, difficulty breathing, and fatigue, particularly in infants and individuals with compromised health. Chronic nitrite exposure has also been associated with long-term health issues such as chronic obstructive pulmonary disease (COPD) and the development of brain tumors in newborns. Additionally, nitrite can react with dietary amines in the acidic environment of the stomach to form nitrosamines, compounds known for their carcinogenic potential (Chan *et al.*, 2018; Zulkefle *et al.*, 2024). According to Malaysia and China standard (MS 2334: 2011 and CAIQRZ- 2015002), the acceptable nitrite limit in EBN is 30 ppm. These standards aim to ensure the safety of EBN for consumption. All samples in this study exhibited nitrite levels well below the 30 ppm limit, ensuring compliance with safety standards.

Cool air- drying at 17 °C resulted in the highest nitrite content, with sample A1 showing 22.65 ± 0.03 ppm and sample A2 showing 21.37 ± 0.06 ppm. These values were significantly higher compared to samples dried using hot air. Specifically, samples B1 and B2 dried at 65 °C had nitrite levels of 10.72 ± 0.07 ppm and 11.05 ± 0.06 ppm, respectively, while samples C1 and C2 dried at 55 °C had nitrite levels of 10.10 ± 0.08 ppm and 10.35 ± 0.04 ppm, respectively. A previous found that fan-assisted dried samples had significantly higher nitrite content ($p < 0.05$) at 36.95 ppm compared to IR-UVC-assisted dried samples (Gan *et al.*, 2016). The hypothesis was that prolonged drying times facilitate nitrobacteria multiplication, leading to increased nitrite content. Our findings support this hypothesis, demonstrating that longer drying times associated with cool air drying result in higher nitrite levels. Importantly, despite the higher nitrite levels observed in cool-air drying, all values remained well within the safety limits, confirming the suitability of these methods for producing safe EBN hydrolysates.

Total sialic acid content

Sialic acid (SA) is a key bioactive component of edible bird's nest (EBN), contributing significantly to its nutritional value and health benefits. Regulatory standards, CAIQRZ- 2015002, emphasize the presence of sialic acid as a critical indicator of EBN's quality. In this study, all EBN hydrolysates demonstrated detectable levels of sialic acid, meeting these standards and affirming their compliance with nutritional benchmarks. Sialic acid has been widely studied for its health benefits. It plays a critical role in neurological development, supporting brain

growth and cognitive function, especially in infants. Furthermore, sialic acid contributes to immune modulation, potentially enhancing the body's defense mechanisms. Additional benefits include lowering LDL cholesterol, improving fertility, and regulating blood coagulation (Tan *et al.*, 2021). These attributes make sialic acid a valuable compound in functional foods, dietary supplements, and nutraceutical applications.

Table 4 provides the SA content for samples subjected to various drying methods, while Table 5 presents comparative data from previous studies. The aim was to determine if cool air and hot air-drying methods effectively retain SA compared to freeze and spray drying. The total SA content for EBNHs varied significantly

depending on the drying method used. Cool airdrying at 17 °C resulted in SA contents of $19.24 \pm 0.18\%$ for sample A1 and $18.96 \pm 0.11\%$ for sample A2. In contrast, hot air drying at 65 °C yielded SA contents of $20.38 \pm 1.06\%$ for sample B1 and $19.88 \pm 0.16\%$ for sample B2, while hot air drying at 55 °C resulted in lower SA contents of $17.84 \pm 0.12\%$ for sample C1 and $17.23 \pm 0.56\%$ for sample C2. These results are consistent with the findings of Yeo *et al.* (2023), who reported that the SA content in EBN hydrolysates dried using cool air at 18-20 °C ranged from 16.03% to 20.49%. Our study's cool-air drying results fall within this range.

Table 4. Chemical properties of EBN hydrolysates (Mean $\pm$ SE)

Drying Methods	Sample ID	Nitrite (ppm)	Concentration	Total Sialic Acid Content (%)
Cool air drying at 17°C	A1	22.65 ± 0.03^a		19.24 ± 0.18^a
	A2	21.37 ± 0.06^b		18.96 ± 0.11^a
Hot air drying at 65°C	B1	10.72 ± 0.07^d		20.38 ± 1.06^{ab}
	B2	11.05 ± 0.06^c		19.88 ± 0.16^b
Hot air drying at 55°C	C1	10.10 ± 0.08^f		17.84 ± 0.12^c
	C2	10.35 ± 0.04^e		17.23 ± 0.56^c

Different superscript letters *a-f* in the same column represent significant differences at $p < 0.05$.

Table 5. Total Sialic Acid Content of EBN Hydrolysate with Different Drying Technologies

References	Raw Material	Drying Technology	Total Sialic Acid (%)
(Ling <i>et al.</i> , 2020)	EBN wastage	Freeze Dried	19.30 ± 3.30
	Raw cleaned EBN		20.30 ± 3.10
(Hui Yan <i>et al.</i> , 2022)	Raw cleaned EBN	Freeze Dried	15.00- 22.40 (with different hydrolysis time 1 -4 hour)
(Chong <i>et al.</i> , 2022)	Raw cleaned EBN	Freeze Dried	19.42 ± 3.49
		Spray Dried	15.87 ± 2.35
(Yeo, Wong, Tan, Yaya Rukayadi, <i>et al.</i> , 2023)	Raw- uncleaned EBN	Freeze Dried	19.13 ± 0.43
		Freeze Dried	18.67 ± 0.20
		Spray Dried	17.47 ± 0.21
(Yeo, Wong, Tan, Rukayadi, <i>et al.</i> , 2023)	EBN by- product	Cool airdried 17 °C (Fan plus air- conditioner)	19.86 ± 0.86
This study	EBN by- product	Cool airdried 17 °C (Fan plus air- conditioner)	18.96, 19.24 (initial volume of hydrolysate: 70 mL, 200 mL)
		Hot airdried 55 °C (Dehydrator)	17.23, 17.84 (initial volume of hydrolysate: 70 mL, 200 mL)
		Hot airdried 65 °C (Dehydrator)	19.88, 20.38 (initial volume of hydrolysate: 70 mL, 200 mL)

*EBN wastage consists of EBN residue, feathers, dirt, and foreign matter, as waste after primary processing of edible bird nest; raw cleaned (RC) EBN is the sample after primary processing (cleaning) of raw uncleaned EBN, as a final product to sell to customers; EBN by-product refers to fragments of EBN with tiny feathers, obtained after the quality control step, needing re-cleaning to get RC EBN.

Comparing these results with those of previous studies (Table 5), freeze- drying showed SA contents ranging from 15.00% to 22.40% depending on the raw EBN material and hydrolysis conditions, while spray- drying resulted in SA contents of 15.87% and 17.47%. Poh *et al.* (2022) reported no significant difference in SA content between freeze-dried and spray-dried samples, indicating the heat stability of SA across different drying methods. While another study investigated RC EBN and discovered that IR-UVC drying at low temperatures resulted in better SA retention in EBN than hot air- drying at higher temperatures (Gan *et al.*, 2017). Therefore, the effect of temperature on SA retention needs to be studied more broadly to draw conclusive results. Our study shows that the SA content in EBNH ranges from 17.23% to 20.38% with cool air and hot air- drying methods. Comparative analysis indicates that cool air drying and hot air drying both effectively retain SA content, with values comparable to those obtained through freeze drying. This supports the idea that both cool and hot air drying can be viable alternatives to freeze drying for retaining SA in EBNH. These findings are significant for the industry, as they suggest that both drying methods can be used as cost-effective alternatives to freeze- drying while ensuring the nutritional and functional quality of EBNH.

Antioxidant activity

This study examined the antioxidant activity of EBNH samples using DPPH and ABTS assays to evaluate their free radical scavenging capabilities. Table 6 presents the antioxidant activity data for the different drying methods used. The antioxidant activity of EBNHs, as measured by DPPH and ABTS assays, varied with the drying method used. The DPPH activity ranged from 25.68% to 28.76%, while the ABTS activity ranged from 62.61% to 68.37%. For DPPH activity, cool air- drying at 17 °C resulted in $27.67 \pm 0.55\%$ for sample A1 and $28.76 \pm 0.28\%$ for sample A2. Hot air- drying at 65 °C yielded $27.91 \pm 0.65\%$ for sample B1 and $26.59 \pm 0.47\%$ for sample B2. Hot air- drying at 55 °C resulted in lower DPPH activity, with $25.68 \pm 0.86\%$ for sample C1 and $25.69 \pm 1.72\%$ for sample C2. These values indicate that cool air drying and hot air- drying at higher temperatures (65 °C) generally retain higher DPPH activity compared

to hot air drying at lower temperatures (55 °C). Regarding ABTS activity, hot air- drying at 65 °C for sample B2 showed the highest activity at $68.37 \pm 0.23\%$, followed by hot air- drying at 55 °C for sample C1 with $66.97 \pm 0.47\%$. Cool air- drying at 17 °C resulted in ABTS activities of $65.87 \pm 0.76\%$ for sample A1 and $62.61 \pm 0.10\%$ for sample A2. The results of this study demonstrate that both cool air and hot air-drying methods effectively retain antioxidant activity in EBN hydrolysates. These findings are significant for the industry as they provide cost-effective alternatives to freeze- drying while ensuring the preservation of the antioxidant properties of EBN hydrolysates.

Drying cost between different drying technologies

This drying cost analysis methodologically compares the four drying technologies based on equipment cost, preparation and drying time, and operational cost, using up-to-date market data and standardized calculation tools. This approach provides a thorough and practical evaluation of the economic feasibility of each drying method for producing EBN hydrolysate, contributing valuable insights for SEMs in selecting the most cost-effective drying technology.

Table 7 compares the equipment cost, preparation and drying time, and operational cost for drying 1 L of liquid EBN hydrolysate using different drying technologies: freeze- drying, spray- drying, cool air- drying, and hot air- drying (at 65 °C). The analysis revealed significant cost differences among these methods. Freeze- drying, requiring a freezer and a freeze dryer, involves substantial initial investment with an equipment cost of RM 116,500.00, and a lengthy preparation and drying time of 96 hours, resulting in a high operational cost of RM 23.33 per L. Despite its ability to retain the most nutrients, as noted by Chok *et al.* (2021), the extremely high equipment cost, and prolonged drying time make it impractical for SMEs production. Spray- drying, utilizing a spray dryer, is the fastest method with a drying time of just 1 hour. However, it is associated with the second highest operational cost of RM 1.96 per L and a very expensive machine cost of RM 627,200.00, leading to high overall costs and energy consumption.

Table 6: Antioxidant activity of EBN hydrolysates (Mean $\pm$ SE)

Drying Methods	Sample ID	Antioxidant activity	
		DPPH (%)	ABTS (%)
Cool air drying at 17°C	A1	27.67 $\pm$ 0.55 ^{ab}	65.87 $\pm$ 0.76 ^b
	A2	28.76 $\pm$ 0.28 ^a	62.61 $\pm$ 0.10 ^c
Hot air drying at 65°C	B1	27.91 $\pm$ 0.65 ^{ab}	66.62 $\pm$ 0.49 ^b
	B2	26.59 $\pm$ 0.47 ^b	68.37 $\pm$ 0.23 ^a
Hot air drying at 55°C	C1	25.68 $\pm$ 0.86 ^b	66.97 $\pm$ 0.47 ^b
	C2	25.69 $\pm$ 1.72 ^{ab}	63.01 $\pm$ 0.49 ^c

Different superscript letters *a-c* in the same column represents significant differences at $p < 0.05$.

Table 7: Equipment cost, and operational process 1 L liquid hydrolysate.

Drying Technology	Description of Product	Equipment Cost (RM)	Time for preparation and drying (hour)	Operational Cost for 1 L
Freeze	Freezer (GN 2866, Liebherr, Switzerland)	**8500.00 (price showed in the lab)	24	259 Watt RM 1.36
	Freeze Dryer (Coolsafe Pro 95-15, LaboGene, Denmark)	108,000.00 (updated price from Chemopharm Sdn Bhd. 10 June 2024)	72	1400 watt RM 21.97
Spray	Spray Dryer (Mobile Minor, GEA Niro A/S, Germany)	627,200.00 (updated price given by GEA Process Engineering Pte. Ltd on 20 June 2024)	1	9000 Watt RM 1.96
Cool air	Fan (ISONIC ISF-1800, Malaysia)	229.00 (https://senghuat.com.my/isonic-18-industrial-stand-fan-isf-1800-green . 1 June 2024)	9.10	110 Watt RM 0.22
	Rack (to put the tray with liquid hydrolysate)	245.00 (https://www.lazada.com.my/products/stainless-steel-15-tray-cooling-rack-bakery-trolley-rack-i3341829985-s17937250711.html . 1 June 2024)		N/A
	Air- conditioner (Acson R410A-A5WM15S, Malaysia)	1530.00 (https://www.lazada.com.my/products/acson-avory-series-non-inverter-standard-r32-air-conditioner-10hp15hp20hp25hp-i4017920718-s22877848303.html . 1 June 2024)		1105 Watt RM 2.19
Hot air	Dehydrator (Excalibur 9 tray dehydrator, USA)	2599.00 (https://cookerlicious.com/collections/excalibur-dehydrator-1/products/excalibur-dehydrator-9-trays-4948 . 1 June 2024)	3.35	600 Watt RM 0.44

*The equipment models for all the drying methods are from Yeo, Wong, Tan, Yaya Rukayadi, *et al.* (2023) and this study. The updated equipment costs were obtained from publicly available prices listed on the internet. If the price was not readily listed on public websites, suppliers were contacted directly for an updated equipment price.

*The operational cost was calculated using the bill calculator provided by Tenaga Nasional (<https://www.mytnb.com.my/residential/understand-your-bill/bill-calculator>)

** The model of this freezer is no longer available on the market, so the price was obtained from the lab that has this freezer.

Cool air- drying, employing a fan, a stainless-steel rack, and an air conditioner, is more affordable than freeze and spray drying, with an equipment cost of RM 2,004.00 and a drying time of 9.1 hours. However, its operational cost is higher at RM 2.41 per L compared to hot airdrying. Hot air drying, using an optimized dehydrator, emerges as the most cost-effective option with the second lowest equipment cost of RM 2,599.00 and the lowest operational cost of RM 0.44 per L. It also has the second fastest drying time of 3.35 hours and retains a comparable total sialic acid content to freeze-drying, as demonstrated in this study. This makes hot air drying the most practical and cost-effective method for the industrial scale drying of EBNH. The equipment and operational costs significantly impact SMEs in the processing industry, influencing their competitiveness and sustainability. High equipment costs can pose a barrier to entry for SMEs, limiting their ability to invest in advanced technologies that enhance productivity and efficiency. For instance, the initial capital required to purchase sophisticated drying equipment can strain the financial resources of SMEs, which often operate with tight budgets and limited access to financing options. This financial constraint can lead to a reliance on outdated or less efficient technologies, ultimately affecting product quality and operational efficiency (The impact of SMEs in the global economy – iQualify UK – Modern British Qualification)

Operational costs further complicate the financial landscape for SMEs. These costs encompass energy consumption, labor, maintenance, and other ongoing expenses necessary for running equipment. As SMEs strive to maintain profitability, high operational costs can erode margins, making it challenging to compete with larger firms that benefit from economies of scale. For example, the energy costs associated with running drying equipment can vary significantly between technologies, impacting the overall cost of processing products like EBNH. SMEs must carefully evaluate these costs to ensure they can sustain operations while remaining competitive in the market (Kocakulah *et al.*, 2017). In this cost analysis, only energy consumption was included in the operational cost because this parameter can be calculated more

consistently across all drying technologies. However, labor, maintenance and other ongoing expenses are discussed below in the feasibility analysis.

In conclusion, while freeze- drying is recognized for its superior nutrient retention, its high costs and extended processing time limit its industrial feasibility. Spray- drying, though efficient in time, is expensive and energy intensive. Cool air- drying is more economical than freeze and spray drying but still more costly than hot air drying. Hot air- drying with an optimized dehydrator stands out as the most viable and economical method, ensuring the nutritional and functional quality of EBNH. Further research is recommended to fully characterize the effects of hot air- drying on the nutritional profile of EBN hydrolysate to optimize its industrial application.

Feasibility between different drying technologies

The feasibility analysis results presented in Table 8 compares four drying methods—freeze- drying, spray- drying, hot air- drying at 65 °C, and cool air- drying at 17 °C. The feasibility analysis, as summarized in Table 8, incorporates key criteria relevant to SMEs, such as cost, scalability, ease of use, and maintenance. Each parameter has been scored to reflect its relative importance for SME operations. Cost-related factors, including initial capital and operation costs, are weighed more heavily in the scoring, given their critical role in SME decision-making. Practical aspects like ease of cleaning, maintenance, and operation are also prioritized to ensure that the methods are accessible and manageable for small-scale producers. While scalability is considered, it is balanced against cost and operational simplicity, as these are often more immediate constraints for SMEs.

Hot air- drying (65 °C) emerged with the highest overall feasibility score of 34 out of 40. This method requires the least space (483 × 432 × 318 mm for the dehydrator), has the lowest initial capital cost (RM 2,599.00), and is the easiest to operate, clean, maintain, and scale up. The compact size and simplicity of operation reduces both the spatial footprint and the need for specialized skills, while the low initial cost makes it accessible for SMEs. Additionally, the

straightforward maintenance and easy availability of replacement parts enhance its practicality and reliability.

Cool air- drying (17 °C) scored 26. Compared to hot-air drying, it requires more steps in the cleaning process, as the fan, rack, and air conditioner, as well as the room containing this equipment, must be cleaned regularly to avoid contamination. The operational skills required for cool-air drying are higher than those for hot-air drying because this method involves controlling the fan, air-conditioner position, speed, and temperature. Additionally, the placement of samples on the rack is crucial to ensure they receive maximum airflow velocity from the fan. Cool-air drying also requires more space for the fan and rack setup and involves higher capital and operational costs compared to hot-air drying. Despite these drawbacks, it remains a feasible option for SMEs, offering moderate initial and operational costs (although higher than those of hot-air drying) and reasonable scalability.

Spray- drying received a score of 13, requiring the most space (2500 × 2000 × 2300 mm) and having the highest capital cost (RM 627,200.00). The advantage of this method is the scalability, as this equipment can work with big volume without adding another dryer or equipment. Despite its fastest drying time of 1 hour, the high initial and operational costs limit its feasibility for SMEs.

The complexity of the equipment and the need for specialized skills to operate it further diminish its practicality for smaller enterprises. The GEA Niro A/S spray dryer, for example, incorporates several key components essential for its operation, including a drying chamber, atomization system (rotary atomizer or nozzle systems), air disperser, cyclone separator, bag filter, and an optional fluid bed for secondary drying or cooling. Additionally, a control system is necessary to monitor and adjust parameters such as temperature and airflow. These components require regular cleaning, maintenance, and part replacement, particularly the atomizer, nozzles, and filters. This adds to the operational complexity and costs, as specialized skills (higher operator cost) are needed to manage the atomization system and airflow controls effectively. Moreover, cleaning the drying chamber, cyclone separator, and bag filter is labor-intensive, increasing downtime and overall operational expenses. These drawbacks, including cleaning, maintenance, part replacement (which often involves ordering from specific suppliers, with some parts requiring a wait time for overseas shipment), and the required operational skills, further reduce spray drying's practicality for SMEs. Its low score in feasibility analysis reflects the significant barriers to adoption in SME settings, primarily due to cost, space constraints, and operational challenges.

Table 8: Feasibility analysis for different drying technologies

Drying technology	Freeze	Spray	Hot air(65°C)	Cool air (17 °C)
Space requirement (L × W × H in mm)	2 Freeze dryer (620 × 495 × 745) Freezer (632 × 600 × 1644)	1 (2500 × 2000 × 2300)	4 (483 × 432 × 318)	3 Fan (500 × 280 × 1400) Rack (600 × 460 × 1650)
Initial capital cost	2	1	3	4
Operation cost	1	2	4	3
Installation	2	1	4	3
Cleaning	3	1	4	2
Maintenance	2	1	4	3
Equipment/ part replacement	2	1	4	3
Operation / skill	2	1	4	3
Scalability	1	4	3	2
Total score of feasibility	17	13	34	26

The highest score of feasibility represents the most feasible of the drying methods for a SME company.

Freeze-drying scored 16, requiring the most equipment (freezer and freeze dryer) and having very high capital costs (RM 116,500.00) and operational costs (RM 23.33 per L). It also has the longest drying time of 96 hours, making it impractical for SME-scale production. While freeze drying is known for preserving nutritional quality, the extended drying time and substantial costs make it a less feasible option for SMEs. The necessity for both a freezer and a freeze dryer increase the spatial and financial burden, making this method suitable only for operations with sufficient resources and space. The freeze dryer consists of several essential components. These include a condenser that can reach temperatures as low as -95 °C, a drying chamber, a two-stage vacuum pump, a control panel for monitoring temperature and pressure, an electrical drain taps for condensate removal, a vacuum valve for pressure control, and lockable castors for mobility. While these components ensure efficient and effective freeze-drying, they also introduce drawbacks such as the need for regular cleaning, maintenance, and part replacement, with some parts requiring orders from overseas suppliers. Additionally, operational skills are necessary to manage the vacuum system, control panel, and temperature settings. Scalability is another limitation, as the dryer can only manage a maximum of 1.2 L of liquid hydrolysate per cycle, further reducing its practicality for SMEs. These factors, combined with the high costs and lengthy drying time, make freeze drying a less viable option for smaller-scale operations.

The feasibility analysis indicates that hot air drying is the most suitable drying method for EBNH production in an SME setting. It meets the key criteria proposed by (Chua and Chou, 2003) for ideal dryers:

- **Low Initial Capital Cost:** The Excalibur dehydrator costs RM 2,599.00 significantly less than the equipment for spray and freeze drying.
- **Ease of Construction and Fabrication:** The dehydrator is a simple, self-contained unit requiring minimal installation.
- **Ease of Operation:** The dehydrator has a basic control panel and does not require high expertise.
- **Better Drying Kinetics:** While hot air drying may degrade some nutrients more than freeze

drying, the operational benefits outweigh this drawback.

- **Ease of Maintenance:** The dehydrator has few moving parts and is straightforward to clean and service.
- **Simple Replacement of Parts:** Common components like the heating element and trays can be easily sourced if needed.

In conclusion, the results demonstrate that hot air-drying is the most feasible method for SMEs, achieving the highest total score (34) due to its low costs, ease of operation, and practical scalability. Cool air-drying (26) ranks second, offering reasonable feasibility with higher operational requirements. Freeze-drying (17) and spray-drying (13), despite their advantages in product quality and scalability, score lower due to their high costs and operational complexities. Hot air-drying with the dehydrator provides an optimal balance of cost, efficiency, and product quality. Further research is needed to fully characterize the effects of hot air drying on the nutritional profile of EBNH.

CONCLUSION

This study evaluated the efficacy of cool air and hot air-drying methods for converting liquid EBN hydrolysate into powder form, focusing on quality and feasibility for SMEs. The findings indicate that both cool air and hot air-drying methods are viable alternatives to more expensive and energy-intensive techniques like freeze-drying and spray-drying. Among the methods studied, hot air-drying at 65 °C emerged as the most efficient and cost-effective, significantly reducing drying time compared to cool air-drying at 17 °C, effectively lowering moisture content and enhancing overall drying efficiency.

The results showed that nitrite content remained within acceptable limits for all drying methods, although cool air-drying resulted in significantly higher nitrite levels due to prolonged drying times. Hot air-drying at 65 °C retained the highest sialic acid content, indicating minimal degradation of nutritional quality, while antioxidant activity, measured by DPPH and ABTS assays, was well retained in both cool-air

and hot-air dried samples. These findings suggest that hot air- drying at 65 °C provides the best balance of nutritional preservation and drying efficiency.

While the bioactive properties of the hydrolysate, such as sialic acid content and antioxidant activity, were largely maintained, the study also highlights the need for future research into sensory attributes, including texture and taste. Hot air- drying may result in slightly coarser textures compared to freeze-drying, which is known to better preserve the structural integrity of the product, based on the literature. However, the high cost and operational complexity of freeze-drying and spray-drying limit their feasibility for SMEs.

In conclusion, hot-air drying at 65 °C not only maintains the nutritional and functional qualities of EBN hydrolysate but also offers lower operational costs and higher feasibility for SMEs compared to freeze- and spray-drying. This method supports the broader adoption of cost-effective drying technologies in the EBN processing industry, providing SMEs with a practical means to produce high-quality EBN hydrolysate powder. Further studies on sensory evaluation, including taste and texture analysis, are recommended to complement these findings and guide future selection of optimal drying methods for various applications, such as skincare, cosmetics, beverages, and supplements.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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