Journal of Health and Nutrition Research
Vol. 5, No. 2, 2026, pg. 700-711, https://doi.org/10.56303/jhnresearch.v5i2.1329
Journal homepage: https://journalmpci.com/index.php/jhnr/index

e-ISSN: **2829-9760**

Effect of Freeze-Drying on the Sensory, Physicochemical, and Microbiological Characteristics of a Dadih-Based Functional Beverage

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ORIGINAL ARTICLES

Submitted: 06 March 2026

Accepted: 14 April 2026

Published: 01 August 2026

Keywords:
Dadih; Freeze-drying; Fermented milk; Physicochemical characteristics; Sensory properties; Lactic acid bacteria

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ABSTRACT

Dadih, a traditional fermented buffalo milk product from West Sumatra, Indonesia, is widely recognized for its probiotic properties and functional health benefits. This study aimed to evaluate the effect of freeze-drying on the physicochemical, physical, sensory, and microbiological characteristics of dadih. An experimental design was employed by comparing fresh dadih with freeze-dried dadih powder, in which samples were pre-frozen at −20°C prior to drying. Physicochemical properties were analyzed through proximate composition and water activity, while physical characteristics were assessed using color parameters (L*, a*, b*) measured by a chromameter. Sensory attributes were evaluated using descriptive analysis by ten trained panelists, and microbiological quality was determined based on total lactic acid bacteria (LAB). The results showed that freeze-drying significantly reduced moisture content (from 80.56% to 2.57%) and water activity (from 0.92 to 0.21), indicating enhanced product stability and extended shelf life. The relative concentrations of macronutrients increased due to water removal. No significant changes were observed in color parameters, suggesting that visual quality was preserved. Sensory evaluation revealed a slight increase in sourness and a minor decrease in milk and fermentation aroma. The total LAB decreased from 1.0×10^8 CFU/g to 7.5×10^5 CFU/g after freeze-drying, representing approximately a 3-log reduction. This final count is slightly below the commonly recommended minimum level (10^6–10^7 CFU/g) required for probiotic efficacy, indicating a partial loss of functional viability. In conclusion, freeze-drying effectively improves the stability of dadih by significantly reducing moisture and water activity while preserving color and sensory characteristics. Unlike conventional thermal drying methods, this technique maintains product quality and concentrates nutrients, although some reduction in viable LAB occurs.

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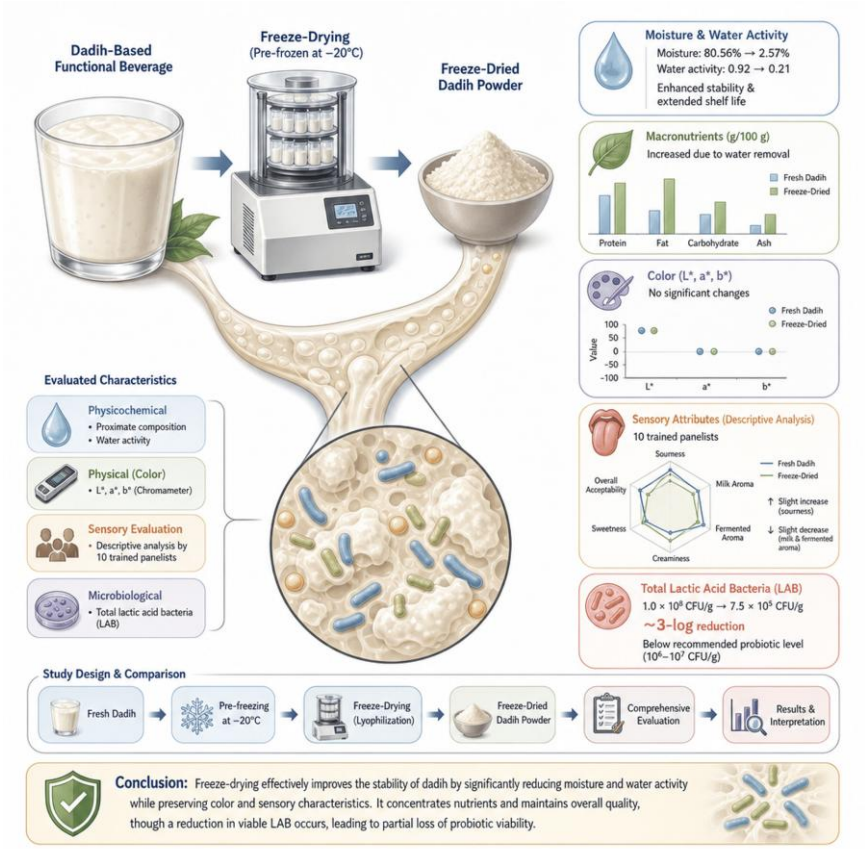
Key Messages:

- Freeze-drying significantly improves the stability of dadih by reducing moisture content and water activity while maintaining its physical color characteristics and concentrating its nutritional components. •
- This study highlights the potential of freeze-drying as an innovative preservation method for traditional dadih, enabling the development of shelf-stable functional products with wider commercial and market opportunities.

GRAPHICAL ABSTRACT



INTRODUCTION

Dadih is a traditional functional beverage originating from West Sumatra, Indonesia, produced from buffalo milk that undergoes natural fermentation inside bamboo tubes(1). Dadih is considered beneficial for human health because it contains probiotic bacteria such as *Leuconostoc paramesenteroides, Levilactobacillus brevis, Lacticaseibacillus casei, and Lactiplantibacillus plantarum*, which play an important role in maintaining gastrointestinal health(2). In addition, dadih contains a relatively complete amino acid profile and exhibits considerable antioxidant activity(3). Despite these health benefits, one of the major challenges associated with dadih is its relatively short shelf life due to its susceptibility to microbiological and chemical deterioration(4).

The limited shelf life of dadih poses a major challenge for large-scale distribution and commercialization. At the same time, global demand for functional beverages with extended shelf stability continues to increase, particularly in the form of shelf-stable and clean-label probiotic powders that offer convenience, longer storage, and minimal additives (5). Therefore, the development of appropriate processing technologies is essential to enhance the stability of dadih without compromising its nutritional and functional properties. One promising approach is freeze-drying (lyophilization), a non-thermal drying technique that removes water through freezing followed by sublimation under controlled temperature and pressure conditions (6). This approach is particularly relevant for dadih, as its probiotic bacteria and volatile aroma compounds (e.g., diacetyl/2,3-butanedione) are highly sensitive to heat and are likely to degrade during conventional thermal drying processes such as spray drying. Compared to thermal methods, freeze-drying better preserves these heat-sensitive components, thereby maintaining product quality (7,8).

Previous studies have demonstrated that freeze-drying can maintain the viability of lactic acid bacteria in fermented products, such as *Lactobacillus delbrueckii*, *Lactococcus lactis*, and *Leuconostoc mesenteroides*, with survival rates ranging from 60% to 95% (9). In addition, freeze-drying has been reported to preserve cell membrane integrity and improve microbial stability during storage, particularly

https://doi.org/10.56303/jhnresearch.v5i2.1329

when combined with stress adaptation treatments, leading to enhanced survival rates of probiotic bacteria (10). Furthermore, recent studies on fermented dairy products indicate that freeze-drying technology can enhance microbiological stability and extend product shelf life without significantly compromising nutritional quality and food safety. For example, research on freeze-dried millet-based yogurt demonstrated that the resulting product maintained good microbiological stability and remained safe for consumption after several weeks of storage(11).

Despite these advantages, the application of freeze-drying to dadih has not been extensively studied. Most previous research on dadih preservation has focused on conventional drying methods, such as spray drying, which may negatively affect heat-sensitive components and probiotic viability (12). Moreover, there is a lack of detailed investigation into how the unique bamboo-tube fermentation matrix of dadih influences the freeze-drying process, including its impact on mass transfer mechanisms, ice crystal formation, and structural changes during sublimation(13). Unlike standardized fermented products such as yogurt or kefir, dadih is produced through spontaneous fermentation, resulting in a heterogeneous matrix and diverse indigenous microbial communities that may respond differently to freeze-drying. This distinctive combination of raw material (buffalo milk), fermentation environment (bamboo tubes), and microbial ecology represents a unique system that has not been systematically evaluated under freeze-drying conditions. Consequently, there is still limited scientific evidence regarding the impact of freeze-drying on the physicochemical, sensory, and microbiological characteristics of dadih. The findings of this study are expected to provide significant contributions to the development of dadih products with improved shelf life, practicality, and economic value, while also supporting the preservation and global promotion of traditional Indonesian foods.

Therefore, this study aims to evaluate the effect of freeze-drying on the physicochemical, physical, sensory, and microbiological properties of dadih. This study provides new insights into the application of freeze-drying for traditional fermented dairy products, supporting the development of dadih with improved shelf life, practicality, and commercial value, while contributing to the preservation and global recognition of Indonesian traditional foods.

METHODS

Research Design

This experimental study evaluated the chemical, sensory, physical, and microbiological characteristics of dadih before and after the freeze-drying process. Chemical characteristics included nutritional composition and pH measurement. Sensory characteristics were assessed using a descriptive sensory evaluation. Physical characteristics were determined by observing color changes, while microbiological characteristics were evaluated by determining the lactic acid bacteria (LAB) count. All measurements were performed in triplicate (n = 3).

Materials and Equipment

Dadih samples were obtained from Bukittinggi, West Sumatra, Indonesia, with a fermentation period of two days. The sampling location was at latitude −0.303272 and longitude 100.357928, with an altitude ranging from 901 to 941 meters above sea level. The dadih samples were collected directly in their original bamboo containers. Prior to the freeze-drying process, the samples were stored under chilled conditions at temperatures below 8 °C.

The equipment used in this study included a GEA Dlf-04 Freeze Dryer located at the laboratory of the National Research and Innovation Agency (BRIN), a crusher, and a 20-mesh sieve. The freeze-drying process was conducted using a GEA Dlf-04 freeze dryer (GEA Group, Germany). Analytical instruments included a chromameter (CR-400, Konica Minolta, Osaka, Japan), Kjeldahl apparatus (Buchi, Switzerland), Soxhlet extractor (Gerhardt, Germany), muffle furnace, moisture analyzer, autoclave, and incubator.

No cryoprotectants (e.g., skim milk powder, sucrose, or maltodextrin) were added prior to freeze-drying. This approach was selected to evaluate the direct effect of freeze-drying on the native characteristics of dadih.

https://doi.org/10.56303/jhnresearch.v5i2.1329

Preparation of Freeze-Dried Dadih

The freeze-drying process was conducted at the BRIN laboratory. The preparation of freeze-dried dadih involved several stages, as illustrated in Figure 1. The freeze-drying process was conducted at the BRIN laboratory. Samples were pre-frozen at −20 °C for 24 hours prior to drying. The frozen samples were then subjected to freeze-drying under vacuum pressure below 4.58 mmHg. Primary drying was conducted under controlled low-pressure conditions to allow sublimation, followed by secondary drying at a final temperature of 35 °C to remove residual moisture. The total drying time was approximately 48 hours until constant weight was achieved.

Figure 1. Flow diagram of freeze-dried dadih production process

Product Analysis

Physicochemical Analysis

Proximate analysis was carried out to measure dadih chemical characteristics. Specifically, it measured water, ash, protein, fat, carbohydrate, and fiber content. The proximate analysis procedure for each parameter is described as follows:

Moisture Content

This procedure began by cleaning and drying the dish at 105^{o} C for 3 hours. After that, it was cooled in a desiccator. The empty dish container (W1) was weighed. Next, 3 grams of the sample was weighed and placed in the oven at 105^{o} C for 3 hours. After drying, the sample was transferred to a desiccator and cooled. Then, the sample did the final weighing (W2) (14).

$$\text{Moisture content (\%)} = (W1 - W2)/W1 \times 100\%$$

Ash Content

The next procedure was to measure the ash content. It was started by drying the cup in the oven at 105°C for 1 hour. The cup was cooled for 15 minutes in a desiccator and weighed. The sample weighed 1.5-2 grams. The sample was put in a furnace whose temperature was 600°C for 3 hours. When the outside of the furnace was cooled to ± 120 ° C, a desiccator was put in. The cup and ash were weighed again to obtain a constant weight (14).

$$\text{Ash content (\%)} = \text{ash weight/sample weight} \times 100\%$$

https://doi.org/10.56303/jhnresearch.v5i2.1329

Protein Content

Protein content was tested using the Kjeldahl method of the AOAC method (15). It was tested through the following stages:

a. Deconstruction stage

Samples were taken and then mashed thoroughly. They were weighed as much as 1 gram and put into the Kjeldahl flask. To facilitate the destruction of the sample, 2 grams of mixed catalyst and 25 ml of concentrated H_2SO_4 were added while stirring gently until the solution was homogeneous. Then, the solution was heated to boiling, and the color changed to clear green.

b. Distillation stage

In this stage, the cooled deconstructed solution was diluted with 100 ml of distilled water in a 100 ml volumetric flask. The solution was pipetted 5 ml into a distillation flask. To separate ammonia from the sample solution, 30% NaOH was added to the alkaline solution. In addition, some boiling stones were added. The solution was distilled. After that, the distillate was collected in an Erlenmeyer containing 10 ml of 2% boric acid solution and a few drops of mixed indicator (methylene red + bromothymol blue). The distillation occurred for approximately 5-10 minutes.

c. Titration stage

The distillate was titrated with 0.01 N hydrochloric acid standard solution. The titration point is reached if there is a blue-to-orange color change. In this stage, the blank was made, like the treatment of the sample. The percentage of the protein was calculated by the following formula:

$$\%N = \frac{(\text{ml NaOH blank - ml NaOH sample}) \times \text{NaOH normality} \times 14.008 \times 100\%}{\text{gr sample} \times 1000}$$

$$\% \text{ Protein} = \% \text{ N} \times 6.25$$

Fat Content

The test of fat content began with drying the fat flask in an oven at 105°C for about 15 minutes. The sample was weighed as much as 5g and then put into the fat sleeve. The filter paper containing the sample was placed in a soxhlet extraction device assembled with a condenser. Hexane solvent was added to the fat flask, and the sample was refluxed for 5 hours. The remaining solvent in the fat flask was removed by heating it in an oven and then weighed. (16). The formula for measuring the fat content is described as follows.

$$\text{Fat content (\%)} = \text{fat weight/sample weight} \times 100\%$$

Fiber Content

Fiber content was measured by weighing a total of 1 gram of sample. It was then put into a 250 mL beaker. Next, 50 mL of H2SO4 solution with a concentration of 0.3 N was added, and the mixture was heated at 70°C for 1 hour. After that, 25 mL of NaOH solution with a concentration of 1.5 N was mixed. The mixture was heated again for 30 minutes at 70°C. The solution was then filtered using a Buchner funnel. During filtration, the precipitate was washed sequentially with sufficient hot distilled water, 50 mL of H2SO4 solution with a concentration of 0.3 N, and 25 mL of acetone. The residue left in the filter paper was placed in a petri dish and dried in an oven for 1 hour at 105°C. After cooling, the residue was weighed (15).

Carbohydrate Content

Carbohydrate content was determined using the total carbohydrate method by difference (15). The calculation is illustrated as follows.

$$\text{Carbohydrate content (\%)} = 100\% - (\text{moisture} + \text{ash} + \text{protein} + \text{fat contents}).$$

Color Analysis

Color measurements were conducted using a chromameter to determine the color intensity of the dadih samples. The samples were placed on the measurement area of the instrument, which emitted light onto the sample surface and detected the reflected light. The color parameters were recorded based on the CIE L*a*b* color system, including L* (lightness), a* (green–red), and b* (blue–yellow) values)(17).

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Sensory Analysis

Sensory evaluation was conducted using a descriptive test method. A total of 10 trained panelists were asked to assess the product characteristics using an intensity scale of 1–10 (very low to very strong). The sensory attributes evaluated included sour taste, milk aroma, dadih aroma, white color, and smooth texture of the product before and after being dried using the freeze-drying technique. The data obtained were then averaged and presented in a spider chart(18).

Microbiology Analysis

Total LAB was determined using the pour plate method on MRS agar. Samples were diluted using 0.1% peptone water. One milliliter of diluted sample was plated and incubated at 41 °C for 48 hours under anaerobic conditions using an anaerobic jar. Colonies were counted and expressed as CFU/g.. First, 1 ml of the sample was placed in a sterile petri dish. The cooled sterile MRS agar medium was carefully poured into the petri dish containing the sample. After filling, the petri dish was closed again and gently moved to distribute the bacteria evenly on the medium. Petri dishes were then incubated at 41°C for 48 hours in an inverted position. After incubation, the growing bacterial colonies were counted using a colony counter to determine the approximate number of bacteria in the sample (19).

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 21. Differences between fresh and freeze-dried samples were evaluated using an independent samples t-test. All results are presented as mean ± standard deviation. Statistical significance was set at $p < 0.05$.

RESULTS

Freeze-Dried Product Characteristics

The freeze-drying process applied to *dadih* produced a dried powder with relatively good quality. As shown in Figure 2, the raw *dadih* (a) was transformed into dried *dadih* powder with a slightly dull white color (b). The product also underwent a rehydration process during further processing. The freeze-drying process consists of four stages: food preparation, freezing, primary drying, and secondary drying. During the preparation stage, *dadih* was placed in a tray and evenly spread to a thickness of approximately 1 cm. In the freezing stage, the sample was stored in a freezer for 24 hours at a target temperature of approximately −20°C(20).

After freezing, the drying stage was conducted through primary drying and secondary drying. Primary drying aimed to remove frozen water from the product through sublimation by increasing the temperature to around 0°C and reducing the pressure below the triple point (4.58 mmHg, 0°C). Secondary drying was then performed by gradually increasing the temperature and pressure until the product reached a normal temperature of approximately 35°C to stabilize the dried *dadih*.

Physicochemical Characteristics After Freeze-Drying

The physicochemical properties of dadih before and after freeze-drying are presented in Table 1. Significant differences ($p < 0.05$) were observed across all measured parameters. Moisture content decreased markedly from $80.56\%^{a} \pm 0.13$ to $2.57\%^{b} \pm 0.12$, corresponding to an approximate 96.8% reduction, while water activity decreased from $0.92^{a} \pm 0.01$ to $0.21^{b} \pm 0.02$, indicating substantially improved product stability.

Conversely, the removal of water resulted in a pronounced concentration effect. Protein content increased approximately 4.1-fold (from $7.66\%^{a}$ to $31.75\%^{b}$), while fat content increased nearly 4.9-fold (from $8.01\%^{a}$ to $39.56\%^{b}$). Similar trends were observed for carbohydrate and ash contents ($p < 0.05$). These findings demonstrate that freeze-drying effectively concentrates macronutrients while significantly reducing water availability, thereby enhancing shelf stability.

https://doi.org/10.56303/jhnresearch.v5i2.1329

((a) Fresh dadih before drying (b) Dadih after the freeze-drying process.

Figure 2. Product before and after freeze-drying

Table 1. Chemical characteristics of dadih before and after freeze-drying

No	Parameter	Unit	Before Drying	After Drying
1	Protein Content	%	7.66^{a}±0.01	31.75^{b}±0.23
2	Total Fat	%	8.01 ^{a} ±0.16	39.56 ^{b} ±1.25
3	Carbohydrate	%	2.76 ^{a} ±0.01	21.47 ^{b} ±0.54
4	Calories from Fat	Kcal/100 g	72.09 ^{a} ±1.40	356.04 ^{b} ±3.45
5	Total Calories	Kcal/100 g	113.75^{a} ±1.37	568.92 ^{b} ±1.25
6	Moisture Content	%	80.56^{a} ±0.13	2.57 ^{b} ±0.12
7	Ash Content	%	1.02 ^{a} ±0.01	4.65 ^{b} ±0.02
8	Water Activity (aw)	-	0.92 ^{a} ±0.01	0.21 ^{b} ±0.02

*Values are expressed as mean ± standard deviation (n = 3). Different superscript letters (^{a}, ^{b}) within the same row indicate significant differences between treatments ($p < 0.05$), where ^{a} represents before drying and ^{b} represents after drying.

Physical observation was conducted by comparing the color of *dadih* before and after freeze-drying using a chromameter. The color parameters of dadih before and after freeze-drying are presented in table 2. No significant differences ($p > 0.05$) were observed in L (lightness), a (green–red), and b (blue–yellow) values, indicating that freeze-drying preserved the visual appearance of the product without inducing browning or pigment degradation.

Table 2. Color characteristics of dadih before and after freeze-drying

No	Parameter	Before Drying	After Drying
1	L (Lightness)	81.2 ± 7.1	80.3 ± 6.9
2	a (Green–Red)	−1.4 ± 2.5	−1.3 ± 1.5
3	b (Blue–Yellow)	7.5 ± 3.2	7.5 ± 4.2

Sensory Characteristics

Sensory evaluation revealed measurable changes in key attributes following freeze-drying (Figure 3). The intensity of sour taste increased from 5.2 to 7.1, while milk aroma decreased from 6.8 to 5.4, and fermentation aroma decreased from 7.0 to 6.0 on a 10-point scale. These results suggest that freeze-drying may concentrate organic acids while reducing volatile aroma compounds, leading to perceptible changes

https://doi.org/10.56303/jhnresearch.v5i2.1329

in sensory profile. Nevertheless, the product maintained acceptable overall sensory characteristics.

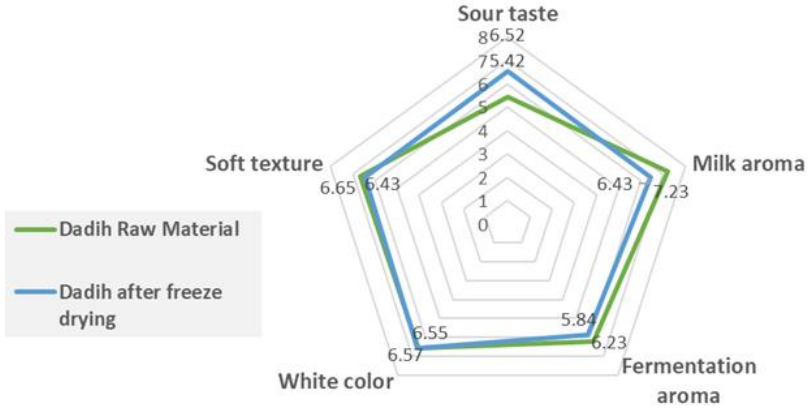

Figure 3. Sensory descriptive analysis of freeze-dried dadih

Microbiological Characteristics After Freeze-Drying

The total lactic acid bacteria (LAB) count decreased significantly ($p < 0.05$) from 1.0×10^8 CFU/g to 7.5×10^5 CFU/g, corresponding to an approximate 2.13-log reduction (Table 3). Despite this reduction, a substantial population of viable LAB was maintained in the final product, with counts approaching the minimum threshold commonly recommended for probiotic efficacy (10^6 CFU/g), indicating that freeze-drying was able to preserve a considerable proportion of functional microbial viability.

Table 3. Changes in microbiological characteristics during freeze-drying

No	Parameter	Unit	Before Drying	After Drying
1	Lactic Acid Bacteria	colony/g	1.0×10^8	7.5×10^5

DISCUSSION

The freeze-drying process effectively reduced the moisture content of dadih while maintaining the nutritional quality of the product. The observed reduction in moisture content is consistent with the mechanism of sublimation under low temperature and pressure conditions.The decrease in water activity to 0.21 indicates that the freeze-dried dadih is microbiologically stable because most pathogenic microorganisms cannot grow at aw values below 0.6(21)(22). Previous studies have reported that freeze-drying can remove 80–95% of the water content in food materials, resulting in a more stable product with extended shelf life(23). Therefore, the low aw value obtained in this study suggests that freeze-drying may significantly extend the shelf life of dadih products. This finding is in agreement with previous studies reporting that freeze-drying produces highly stable, low-moisture food systems

The increase in macronutrient content, including protein, fat, and carbohydrates, can be attributed to the concentration effect caused by water removal, rather than the synthesis of new nutrients (24). Freeze-drying process is considered to cause minimal damage to nutrients(25). Freeze-drying is widely recognized as a non-thermal preservation method that minimizes nutrient degradation. Unlike thermal drying techniques, freeze-drying limits protein denaturation and preserves heat-sensitive nutrients, thereby maintaining nutritional quality. Protein denaturation during the drying process can alter the solubility, digestibility, and availability of amino acids in proteins, thereby potentially reducing their nutritional value(26). Similar findings have been reported in other freeze-dried dairy and fermented products(27). Compared with other drying techniques such as spray drying, oven drying, or hot air drying, freeze-drying is considered superior in preserving nutritional quality because it uses low temperatures, which minimize nutrient degradation. High-temperature drying methods often lead to protein denaturation and nutrient loss (28).

The color analysis showed that freeze-drying did not significantly affect the color parameters of dadih. This result contrasts with other drying techniques that involve heat treatment, where Maillard

https://doi.org/10.56303/jhnresearch.v5i2.1329

reactions between lactose and milk proteins often lead to browning. Because freeze-drying operates at low temperatures, the Maillard reaction is minimized, allowing the product to retain its original color(29). Other studies have reported that high-temperature drying causes the lightness value (L) to decrease (30).

Sensory profile changes were observed in dadih after the freeze-drying process, particularly in terms of taste and aroma. Dadih experienced an increase in sour taste after undergoing freeze-drying, which is related to the loss of water during the drying process. The reduction in water content increases the pH of the product. The sour taste in dadih originates from organic acids, particularly lactic acid, which is a compound that is relatively non-volatile during the freeze-drying process(31). Furthermore, the aroma profile also changed. Dadih generally has a strong buffalo milk aroma combined with a characteristic fermented sour aroma. The aroma composition is produced from volatile compounds influenced by microorganisms present in the product. Compounds such as 2,3-butanedione, 2-butanone, acetaldehyde, ethanol, and acetone contribute to the characteristic aroma(32). During the freeze-drying process, the level of volatile compounds in the product decreases due to evaporation. These compounds are known to be sensitive to processing conditions and may be partially lost during freeze-drying, leading to a reduced aroma profile.(33).

After freeze-drying, the number of lactic acid bacteria in dadih decreased drastically from 1.0×10^8 colony/g to 7.5×10^5 colony/g. The reduction in bacterial count during processing is mainly caused by the freeze-drying process, which significantly decreases the moisture content and water activity (aw) in dadih. These conditions can lead to cellular damage, including damage to bacterial DNA and RNA, as well as disruption of the bacterial cell wall. This damage is also associated with increased acid concentration, which may further contribute to bacterial cell injury(34). A significant reduction in lactic acid bacteria (LAB) was observed after freeze-drying. This reduction can be explained by multiple stress factors, including ice crystal formation during freezing, osmotic stress during dehydration, and structural damage to cell membranes and nucleic acids. These stress conditions can impair bacterial viability and metabolic activity. Similar reductions in LAB viability have been widely reported in freeze-dried probiotic products.

These findings are consistent with the study by Cui et al., which reported that freeze-drying leads to a reduction in the number of lactic acid bacteria, particularly in *Lactobacillus plantarum*(35). However, the decline in microbial populations during freeze-drying is generally considered lower compared to other drying techniques such as air drying, explosion puffing drying, and microwave drying(35). Lactic acid bacteria play an important role in the food and biotechnology industries. These bacteria are widely used as starter cultures in the production of fermented foods such as yogurt, cheese, dadih, and fermented vegetables, as well as in probiotic products. Freeze-drying, or lyophilization, is a convenient method for preserving bacteria. By reducing water activity to levels below 0.2, this method enables long-term storage and distribution at relatively low cost because it does not require strict temperature control, while also minimizing the loss of microbial viability and functionality(36). Several factors influence the stability of lactic acid bacteria, including: (a) formulation factors, such as probiotic bacterial strains, microbial interactions, pH, titratable acidity, oxygen levels, moisture content, food matrix, and additives; (b) process factors, including incubation temperature, heat treatment, type of inoculation, and storage temperature; and (c) packaging materials and systems(37).

The findings of this study provide important implications for the development of traditional fermented dairy products with improved stability and commercial value. The application of freeze-drying technology in dadih processing demonstrated its ability to significantly reduce moisture content and water activity while maintaining most of the nutritional components, physical characteristics, and functional properties of the product. These changes contribute to improved product stability and extended shelf life, which are essential for wider distribution and commercialization of traditional fermented foods.

However, this study also has several limitations. First, the microbiological analysis focused only on the total count of lactic acid bacteria without identifying specific probiotic strains that may survive the freeze-drying process. Second, the study evaluated the characteristics of dadih immediately after drying, and did not assess the stability of physicochemical properties and microbial viability during long-term storage. Third, the sensory evaluation involved a limited number of trained panelists, which may not fully represent consumer acceptance in a broader market context. Therefore, further studies are needed to

https://doi.org/10.56303/jhnresearch.v5i2.1329

evaluate the stability of freeze-dried dadih during extended storage, optimize processing conditions to improve probiotic survival, and conduct consumer acceptance testing on a larger population. Such studies will strengthen the scientific basis for developing freeze-dried dadih as a sustainable and commercially viable functional food product.

CONCLUSION

In conclusion, freeze-drying significantly influenced the physicochemical, sensory, and microbiological characteristics of dadih. The process effectively reduced moisture content and water activity, contributing to improved product stability and extended shelf life, while preserving color attributes as a non-thermal technique. However, notable sensory changes were observed, including increased sourness and reduced aroma intensity due to the concentration of organic acids and loss of volatile compounds. Importantly, although lactic acid bacteria remained viable after freeze drying, their population decreased substantially from 10^8 to 10^5 CFU/g, which may limit the product's probiotic functionality.

Therefore, while freeze-drying shows potential as a preservation method for producing shelf-stable dadih, further optimization is required to enhance microbial survival. Future studies should focus on refining drying parameters and exploring protective strategies, such as the application of cryoprotectants, to achieve higher viable cell counts and improve the functional properties of the product.

DECLARATION OF THE USE OF AI

The authors declare that no artificial intelligence (AI), AI-assisted technologies, or large language models (LLMs) were used in the conception of the study, data analysis, or the drafting, writing, and editing of this manuscript. The only exception is the graphical abstract, which was created using the design platform Illustrae (https://illustrae.co/). The authors take full responsibility for the content and accuracy of the graphical abstract and the entire manuscript.

FUNDING

This research was funded by the Ministry of Health of the Republic of Indonesia through the Beginner Research Grant Scheme. The APC was funded by the same grant.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the Ministry of Health of the Republic of Indonesia for funding this research through the Beginner Research Grant Scheme. The authors also acknowledge the support of the BRIN Laboratory for providing research facilities and technical assistance during the implementation of this study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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