

**IMPACT OF DIFFERENT VACUUM OVEN DRYING TEMPERATURE
ON THE CHEMICAL, PHYSICAL, AND MICROBIAL
CHARACTERISTICS OF A DRIED PROBIOTIC YOGURT PRODUCT
DURING COLD STORAGE**

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Abstract

The objective of this research was to prolong the storage life of probiotic yogurt and facilitate its handling and transportation, in addition to preserving the physicochemical characteristics and vitality of probiotic bacteria within the permissible ranges, during storage periods. The results showed the possibility of preserving probiotic yogurt dried at three different temperatures of 40, 50, and 60 °C and pressures of 0.5 millibars to maintain its physical and chemical characteristics within acceptable ranges. The number of bacteria decreased during the storage period, but the number of bacteria that enabled them to perform their therapeutic function remained maintained. The moisture, protein, fat, carbohydrate, ash contents, pH levels, and total acidity of probiotic yogurt of 4 %, 30.01%, 1.4%, 5 4.68%, 7.89%, 4.2, and 1.1%, respectively, were within acceptable ranges at different temperatures and storage periods. Several tests were conducted on dried yogurt samples, including X-ray diffraction, electron microscopy, and thermal analysis. This demonstrated the extent of the effect of temperature and the drying process on the structure of dried probiotic yogurt after reconstitution and the extent of its effect on the bonding of the yogurt components with each other.

Keywords: vacuum oven, probiotic yogurt, X-ray diffraction, EMS, lactic acid bacteria

Introduction

Various methods have been employed to obtain dry yogurt, addressing manufacturing challenges and facilitating its transportation and marketing. These methods include freeze drying, spray drying, hot air drying, and other techniques. The efficiency of the drying process depends on the technology used and the drying conditions (Kumar and Mishra, 2004). In recent years, there has been an increase in

trends towards developing various technologies for producing dried products. However, their applications are often limited due to their impact on the final dried product's physicochemical, rheological, and microbial characteristics (Qiu *et al.*, 2019).

Today, many dried dairy products are available in the world's markets. Researchers have conducted numerous studies to address production challenges, including transportation and storage. They aimed to preserve the components and nutritional value of dairy products during the drying process, ensuring they meet quality standards and maintain their healthy microbiological activity (Erbay *et al.*, 2023).

Yogurt is one of the most prominent food products resulting from the fermentation of milk. More than being a good source of nutrients, yogurt is a healthy food that contains lactic acid bacteria. These bacteria produce many compounds that improve the health of the host. Yogurt is made using lactic acid bacteria, which are beneficial to consumers' health. The yogurt starter consists of two types of bacteria: *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Recently, various probiotic bacteria have been incorporated into yogurt production to enhance its health and functional benefits. Among these bacteria are *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Al-Sahlany *et al.*, 2022; Niamah *et al.*, 2016).

Probiotic yogurt is one of the most popular functional foods in the world. There is great importance in the availability of appropriate numbers of probiotic bacteria, which play a crucial role in determining the properties of yogurt that enhance human health (Meybodi *et al.*, 2020). The viability and activity of yogurt bacteria (lactic acid bacteria) and their survival throughout the shelf life are important commercial issues. Considering that food legislation in many countries requires these bacteria to be viable and available until consumption, it is crucial to use appropriate temperatures to maintain these characteristics. The survival of a significant number of microorganisms is essential due to their significant impact on the therapeutic effectiveness of yogurt. Several researchers have emphasized the necessity of having therapeutic bacteria in sufficient live quantities in therapeutic dairy products to ensure their therapeutic benefits. Studies have shown that the presence of *B. bifidum* and *L. acidophilus* bacteria should be at least 10^6 CFU/gram of product (Sfakianakis and Tzia, 2014).

Dried yogurt powder is added to various foods to provide the desired technical and functional properties and to improve their nutritional and sensory characteristics. These components undergo various changes during storage. The extent of these changes depends on the powder formulation, processing parameters, and storage conditions. Most changes in powder properties are related to variations in free moisture content, which is affected by fluctuations in relative humidity and temperature in the overhead space. Hygroscopicity can lead to a variety of undesirable changes, including lactose crystallization, color changes (Maillard browning), lipid oxidation, changes in protein composition, changes in surface composition, and changes in particle morphology (Phosanam *et al.*, 2021).

Fresh yogurt has a short shelf life, especially when stored at 25°C. Therefore, it requires storage at 4°C, which results in high economic costs. Storage,

transportation, and marketing also contribute to increased production costs (Kumar and Mishra, 2004).

Vacuum drying effectively reduces drying temperatures and improves drying rates, producing products with the best sensory and nutritional properties compared to those obtained using convention drying methods (Bao *et al.*, 2023). This technology utilizes reduced pressures, allowing food to be dried at lower temperatures. This approach minimizes oxidation reactions caused by air exclusion, while simultaneously preserving the flavor, color, and texture of dried food products (Alp and Bulantekin, 2021). The vacuum environment facilitates the expulsion of gases from the porous structure of the food, increasing the surface area conducive to mass transfer. This process promotes water extraction at lower temperatures, which is particularly beneficial for heat-sensitive foods, such as yogurt (Anli, 2020).

The objective of the current investigation was to transform probiotic yogurt into dried probiotic yogurt and evaluate the physical, chemical, and microbiological properties of the dried probiotic yogurt product by vacuum drying it at different temperatures while maintaining viable numbers of probiotic bacteria during storage periods. Since the drying process removes a large amount of water, it decreases the expenses associated with packaging, processing, and transportation.

Materials and methods

Raw Materials

The milk used in this study was a commercial skimmed milk powder purchased from a local supermarket. The composition of the skimmed milk powder was determined to be 5% water, 53% carbohydrates (lactose), 32% protein, and 9% ash (comprising minerals). The probiotic starter culture was acquired from the laboratories of Chr. Hansen A/S, Denmark. It was composed of a combination of *Lactobacillus acidophilus* La-5, *Bifidobacterium bifidum* Bb-12, and *Streptococcus thermophilus* in equal proportions (1:1:1). MRS-sorbitol (MRS basal medium without glucose) was prepared by mixing 10 mL of membrane-filtered sterile solutions containing 10% D-sorbitol with 90 mL of the basal medium, resulting in a final concentration of 1% sorbitol. Filter-sterilized MRS-NNLP (stock solutions of the NNPL components, namely neomycin sulfate at a concentration of 100 mg/L, paromomycin at 200 mg/L, nalidixic acid at 15 mg/L, and LiCl at 3 g/L) is commercially available from Sigma-Aldrich in Germany. Additionally, MRS agar and M17 culture media were used to activate the starter culture and enumerate the bacterial population during the production of yogurt and the subsequent storage periods.

Probiotic-Yogurt Production

An amount of 150 g of skimmed milk powder was mixed with 850 ml of distilled water to produce yogurt. The milk was heated at 95 °C for 15 min. After lowering the temperature to 40°C, a 5% concentration of an activated starter culture containing 109 CFU/g of lactic acid bacteria was added. The mixture was then incubated at 40°C until it reached a pH of 4.6. Then, the sample was cooled at 6±2°C for 24 hours, until the next tests were performed (Chandan, 2017).

Vacuum Oven Drying of Probiotic Yogurt

An amount of 50 grams of fresh yogurt was spread evenly on a glass plate (50×10×10) cm. The thickness of the yogurt layer was 0.5 cm, and the pressure applied was 0.5 bar at 40, 50, and 60°C (Abbasi and Azizpour, 2016). The dried yogurt samples were stored in vacuum bags and placed in the refrigerator at a temperature of 6±2 °C for a period of 1, 30, 60, and 90 days.

Reconstitution of Dried Yoghurt

The dried yogurt was dissolved in warm water (50°C) at a ratio of 1:6 (w:v). After stirring, the mixture was cooled to 40°C and allowed to equilibrate for 5 minutes. The mixture was then poured into a cup and let to cool down.

Yoghurt Analysis

Chemical Composition

The fresh yogurt and dried yogurt samples were analyzed for chemical composition after storage for a period of 1, 30, 60, and 90 days. Moisture content was determined by air oven at 105±2°C until constant weight (Nielsen, 1998). Total protein (N×6.38) was estimated using the Micro-Kjeldahl method (Brodziak *et al.*, 2020). The ash was estimated by a muffled furnace at 621°C for 16-20 hours (Folch *et al.*, 1957). The percentage of carbohydrates was estimated using the following equation: total carbohydrates % = 100 - (moisture % + protein % + fat % + ash %).

Physicochemical Test

The total titratable acidity and pH of fresh yogurt and dried yogurt were estimated as reported by AOAC. (2019). Water-holding capacity (WHC) was estimated for fresh and dried yogurt samples according to the method described by Niamah, (2017). Thermal analysis was performed to determine the behavior of dried yogurt samples using differential scanning calorimetry (DSC). 1 g of dried yogurt samples was taken and placed in Petri dishes inside a desiccator containing silica gel at 25°C. The samples were transferred to a sealed aluminum container and heated at 10°C/min. The temperature and heat flow were measured using zinc and indium. (Kennas *et al.*, 2020). XRD analysis was performed on dried yogurt samples using a voltage of 40 kV and a 2θ angle ranging from 10 to 30 degrees (Jaya, 2009). Scanning electron microscope (SEM) analysis of dried yogurt samples was performed by the method described by Oliveira *et al.* (2007). Dried yogurt particles were placed on double-sided carbon tape mounted on aluminum legs. Images were captured at an accelerating voltage of 5 kV and a current of 1750 mA.

Microbiological analysis

The total number of bacteria and lactic acid bacteria was estimated in the starter, probiotic yogurt, and dried probiotic yogurt samples using the casting method. The total number of lactic acid bacteria was cultured on MRS agar medium and incubated at 37°C for 24 hours under anaerobic conditions. The number of *S. thermophilus* was cultured on M17 medium under aerobic conditions at 42°C for 48 hours. As for *L. acidophilus* and *B. bifidum*, it was grown on MRS-sorbitol and MRS-NPNL medium under anaerobic conditions at 37°C for 48-72 hours (Niamah, 2019; Ashraf and Smith., 2015). *E. coli* bacteria were estimated using MacConkey agar medium, after

incubation over 24 hours at 37°C. Staphylococci count was estimated using mannitol salt agar, after incubation at 37°C for 24-48 hours. Endospore-forming bacteria were grown on Nutrient agar medium at 37 °C for 24-48 hours, following the heat treatment of samples at 85 °C for 30 min (Halim *et al.*, 2022).

Statistical Analysis

Mean values and standard deviations were calculated as part of the statistical analysis. Using GenStat 12 microarray examples for Microsoft Windows 11, the data were then submitted to one-way analysis of variance (ANOVA) and to unpaired least significant difference (L.S.D.). A *p*-value less than 0.05 was considered statistically significant.

Results and discussion

Probiotic Yoghurt Properties

Table 1 shows the chemical, physical, and microbiological properties of fresh yogurt after 24 hours of production. The contents of proteins, carbohydrates, ash, moisture, and fats were 5.21%, 5.04%, 1.10%, 87.41%, and 0.24%, respectively.

Table 1. Chemical, physical, and microbial properties of produced probiotic yogurt

	Tests	Value*
Chemical	Moisture (%)	87.91 ±2.22
	Protein (N × 6.38) %	5.41 ±0.01
	Carbohydrates (lactose)%	5.04 ±0.03
	Fat (%)	0.24 ±0.02
	Ash (%)	1.40 ±0.05
Physical	pH	4.64 ±0.08
	Total acidity (%)	1.10±0.01
	Water-holding capacity (%)	55.79 ± 3.36
Microbial	Total lactic acid bacteria (log. CFU/g)	9.66±0.11
	<i>S. thermophilus</i> (log. CFU/g)	8.80±0.25
	<i>L. acidophilus</i> (log. CFU/g)	8.75±0.36
	<i>B. bifidium</i> (log. CFU/g)	8.65±0.16
	Staphylococci (log. CFU/g)	Nil
	<i>E. coli</i> (log. CFU/g)	Nil
	Endospore-forming bacteria (log CFU/g)	Nil

*± SD: standard deviation, the result is the average of three replicates.

The increase in the proportions of yogurt components compared to raw milk was surprising, as there is no convincing explanation for it. The pH levels show a decline to 4.64, while the overall acidity exhibited an elevation of 1.10%. The reason may be due to the production of organic acids (lactic and acetic) by starter bacteria that ferment the lactose sugar present in milk. The starter bacteria counts were 9.66 log CFU/g, 8.80 log CFU/g, 8.75 log CFU/g, and 8.65 log CFU/g for total lactic acid bacteria, *S. thermophilus*, *L. acidophilus*, and *B. bifidum*, respectively. The dominance of *St. thermophilus* can be attributed to the optimal growth conditions provided by the incubation temperature of the yogurt. And no growth of staphylococci, *E. coli*, and endospore-forming bacteria was observed. The results presented in this study are consistent with those of previous research highlighting the formation of a gelatinous matrix within yogurt after the cooling stage. Furthermore, there is strong evidence indicating a decrease in pH levels and a simultaneous increase in total acidity because of the hydrolysis of lactose and its subsequent conversion to organic acids through the metabolic activities of lactic acid bacteria. This process highlights the complex biochemical changes that occur during the fermentation and maturation stages of yogurt production, highlighting the intricate interactions between microbial activity and the physicochemical properties of fermented dairy products (Alirezalu *et al.*, 2019; Ayivi and Ibrahim, 2022).

Dried Probiotic Yogurt Properties

Tables 2 and 3 show the chemical composition and physicochemical properties of dried yogurt at 40, 50, and 60°C and pressure of 0.5 bar during storage periods of 1, 30, 60, and 90 days. The effect of drying concentration on moisture content (%) was observed, with the results showing a significant decrease in moisture content, which led to an increase in the percentage of other components (protein, carbohydrates, ash, fat) as a result of heat treatment and storage. The protein and ash content values of dried yogurt at 40, 50, and 60°C and a pressure of 0.5 bar ranged between 30.01% and 32.48%, and between 7.51% and 8.82%, respectively. The results of the current research showed an increase in protein content and a decrease in ash content compared to previous studies (Kumar and Mishra, 2004; Jaafar *et al.*, 2024). The carbohydrate (lactose) values ranged between 55.52 and 51.07%. It was found that there is an inverse relationship between temperature and carbohydrate value in dried yogurt. The decrease in lactose content during storage can be attributed to the metabolic processes of viable lactic acid bacteria present in the dried yogurt product, which continue to grow slowly and secrete their hydrolytic enzymes during fermentation, which remain active during storage, thus breaking down this sugar. (Gallardo-Rivera *et al.*, 2021). The fat content of dried yogurt samples during storage ranged between 1.39% and 1.46%, despite the use of dried milk in production. In addition, most dried milks contain between 0.1% and 0.5% fat.

Table 2. Chemical ingredients of dried probiotic yogurt product at 40, 50, and 60 °C storage periods.

Chemical Ingredients	Treatment (°C)	Storage Periods (day)*			
		1	30	60	90
Protein % (N × 6.38)	40	30.01 ^a ±1.11	30.23 ^a ±1.32	30.27 ^a ±1.91	30.85 ^a ±1.88
	50	31.92 ^a ±1.09	31.96 ^a ±1.63	31.25 ^a ±1.44	31.88 ^a ±1.22
	60	32.48 ^a ±1.10	32.40 ^a ±1.99	32.43 ^a ±1.71	32.28 ^a ±1.19
Lactose %	40	54.68 ^a ±1.92	55.00 ^a ±1.92	52.60 ^a ±1.92	51.82 ^a ±1.52
	50	52.51 ^a ±1.92	55.52 ^a ±1.92	55.3 ^a ±1.92	51.07 ^a ±1.00
	60	52.05 ^a ±1.92	53.85 ^a ±1.92	53.11 ^a ±1.92	51.36 ^a ±1.87
Ash (%)	40	7.89 ^b ±0.88	7.82 ^b ±0.76	7.78 ^a ±0.77	7.67 ^a ±0.68
	50	8.22 ^a ±0.28	8.10 ^a ±0.94	7.52 ^a ±0.61	7.51 ^a ±0.81
	60	8.08 ^a ±1.00	7.98 ^a ±0.53	7.92 ^a ±0.55	7.85 ^a ±0.31
Moisture (%)	40	5.97 ^a ±0.65	5.50 ^a ±0.22	7.90 ^a ±0.62	8.20 ^a ±0.54
	50	5.90 ^a ±0.11	6.00 ^a ±0.59	4.50 ^b ±0.92	8.11 ^a ±0.71
	60	4.46 ^b ±0.80	6.00 ^a ±0.35	5.12 ^b ±0.44	7.16 ^b ±0.96
Fat (%)	40	1.45 ^a ±0.01	1.45 ^a ±0.07	1.45 ^a ±0.03	1.46 ^a ±0.01
	50	1.44 ^a ±0.06	1.43 ^a ±0.00	1.43 ^a ±0.09	1.43 ^a ±0.01
	60	1.39 ^a ±0.08	1.40 ^a ±0.01	1.42 ^a ±0.02	1.40 ^a ±0.05

*± SD: standard deviation, the result is the average of 3 replicates, ^{a,b,c} Different superscripts in a column indicate significant differences among means ($p \leq 0.05$).

As shown in Table 3, the pH values and total acidity levels ranged between 3.2 and 4.6 and between 1.20% and 1.82%, respectively, for the dried yogurt samples at 40°C, 50°C, and 60°C during storage. The current study showed no significant effect of temperature on the pH and total acidity of dried yogurt. A decrease in pH values was also observed during storage, which may be due to the increased acidity caused by the presence of starter bacteria. A pH value above 4.00 is considered suitable for preparing yogurt powders, as pH values below this limit have been consistently rejected by consumers (Ebrahim *et al.*, 2021). The water holding capacity (WHC) values of the reconstituted dried yogurt samples ranged from 34.02% to 37.28%. It is worth noting that drying at 40°C yielded the lowest WHC value. The WHC increased with increasing drying temperatures. After a storage period of 3 months, the WHC of the reconstituted dried yogurt remained stable throughout storage. The observed differences in the rehydrated samples can be attributed to increased protein aggregates in the dried yogurt samples prepared at 60°C. The formation of larger protein aggregates resulted in the most stable gel, which showed an improvement in the water content of the gel matrix. The WHC of the reconstituted dried yogurt decreased significantly compared to the fresh yogurt (Favilla *et al.*, 2022; Jaafar *et al.*, 2024).

Table 3. Physical properties of dried and reconstituted dried probiotic yogurt products during storage periods

Physical Properties	Treatment (C)	Storage Periods (day)*			
		1	30	60	90
pH	40	4.50 ^a ±0.02	4.60 ^a ±0.01	4.26 ^a ±0.00	4.10 ^a ±0.11
	50	4.40 ^a ±0.08	4.45 ^a ±0.05	4.11 ^a ±0.01	3.80 ^b ±0.01
	60	4.20 ^a ±0.07	4.35 ^a ±0.03	3.81 ^b ±0.02	3.20 ^c ±0.09
Total acidity (%)	40	1.20 ^b ±0.01	1.30 ^a ±0.05	1.36 ^a ±0.04	1.38 ^a ±0.01
	50	1.40 ^b ±0.06	1.32 ^a ±0.09	1.37 ^a ±0.00	1.42 ^a ±0.01
	60	1.82 ^a ±0.03	1.4 ^a ±0.02	1.42 ^a ±0.05	1.45 ^a ±0.01
WHC (%)	40	34.02 ^a ±1.12	34.82 ^a ±1.00	34.86 ^a ±1.66	34.91 ^a ±0.97
	50	35.22 ^a ±1.92	35.39 ^a ±1.31	35.42 ^a ±1.16	35.47 ^a ±1.11
	60	36.86 ^a ±2.01	37.25 ^a ±1.09	37.28 ^b ±1.34	37.32 ^b ±1.05

*± SD: standard deviation, the result is the average of 3 replicates, ^{a,b,c} Different superscripts in a column indicate significant differences among means ($p \leq 0.05$).

Bacteria Starter Count

The numbers of lactic acid bacteria, *S. thermophilus*, *L. acidophilus*, and *B. bifidum* were estimated in dried yogurt samples after drying in a vacuum oven at 40, 50, and 60 °C, and pressure 0.5 bar during a storage period of (1, 30, 60, and 90) days. The results showed that the number of viable bacteria decreased in *B. bifidum* compared to *S. thermophilus* and *L. acidophilus* due to the high temperatures and pressure used in the drying process. This difference may be due to the ability of *S. thermophilus* and *L. acidophilus* to withstand these conditions compared to *B. bifidum* (Figure 1). As shown in Figure 1, the results showed that the number of viable bacteria witnessed a decrease in the numbers of *B. bifidum* compared to the numbers of *S. thermophilus* and *L. acidophilus* as a result of being affected by high temperatures and pressure used in the drying process. This difference may be due to the ability of *S. thermophilus* and *L. acidophilus* to withstand these conditions compared to *B. bifidum*. The log. numbers of bacteria were measured after 1 day of storage at 40°C-60°C, where the numbers of total lactic acid bacteria, *S. thermophilus*, *L. acidophilus*, and *B. bifidum* were 8.85-9.01, 7.28-7.62, 5.33-7.35 and 6.81-7.11 log. CFU/g, respectively. After 30 days, their values reached 8.44-8.85, 6.88-7.40, 5.11-7.12, and 6.24-6.95 log. CFU/g respectively, and after 90 days, the numbers of the total count of lactic acid bacteria, *S. thermophilus*, *L. acidophilus*, and *B. bifidum* reached 8.65-7.65, 5.82-6.90, 6.85-4.32, and 5.00-6.72 log. CFU/g, respectively. All probiotic bacteria numbers continued to decline steadily during the storage period but remained within acceptable limits. The number of viable bacteria remaining depends on several factors, such as drying temperatures, the initial bacterial count in the product before drying, the applied pressure, and the exposure time (Ebrahim et al., 2021; Wirjantoro and Phianmongkhon, 2009). The ability of yogurt starter cultures to maintain a high bacterial population after the drying process is attributed to the presence of a protective matrix consisting of solids (proteins and carbohydrates) surrounding the bacterial cells (Niamah et al., 2021). The protective layer mitigates the damaging effects of drying on the cell membrane while enabling

an encapsulation process that effectively maintains bacterial viability. The use of moderate-temperature vacuum oven drying, as an alternative technology for producing yogurt powders, can improve microbial properties (Atta *et al.*, 2025). The protective layer mitigates the damaging effects of drying on the cell membrane while enabling an encapsulation process that effectively maintains bacterial viability. The use of moderate-temperature vacuum oven drying, as an alternative technology for producing yogurt powders, can improve microbial properties. The prebiotics bind to the protein and form a network that protects the lactic acid bacteria contained therein from the effects of the drying process (Kieps and Dembczyński, 2022).

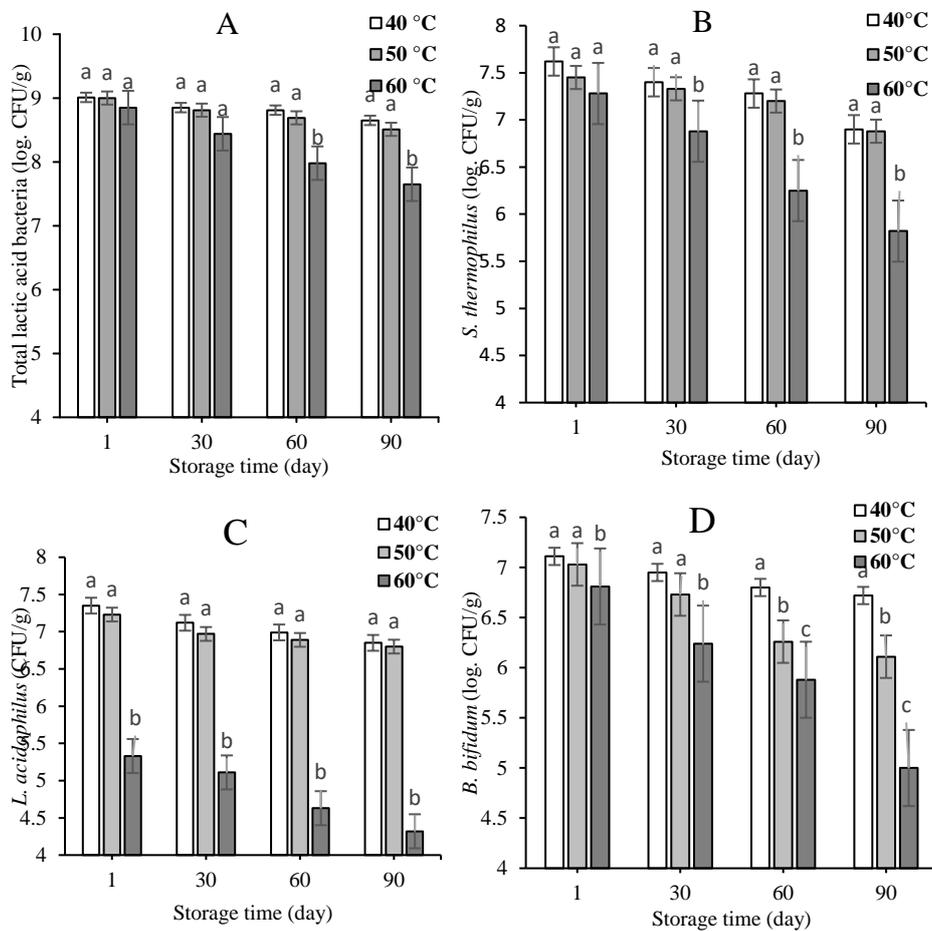


Figure 1. Log. Starter bacteria numbers of dried and reconstituted dried probiotic yogurt products during storage periods. (A) total lactic acid bacteria, (B) *S. thermophilus* numbers, (C) *L. acidophilus* numbers, (D) *B. bifidum* numbers

The results are reported as mean \pm SD. ^{a,b,c} different superscripts indicate significant differences at $p \leq 0.05$.

The results indicated that there was no growth of staphylococci, *E. coli*, and endospore-forming bacteria in the dried yogurt samples that were dried in a vacuum oven during storage periods for three months (90 days). This is attributed to the low water activity of the dried product and the inability of bacteria to grow under these conditions, in addition to the refrigerated storage conditions that limit bacterial growth activity, as well as the inhibitory metabolic products produced by lactic acid bacteria.

Thermal analyses

The dried yogurt samples' glass transition and thermal dissociation temperatures were determined at various temperatures (40, 50, and 60 °C) and a pressure of 0.5 bar, as illustrated in Figure 3.

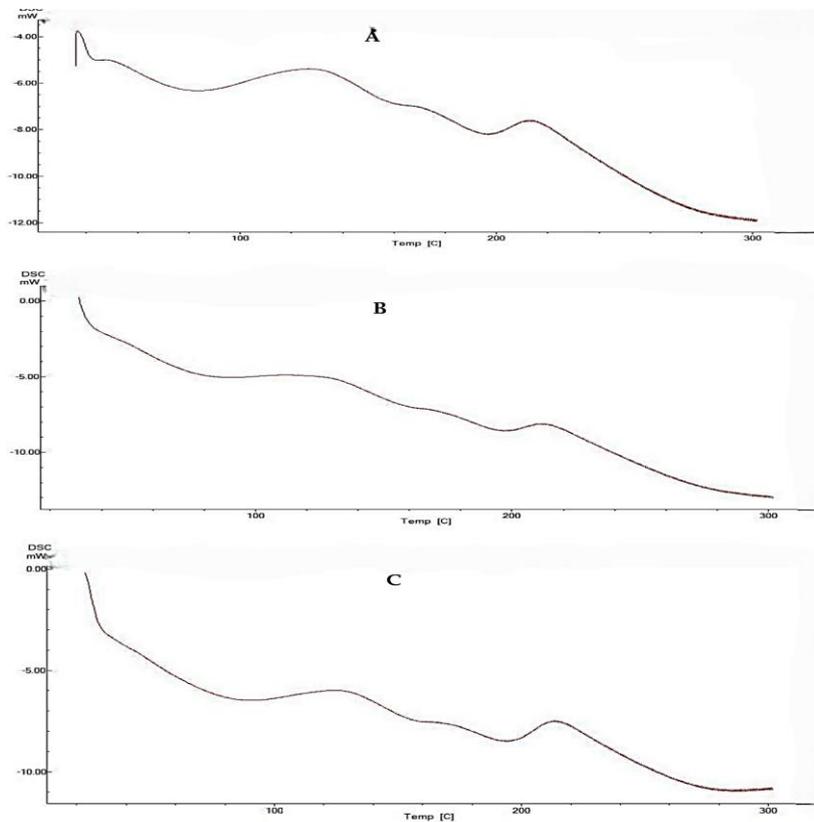


Figure 3. Differential Scanning calorimetry of dried probiotic yogurt using vacuum-oven drying at (A):40°C, (B): 50°C, (C): 60°C

An observable reduction in the DSC level is noted, which can be attributed to the breakdown of lactose resulting from the fermentation process. These results are consistent with the conclusions of Balasubramanian *et al.* (2016), who highlighted that powders containing hydrolyzed lactose exhibited significantly lower glass

transition temperatures. Additionally, past study confirmed that some harmful reactions, such as the Maillard reaction, occur at temperatures higher than those observed in the DSC test (Chávez and Ledebor, 2007). The higher temperature during drying leads to increased molecular mobility and, due to the presence of active sugar and protein groups, the browning color is formed because of the Maillard reaction. Therefore, our yogurt powder should be stored in a dry, cool environment (below 30°C) to avoid any potential structural deterioration or physical changes. The DSC values of the probiotic dried yogurt ranged between 42 and 46°C (de Medeiros *et al.*, 2014). This contrasts with the observations in this current study, where DSC values were recorded between 54 and 60°C.

X-ray diffraction analysis

X-ray diffraction analysis was conducted for dried yogurt samples as shown in Figure 4.

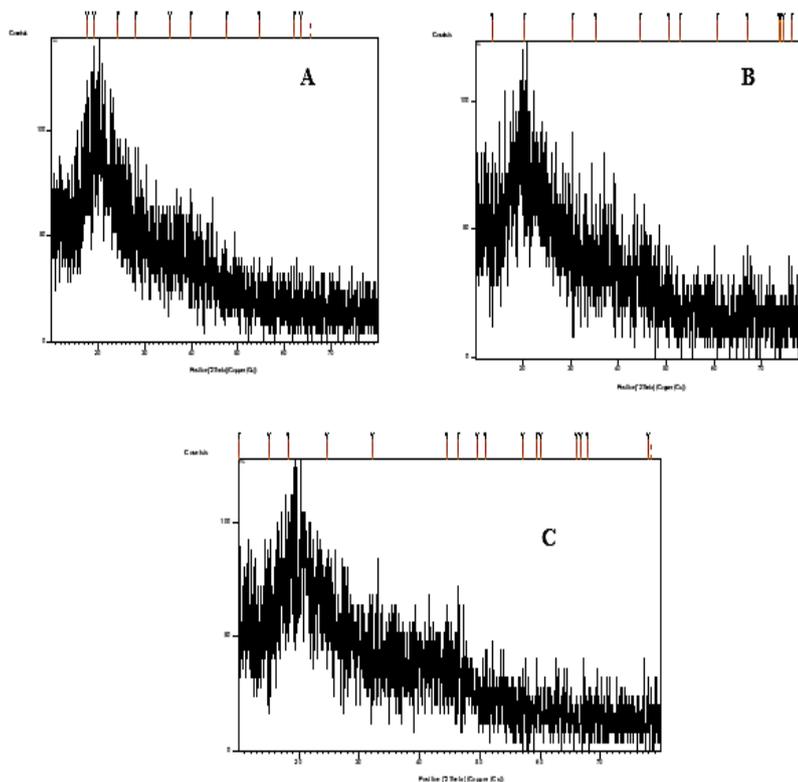


Figure 4. X-ray diffraction patterns of dried probiotic yogurt using vacuum-oven drying at (A):40°C, (B): 50°C, (C): 60°C

When drying sugar-rich products, the structure of the sugar changes from amorphous to crystalline. As a result, the final products differ in particle size, physicochemical properties, chemical stability, water solubility, and hygroscopicity. The study

showed that there were slight differences between vacuum oven-dried yogurt samples, which showed an amorphous structure. These results agreed with studies (Jaya, 2009; Kennas *et al.*, 2020), who agreed that dried yogurt gives an amorphous structure, and this is mainly due to the fermentation of the lactose sugar during the drying process. Lactose is the sugar primarily responsible for providing the crystalline structure. This amorphous structure is desirable in food powders, especially those containing viable cultures such as lactic acid bacteria, as it protects these cells from damage caused by the crystalline structure. The crystallization process of lactose sugar begins at 93.5 °C (Wong and Hartel, 2014), and this temperature is not used in the vacuum oven during the current study.

Scanning electron microscopic analysis (SEM)

Figure 5 shows scanned images of dried yogurt samples using a scanning electron microscope after drying by vacuum oven. Fat globules found in dehydrated yogurt formed pores.

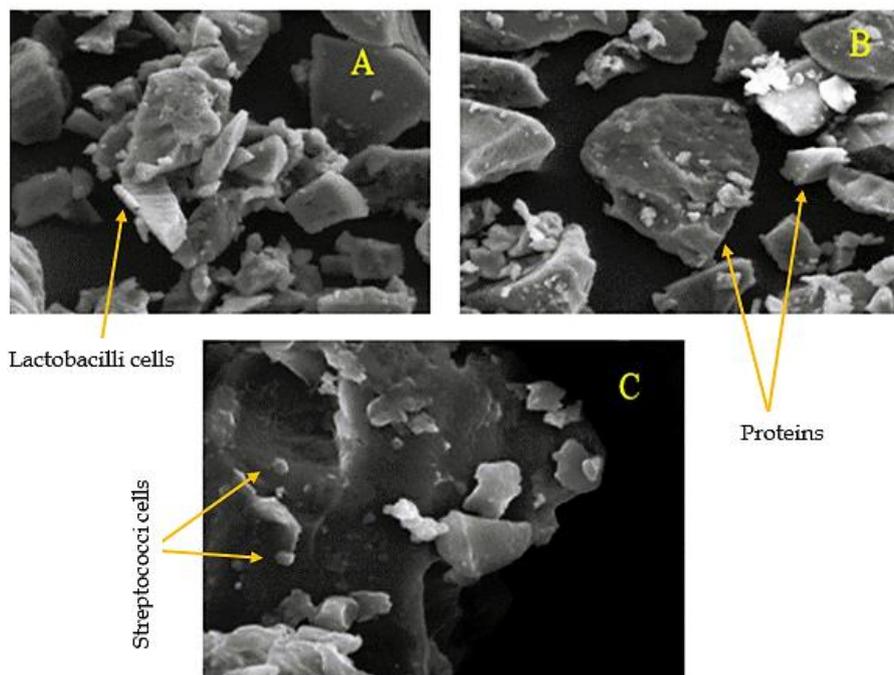


Figure 5. Scanning electron microscopic of dried probiotic yogurt using vacuum oven drying at (A):40°C, (B): 50°C, (C): 60°C

During the vacuum oven drying process, the hydrogen bonds that bind the outer layer of proteins to the bacterial cell wall of yogurt were broken. However, the effect of vacuum oven drying on these bonds is significantly less pronounced when compared to the effect of electric oven drying, microwave drying, and other similar techniques. The reason behind the thermal resistance exhibited by most thermophilic lactobacilli lies in the existence of additional bonds that enhance the stability of the secondary

and tertiary structures of the proteins, thus contributing to their overall thermal stability (Ar and Ocak, 2022). These bonds include disulfide bonds, hydrogen bonds, ionized group interactions, and hydrophobic bonds. The yogurt samples consisted of a three-dimensional network of protein chains and aggregates of casein micelles. This structure was partially preserved in the vacuum-dried yogurt, with fewer chains and more clusters. Usually, proteins aggregate when heat treatment is combined with agitation, which is the case with vacuum drying (Wilbanks *et al.*, 2023). This network of protein chains gives the finished product its strength. The results show that the vacuum-dried samples better retained the overall microstructure of the yogurt at different temperatures compared to other drying methods (Jaya, 2009).

Conclusions

The results demonstrated the feasibility of using a vacuum oven for drying the probiotic yogurt at thermodynamic conditions of 40, 50, and 60°C and preserving it for three months. Of the three thermodynamic conditions applied during the drying process, 50°C emerged as the best. The results also demonstrated the extent to which the samples retained their physical and chemical properties, as well as the stability of probiotic bacteria (starter cultures) at the desired levels throughout the three-month preservation period. Structural changes did not affect the chemical composition of protein, lipids, minerals, and carbohydrates in the dried probiotic yogurt. Microstructural changes in the dried yogurt resulting from vacuum drying were demonstrated through scanning electron microscope (SEM) images. Unlike fresh yogurt, which has a limited shelf life, the dried probiotic yogurt conferred significant biological value, even after three months of preservation. Furthermore, the dried probiotic yogurt was easier to transport and process than fresh yogurt.

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