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EXPLORING THE INCORPORATION OF MULBERRY (*MORUS ALBA* L.) INTO FREEZE-DRIED YOGURT FOR ENHANCED NUTRITIONAL VALUE AND QUALITY

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ABSTRACT

Yogurt, popular worldwide, is nutrient-rich with calcium, potassium, protein, B vitamins, and beneficial microorganisms. Mulberries possess medicinal qualities, notably anthocyanins, which have antioxidant, anti-diabetic, and anti-bacterial effects. Though fruit-flavored yogurt is popular, producing it is challenging because of its limited shelf life. However, freeze-drying technology can address this, allowing prolonged storage without compromising its quality. This study evaluated incorporating mulberry into yogurt to enhance its nutritional value and quality while seeking an optimal production process for freeze-dried mulberry yogurt. Mulberry syrup was added in various ratios, demonstrating statistically significant effects on the yogurt's physicochemical properties. Particularly, a 9:1 mulberry syrup to yogurt ratio exhibited a total polyphenol content of 170.84 mg GAE/100 g, marking a 94% increase compared to the yogurt without mulberry syrup. This substantial enhancement signifies an improvement in yogurt efficiency when mulberry is integrated. Similarly, DPPH and ABTS free radical scavenging activities for the 9:1 ratio were observed at 12.22 mgAAE/100g and 22.55 mgAAE/100g, which is an enhancement of 1.89% and 15.99%, respectively, compared to the reference yogurt sample (10:0 ratio). The quality changes in the freeze-dried mulberry yogurt were monitored over a 28-day storage period. The results revealed relatively stable physicochemical properties, microbiological density, and biological activity throughout the storage, highlighting the potential of freeze-drying technology in creating innovative and nutritious yogurt products.

1. Introduction

Yogurt, a dairy product with high nutritional value and delectable taste, is widely consumed across the globe (Sun-Waterhouse et al., 2012). Produced by fermenting milk with lactic acid bacteria, yogurt boasts a tangy flavor and creamy texture (Miller et al., 2006). It is a rich source of essential nutrients that contribute to overall health, including calcium, potassium, protein, vitamin B, and probiotics (Gilliland, 1989; Rizzoli, 2014). Numerous studies have demonstrated the benefits of yogurt consumption, such as improved digestion, reduced cholesterol levels, and prevention of diarrhea (Desobry-Banon et al., 1999). Consequently, there is a growing interest in developing innovative and nutritious yogurt products to cater to consumer needs.

Fruit-flavored yogurt has become a household favorite worldwide, offering a diverse array of fruity flavors while enhancing the product's visual appeal (Ha et al., 2021; Lutchmedial et al., 2004; Sung et al., 2015). One such fruit is the mulberry (*Morus alba* L.), a member of the *Moraceae* family that is globally cultivated and distributed under varied climatic conditions, ranging from tropical to temperate (Yuan & Zhao, 2017). Traditionally employed as a medicinal remedy for colds, liver protection, joint fortification, and blood pressure reduction, mulberries are a rich source of minerals like potassium, manganese, and magnesium (Butt et al., 2008; Yuan & Zhao, 2017; C. Wang et al., 2019). They also rich in components like as anthocyanins, which have antioxidant, anti-diabetic, and anti-microbial properties. Other, compounds such as albafuran, bergaptan, and cyanidin-3-glucosides in mulberries are known for their antioxidant, antibacterial, and anti-inflammatory effects (H. Wang et al., 1997; Grace et al., 2009; C. Wang et al., 2019). In Vietnam, mulberries are primarily processed into wine or jam, highlighting the need to diversify mulberry-based products, which could benefit farmers and food processing enterprises.

However, the production of fruit yogurt presents the challenge of a relatively short shelf life, necessitating low-temperature storage, and transportation (Deshwal et al., 2021). Freeze-drying presents itself as a potential solution to this issue (Valentina et al., 2016). This process involves the removal of water from a product through sublimation, enabling long-term storage at room temperature while preserving the organoleptic, biological, and nutritional properties of the dried product (McDonough et al., 1982; Barbosa et al., 2015; Ermis, 2022). This method could introduce a new innovation: freeze-dried mulberry yogurt enriched with probiotics. This product would combine the high nutrient content of dairy products with biologically active fruit ingredients, such as anthocyanins.

This study aims to evaluate the potential of integrating mulberries into yogurt to augment its nutritional value and quality and devise an effective production process for freeze-dried mulberry yogurt enriched with probiotics. We will analyze the physicochemical properties, sensory value, biological activity, and stability of the yogurt during storage to assess the feasibility of this approach. This investigation presents a unique solution to the challenges associated with fruit yogurt production and has the potential to significantly impact the food industry by contributing to the development of new functional food products.

2. Materials and methods

2.1. Materials

2.1.1.Samples

All ingredients used for yogurt production, such as whole milk powder, saccharose, modified starch, and gelatin, were purchased from a local company in Ho Chi Minh City, Vietnam. The mulberries utilized in this study was procured from a local market, washed with tap water, drained, and blended using a kitchen blender (HR2223/00, Philip, China). The resultant puree was then transferred to a sealed plastic bag and stored at -18°C subsequent use.

2.1.2.Chemicals

The chemicals used in this study included sodium hydroxide (NaOH) 0.1N, which was purchased from Cemaco (Vietnam); methanol (CH_3OH) 99.7%, phenolphthalein, and sodium bicarbonate (Na_2CO_3) 99.5% which were purchased from Xilong (China); Folin-Ciocalteu 2N, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azisbonis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS), and gallic acid which were purchased from Sigma Aldrich (USA). In addition, culture, media like MRS (De Man, Rogosa, and Sharpe) agar and MRS broth were purchased from HiMedia (India).

2.2. Methods

2.2.1.Manufacture of yogurt

This procedure is based on the work by (Golmakani et al., 2021) and our preliminary test. Specifically, the milk mixture comprised 12% whole milk powder, 7.0% sucrose, 1% modified starch, 0.5% gelatin, and 79.5% hot

water at 85 °C. Subsequently, 150 mL of milk was poured into sterilized glass bottles, sealed, and pasteurized at 85 °C for 10 min in a distillation pot (Tomy, SS-325, Japan). Afterward, the milk was cooled down 37 °C and 0.006% of the biomass of commercial probiotic Chr Hansen YC-X11 Thermophilic Yogurt Culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was added to the mixture. Samples were kept in an incubator (DH5000II Faithful, China) for 24 h at 37 °C. The final yogurt reached a pH of 4.33 ± 0.01, a total acid content of 0.75% ± 0.01, and a bacteria density of ~10.3 log CFU/g. Samples were stored in a refrigerator at 4-6 °C, for no more than 24 hours until further experiments were carried out.

2.2.2.Freeze dried yogurt-mulberry procedure

To formulate the yogurt-mulberry blend, yogurt and mulberry syrup were combined in various ratios, specifically: 10:0, 9:1, 8:2, 7:3, 6:4, and 5:5. The prepared mix was poured into a silicone mold (dimensions: 1 x 2 x 1 cm) and frozen at -20 °C for 4 hours, setting the stage for freeze-drying. The dehydration process was executed using an automatic freeze dryer (HR-3, Harvest right, USA), operating at -20 °C with a pressure of 500 mTorr over 24-26 hours in vacuum. After drying, the samples were brought to room temperature, sealed in plastic bags, and stored between 25-27°C. Notably, samples were retained for no more than 24 hours before further analysis.

2.2.3.Physicochemcial properties

The TSS of the samples was measured using a handheld refractometer (0-32%, Alla, France). The total acidity was measured using the titration method with 0.1N NaOH and 1% phenolphthalein as an indicator (Zahid et al., 2022). The data was reported as lactic acid content (%). The pH of the samples was examined using a pH meter (Hanna, HI2210-02, Romania). Prior to evaluation, all FD yogurts were ground and completely dissolved in distilled water. The moisture content and water activity of the samples were measured using a moisture analyzer (MB90, Ohaus, USA) and a water activity meter (Novasina, LabTouch, Switzerland), respectively.

2.2.4.Color measurement

The color properties of samples such as L*, a*, and b* were measured by using a handheld chroma meter (Konica Minolta, CR-400, Japan). In this context, L* indicated lightness/darkness, a* indicated greenness/redness, and b* indicated blueness/yellowness. The different colors between the freeze-dried yogurt with and without mulberry was also determined following the equation (1) as described by (Tuyen et al., 2015)

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad (1)$$

Where: L_1*, a_1*, and b_1* are the parameters of sample without mulberry and L_2*, a_2*, and b_2* are the parameter of samples that were mixed with mulberry.

2.2.5.Total anthocyanins content (TAC)

The total anthocyanin content was determined using the method by (Lee et al., 2005). Initially, to ascertain the appropriate dilution factor, the test portion was diluted using a pH 1.0 buffer until the absorbance at 520 nm fell within the linear range of the spectrophotometer. Once this dilution factor was established, the test sample was prepared in two different dilutions: one using a pH 1.0 buffer and the other using a pH 4.5 buffer. The absorbance of each of these test portions, diluted with the respective buffers, was measured at two distinct wavelengths, namely 520 nm and 700 nm. A blank cell filled with distilled water was used as a comparative reference for these diluted test portions. All absorbance measurements were conducted within a time frame of 20 to 50 minutes post-preparation.

Following the measurements, the anthocyanin pigment concentration was calculated and expressed in cyanidin-3-glucoside (cyd-3-glu) equivalents. The formula used for this calculation is as follows (2):

$$C = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (2)$$

Where:

A: (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5;

MW (molecular weight): 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu);

DF: the previously determined dilution factor;

l: pathlength in cm; and

ε: 26,900, which is the molar extinction coefficient in L x mol^{-1} x cm^{-1} for cyd-3-glu;

10^3: factor for conversion from g to mg.

2.2.6.Total phenolic content (TPC)

The analysis was conducted according to the method of (Lim et al., 2007), with slight modifications. In a glass tube, the diluted solution (0.3 mL) was thoroughly mixed with 10% Folin–Ciocalteu solution of 1.5 mL. The mixture was then left at room temperature for 5 minutes in dark conditions. Next, the mixture was vortexed with a 7.5% Na_2CO_3 solution of 1.2 mL and incubated at room temperature for 30 minutes in a dark condition. Finally, the sample the absorbance of the sample was then measured using a spectrophotometer (Thermo Scientific, Evolution 60S, USA) at a wavelength of 765 nm. Gallic acid, with the concentration ranges from 0 µg/mL to 70 µg/mL, was used for the standard curve, and the total phenolic content was expressed as mg gallic acid equivalents per 100 grams of dried sample (mg GAE/100g dw). The blank contains acidified methanol and reagent.

2.2.7.Antioxidant activity using DPPH reagent

The analysis followed (Phuong et al., 2020), with slight modifications. Firstly, the DPPH working solution was prepared by dissolving 3.94 mg of DPPH in 100 mL methanol and kept in the dark at -20 ℃ for further use. Then, the 0.1 mL diluted solution was thoroughly mixed with 2 mL DPPH working solution. The mixture was then left at room temperature for 30 minutes in a dark condition. Finally, the sample the absorbance of the sample was subsequently measured using a spectrophotometer (Thermo Scientific, Evolution 60S, USA) at a wavelength of 517 nm. Ascorbic acid with various concentrations from 0 µg/mL to 100 µg/mL was used as a standard. The antioxidant activity of yogurt samples was expressed as mg ascorbic acid equivalent per 100 g dry matter (mg AAE/100g dw). The acidified methanol was used as a blank sample.

2.2.8.Antioxidant activity using ABTS reagent

The analysis was conducted as described by (Phuong et al., 2020), with slight modifications. Firstly, the ABTS stock solution was prepared by mixing an equal amount of 7 mM ABTS solution (10 distilled water containing 0.0384 g ABTS) and 2.45 mM potassium persulfate solution (10 mL distilled water containing 0.0066 g kali persulfate) and left at room temperature for 12 hours. The stock solution was then diluted with distilled water to reach the OD value of 0.7 (± 0.02) by using a spectrophotometer at a wavelength of 734 nm. The ABTS working solution was prepared 15 minutes before use. In a glass tube, the diluted solution (0.1 mL) was combined with 3.2 mL ABTS working solution. The mixture was then left at room temperature for 5 minutes in dark conditions. Finally, the sample the absorbance of the sample was then measured using a spectrophotometer (Thermo Scientific, Evolution 60S, USA) at a wavelength of 734 nm. The total phenolic content was calculated based on the standard curve of ascorbic acid (ranging 0-50 µg/mL). The data was expressed with a unit as ascorbic acid equivalent/100g dry matter (mg AAE/100g dw). The blank contains acidified methanol.

2.2.9.Viable cell count

One gram of the sample was diluted in a 1:10 ratio with sterilized and then spread on MRS agar plates. The dishes were incubated for 48 hours at 37 ℃ in an incubator. The number of viable cells was quantified as log colony-forming units per gram (log CFU/g) (Barbosa et al., 2015).

2.2.10. Sensorial evaluation

The preference for freeze-dried yogurts was scored by 20 trained panelists that comprising ten males and ten females, aged between 20 and 30 years. The selected panelists consumed yogurt at least two times a week and were free of allergic dairy products. The samples were

presented on a white ceramic plate and coded with randomly coded with three-digit numbers. The assessor asked to rinse their mouths odorless water between sampling each product. The sample's preference was described using a 7 points hedonic scale, herein 1: dislike very much and 7: like very much (Peryam & Pilgrim, 1957). The sensory characteristics, including color, aroma, flavor, and texture, were evaluated in this study.

2.2.11.Statistical analysis

The data obtained from the various experiments are presented as mean ± standard deviation. All data processing, calculations, and graphical representations were conducted using Microsoft Excel 2019. To determine the statistical significance of the experimental results, a one-way Analysis of Variance (ANOVA) followed by the Least Significant Difference (LSD) test was performed. These analyses were executed using STATGRAPHICS Centurion XV software, and statistical significance was established at a p-value of less than 0.05.

3. Results and discussions

3.1.Physicochemical properties and bioactive compounds of fresh mulberry fruit

The physicochemical properties and bioactive compounds of fresh mulberry fruit are detailed in Table 1. These attributes include encompass moisture content, water activity, total sugar content, and total acidity, which are crucial to understanding the unique properties of the mulberry fruit. The sweetness of the fruit is measured by total sugar content and its tartness by total acidity. Likewise, the freshness and shelf-life assessed by moisture content and water activity. The study also examines additional parameters, such as total dissolved solids, ash content, and pH values. These correlate with the findings of previous research on mulberries, validating our results (Saensouk et al., 2022; Sangteerakij et al., 2023). The color of mulberry fruit, ranging from burgundy to black, has been quantified precisely using L*, a*, and b* values, serving as indicators of ripeness (Table 1 and Figure 1). This color analysis is in line with the findings of (Sangteerakij et al., 2023). The documents study reports a remarkable total polyphenol content of 1562.2 mg GAE/100 g in mulberry fruit, echoing earlier studies. The observed is greater than the 10.3 mg GAE/100 g found by (Liu et al., 2009), but falls short of the 2570 mg GAE/100 g reported by (Bae & Suh, 2007). Our mulberry samples display a notably higher anthocyanin content of 261.53 mg/g, exceeding the 65.23 mg/g noted by (Sangteerakij et al., 2023). The variance in these findings might stem from differences in analytical methodologies, fruit quality, variety, environmental factors, climatic conditions, and ripeness at harvest (Chen et al., 2022; Saensouk et al., 2022). Furthermore, our evaluation of the mulberry's antioxidant capacity using DPPH and ABTS free radical neutralizing assays yielded values of 74.45 and 88.04 mg AAE/100 g, respectively, supporting Chen et al., (2022) and Saensouk et al., (2022) description of mulberry's potent antioxidant characteristics. The fruit's antioxidant capacity originates mainly from polyphenols, anthocyanins, and flavonoids, and includes riboflavin (vitamin B2), niacin (vitamin B3), and ascorbic acid (vitamin C) (Okatan et al., 2016). In summary, mulberries boast numerous health and nutritional benefits attributable to their high content of phenolic compounds, total flavonoids, anthocyanins, and antioxidant activity (Chen et al., 2022). It's important to note, however, that these compounds' concentrations can fluctuate based on the fruit's ripeness, highlighting the importance of a thorough evaluation of the raw material to ensure the best processing results (Punthi & Jomduang, 2021). Considering mulberries have a relatively low sugar content, pre-processing adjustments may be useful to fine-tune the final product's flavor.

Table 1. Physicochemical properties and bioactive compound of mulberry fruits

Mulberry fruits	Parameters
Physicochemical properties	
Moisture content (%)	77,34±0,06
Water activity (aw)	0,97±0,01
Total sugar content (%)	8,1±0,10
Total acidity (%)	0,42±0,02
Total Soluble Solids (˚Brix)	9,8±0,1
Ash (%)	2,81±0,2
pH	3,84±0,01
Color values	
L*	24,45±0,12
a*	4,96±0,30
b*	-1,31±0,1
Bioactive compound	
TPC (mg GAE/100 g dw)	1562,2 ± 55,19
TAC (mg/L)	261,53 ±2,83
DPPH (mg AAE/100 g dw)	74,45±2,87
ABTS (mg AAE/100 g dw)	88,04±0,06

Values are expressed as mean ± standard deviation.

Figure 1. The mulberry fruits fully ripened stage.

3.2.Effect of mulberry syrup in different proportions on physicochemical properties and bioactive compound of freeze-dried mulberry yogurt

The physicochemical analysis detailed in Table 2 demonstrates that variations in the percentage of mulberry syrup addition do not significantly influence the moisture content and water activity in freeze-dried yogurt. These attributes, namely moisture content and water activity, are crucial indicators for assessing potential microbial growth and the overall stability of dried products (Tapia et al., 2020). Research establishes that a water activity threshold below 0.60, along with a moisture content less than 5.00%, prevents bacterial growth, auto-oxidation, hydrolysis, and enzyme activity, thus ensuring that dried foods can be stored at room temperature without the risk of spoilage (Bosnea et al., 2017). No statistical difference ($p<0.05$) was observed in these two parameters among the various freeze-dried yogurt samples. After freeze-drying, moisture values ranged between 2.08-4.33%, remaining below the 5.00% threshold, while the water activity values hovered around 0.30, well below the 0.60 limit. This suggests that the freeze-dried yogurt samples remained relatively stable. Unlike moisture content and water activity, the color, total acidity (TA), and total soluble solids (TSS) values displayed statistically significant changes ($p<0.05$) with alterations in mulberry syrup addition. The total acidity decreased, while the total dissolved solids increased a steadily increased with the increasing rate of mulberry syrup addition. The sample with the

most mulberry had the lowest TA value at 2.28% and the highest TSS at 93.06%. In contrast, the yogurt sample without added mulberry syrup recorded the highest TA at 3.23% and the lowest TSS at 78.66%.

Furthermore, the addition rates of mulberry syrup significantly impact both the biological and antioxidant activities of the product. As outlined in Table 3, the total polyphenol content (TPC), total anthocyanin content (TAC), and antioxidant activity against DPPH and ABTS free radicals tend to rise with increasing amounts of mulberry syrup. There exist statistically significant differences ($p<0.05$) between samples as the mulberry syrup proportion increases. Based on the addition ratios from 10:0 to 5:5 of mulberry syrup in yogurt samples, the total polyphenol content ranges from 170.84 to 213.30 mg GAE/100 g DM, while the total anthocyanin content varies between 63.24 and 78.91 mg/L. The antioxidant activities of DPPH and ABTS follow the trends seen in TPC and TAC, showing a similar increase as the mulberry syrup ratio increases from 10:0 to 5:5.

Mulberries also contain valuable components like anthocyanins, known for their antioxidant, anti-diabetic, and anti-microbial properties. Other notable compounds in mulberries, such as albafuran, bergaptan, and cyanidin-3-glucosides, have been recognized for their antioxidant properties (Wang et al., 2019). Thus, the incorporation of mulberry syrup enhances polyphenol and anthocyanin contents and improves the yogurt's texture. Prior studies have shown that the addition enhance antioxidant content in yogurt. For instance, an increase in TPC value of 4.17, 5.63, 7.63, and 9.63 mg GAE/100 g was observed corresponding to the addition of 0%, 1%, 3%, and 5% apple pomace in yogurt (Jovanović et al., 2020). Another study revealed that yogurt with 20% dates had a total polyphenol content of 37.00 mg GAE/100 g, a value 4.3 times higher than that of unsupplemented yogurt (Arfaoui et al., 2020).

Table 2. Physicochemical properties of yogurt samples mixed with mulberry syrup

Yogurt: Mulberry syrup (w/w)	Moisture content	Water activity	Total soluble solids (TSS %)	Total acidity (TA %)	pH
5:5	$4,33^{a}\pm 0,11$	$0,35^{a}\pm 0,01$	$91.33^{a} \pm 0.29$	$2,28^{e} \pm 0,02$	$3,38^{f} \pm 0,01$
6:4	$3,95^{b}\pm 0,31$	$0,35^{a}\pm 0,01$	$89.97^{b} \pm 0.06$	$2,47^{d} \pm 0,04$	$3,42^{e} \pm 0,01$
7:3	$3,6^{bc}\pm 0,24$	$0,33^{a}\pm 0,01$	$89.32^{c} \pm 0.28$	$2,54^{d} \pm 0,2$	$3,64^{d} \pm 0,01$
8:2	$3,45^{bc}\pm 0,52$	$0,33^{a}\pm 0,01$	$83.83^{d} \pm 0.29$	$2,68^{c} \pm 0,04$	$3,58^{c} \pm 0,01$
9:1	$3,39^{bc}\pm 0,64$	$0,30^{a}\pm 0,10$	$80.33^{e} \pm 0.58$	$3,00^{b} \pm 0,02$	$3,72^{b} \pm 0,01$
10:0	$2,08^{c}\pm 0,46$	$0,22^{a}\pm 0,01$	$77.67^{f} \pm 0.29$	$3,23^{a} \pm 0,01$	$3,88^{a} \pm 0,01$

Values are expressed as mean ± standard deviation. The digits in the same column indicate a statistically significant difference ($p<0.05$).

Table 3. Bioactive compound of yogurt samples mixed with mulberry syrup

Yogurt: Mulberry syrup (w/w)	TPC (mg GAE/100g)	TAC (mg/L)	DPPH (mgAAE/100g)	ABTS (mgAAE/100g)
5:5	$213,30^{a} \pm 1,63$	$78,91^{a} \pm 0,24$	$15,39^{a} \pm 1,17$	$22,55^{a} \pm 0,23$
6:4	$200,89^{b} \pm 0,72$	$75,09^{b} \pm 0,38$	$15,62^{a} \pm 0,26$	$21,44^{b} \pm 0,18$
7:3	$197,34^{b} \pm 1,16$	$70,64^{c} \pm 0,48$	$14,25^{ab} \pm 0,05$	$20,22^{c} \pm 0,10$
8:2	$186,14^{c} \pm 1,43$	$66,84^{d} \pm 0,65$	$13,16^{bc} \pm 0,07$	$19,76^{c} \pm 0,12$
9:1	$170,84^{d} \pm 4,75$	$63,42^{e} \pm 0,95$	$12,22^{c} \pm 0,52$	$18,98^{d} \pm 0,01$
10:0	$76,29^{e} \pm 1,00$	$0,00^{f} \pm 0,00$	$10,33^{d} \pm 0,25$	$2,99^{e} \pm 0,59$

Values are expressed as mean ± standard deviation. The digits in the same column indicate a statistically significant difference ($p<0.05$).

3.3.Effect of mulberry syrup in different proportions on the color of freeze-dried mulberry yogurt

Table 4 presents the colorimetric analysis of mulberry yogurt produced with varying ratios of mulberry syrup. The results indicate that the L* (lightness), a* (red-green component), and b* (yellow-blue component) values tend to increase with decreasing amounts of mulberry syrup. The sample supplemented with 50% mulberry displayed the lowest L*, a*, and b* values, representing the darkest purple-red color among the surveyed samples. Conversely, the sample without mulberry (ratio 10:0) exhibited the highest L* and b* values, showcasing the typical ivory-white color characteristic of yogurt. A statistically significant difference ($p<0.05$) was observed in the color difference (ΔE) between mulberry-supplemented samples and those without mulberry (control samples). The ΔE value demonstrated a decreasing trend with the reduction in the rate of mulberry addition. However, all ΔE values exceeded 3.00, indicating that the color difference between samples is discernible to the naked eye (Figure 2). Anthocyanin compounds found in mulberries, such as Cyanidin-3-glucoside, Delphinidin-3-glucoside, and Petunidin-3-glucoside, provide the fruit with its characteristic deep purple-red color (Yawadio & Morita, 2007). These pigments are also known for their antioxidant properties, with potential benefits in reducing the risk of cancer and cardiovascular diseases (Grace et al., 2009; Wang et al., 2019). Consequently, the addition of mulberry syrup has a direct impact on the color of freeze-dried mulberry yogurt, with higher ratios giving a darker purple-red hue, reminiscent of mulberries.

Table 4. Color change between samples of mulberry yogurt freeze-dried with different ratios

Yogurt: Mulberry syrup (w/w)	Color			ΔE
	L*	a*	b*	
5:5	49,630^{d}± 1,620	10,037^{e}± 0,521	-2,290^{d} ± 0,171	48,774^{ab}± 1,437
6:4	49,263^{d}± 1,006	18,050d± 1,091	-1,557^{c} ± 0,023	50,669^{a}± 1,158
7:3	53,167^{c}± 0.597	21,703^{c}± 0,836	-0,650^{b} ± 0,300	48,447^{ab}± 1,051
8:2	55,013^{c}± 1.786	24,777^{b} ± 0,682	-0,373^{b} ± 0,188	48,130^{ab}± 2,064
9:1	59,940^{b}± 0,288	28,373^{a} ± 0,242	-0,347^{b} ± 0,170	45,960^{b}± 0,346
10:0	96,017^{a}± 0,386	2.030^{f} ± 0,035	10,470^{a} ± 0,156	-

Values are expressed as mean ± standard deviation. The digits in the same column indicate a statistically significant difference ($p<0.05$).

Figure 2. The visual appearance of freeze-dried mulberry yogurt at different ratios

3.4.Sensory evaluation of freeze-dried mulberry yogurt

The sensory evaluation of the freeze-dried mulberry yogurt uncovered nuanced preferences related to color, aroma, taste, and texture. The 8:2 mulberry syrup to yogurt ratio achieved the highest score in terms of color (6.15 out of 7.00 points), whereas the 5:5 ratio scored the lowest at 2.55 out of 7.00 points (Table 5). Incorporating mulberry syrup enhanced the aroma, adding compounds like esters, aldehydes, ketones, and terpenes, among others (Calín-Sánchez et al., 2013; Mostafa et al., 2022) to the inherent volatile organic acids in yogurt. The yogurt sample without mulberry syrup was the least preferred, earning 3.2 points,

while the 8:2 and 9:1 samples attained the highest ratings (~5.00 out of 7.00 points). Notably, the 5:5 ratio detracted from taste preferences due to its overpowering sweetness and less crisp texture, evident in the sensory scores of 2.55 and 2.00 points out of 7.00, respectively. In opposition, the 9:1 sample earned considerable appreciation for both taste and texture, garnering 6.10 and 6.45 points out of 7.00, respectively. This balance of sweet and sour was linked tothe sublimation drying process, which increases the concentration of lactic acid in the yogurt, thus enhancing its acidity (Fellows, 2017). The porosity of the samples was inconsistent, rising from the 10:0 to 8:2 ratio and subsequently reducing towards the 5:5 ratio. This change is tied to the physical properties of the ingredients and their interactions during fermentation and drying (Dai et al., 2021). The carbohydrates and fibers in mulberries, including sugars, cellulose, hemicellulose, pectin, and lignin, seemed to bolster the yogurt's structure post-drying (Lin & Tang, 2007), esulting in a firmer and more pleasing texture. In contrast, products devoid of mulberry dissolved swiftly in the mouth, and those with excessive mulberry came across as overly hard and less crunchy. Notably, the 9:1 yogurt to mulberry ratio garnered a high sensory evaluation score across most categories, highlighting its positive effect on overall product quality. Consequently, the 9:1 ratio was selected for further exploration.

Table 5. Sensory evaluation of samples of freeze-dried mulberry yogurt

Yogurt: Mulberry syrup (w/w)	**Sensory Evaluation**				
	Color	**Odor**	**Taste**	**Crispness**	**Overall acceptance**
5:5	$2,55^{c}\pm 0,4$	$4,00^{c}\pm 0,72$	$2,55^{d}\pm 0,88$	$2,00^{e}\pm 0,64$	$2,95^{d}\pm 0,60$
6:4	$3,90^{b}\pm 0,85$	$4,95^{b}\pm 0,6$	$3,90^{c}\pm 1,07$	$2,70^{d}\pm 0,65$	$3,60^{c}\pm 0,59$
7:3	$5,50^{c}\pm 0,67$	$5,95^{a}\pm 0,51$	$4,95^{b}\pm 0,82$	$4,35^{c}\pm 0,74$	$4,65^{b}\pm 0,74$
8:2	$6,15^{a}\pm 0,67$	$5,30^{b}\pm 0,65$	$6,20^{a}\pm 0,69$	$5,75^{b}\pm 0,63$	$6,00^{a}\pm 0,64$
9:1	$5,85^{a}\pm 0,74$	$5,45^{ab}\pm 0,68$	$6,10^{a}\pm 0,64$	$5,75^{b}\pm 0,63$	$6,25^{a}\pm 0,0,78$
10:0	$3,40^{b}\pm 0,94$	$3,20^{d}\pm 0,95$	$3,70^{c}\pm 1,12$	$6,45^{a}\pm 0,60$	$4,15^{bc}\pm 0,81$

The values represent the average sensory scores given by the panelists, with higher scores indicating a more positive evaluation. The letters after the values denote statistical significance at $p< 0.05$.

3.5.Evaluation of the change of mulberry yogurt freeze-dried during storage

Upon examining the impact of storage time on the physicochemical properties, microbiological activity, and biological performance of freeze-dried mulberry yogurt, distinct shifts were observed. The moisture content and water activity of the product rose progressively with storage duration, with these changes being statistically significant ($p<0.05$) as shown in Table 6. The moisture content ranged from 2.42% at the outset and reached

3.76% after 28 days of storage, while water activity values saw a slight increase, moving from 0.28 to 0.29. Even though these increases were moderate, they align with expected changes in dried goods (namely, moisture less than 5.00% and a_w less than 0.60). Alongside, L* values declined, while b* values rose, culminating in a statistically significant ($p<0.05$) increase in ΔE. This suggests the product's color shifted towards a darker red-violet hue by the end of 28 days. This color deviation became perceptible after the 21st day, with ΔE values surpassing 3.00. An uptick in moisture content and exposure to light during storage might have triggered oxidation reactions affecting the product's color (Suh et al., 2004). Simultaneously, modest variations in the synthesis of polyphenol content and antioxidant activity emerged, as depicted in Table 6. The initial total polyphenol content of 170.84 mg GAE/100 g dwindled to 161.14 mg GAE/100 g over 28 days. In parallel, the antioxidant activity measured by DPPH and ABTS assays witnessed a slight descent, suggesting a minor decline possibly due to the reduced polyphenol content. In the microbiological realm, a non-significant decrease in beneficial bacterial density was observed, declining from 10.72 log CFU/g to 9.66 log CFU/g over the 28-day span, as illustrated in Figure 3. Such a drop in the count of live microorganisms and biological activity is consistent with findings from previous studies on freeze-dried products (Liu et al., 2015; Ha et al., 2021; Emteborg et al., 2022). Despite this, freeze-dried mulberry yogurt maintained relatively stable physicochemical, microbiological, and antioxidant properties over the 28-day observation period, reinforcing the product's resilience during storage.

Table 6. Effect of storage time on physicochemical properties, microbiological and biological activities of freeze-dried mulberry yogurt.

Parameters	**Storage time (day)**				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Physicochemical properties					
Moisture Content (%)	$2,42^{d} \pm 0,02$	$2,7^{cd} \pm 0,02$	$2,93^{bc} \pm 0,02$	$3,1^{b} \pm 0,01$	$3,76^{a} \pm 0,02$
Water Activity	$0,28^{e} \pm 0,01$	$0,28^{b} \pm 0,01$	$0,28^{c} \pm 0,01$	$0,29^{b} \pm 0,01$	$0,29^{a} \pm 0,01$
ΔE	–	$1,50^{d} \pm 0,06$	$2,90^{c} \pm 0,08$	$4,20^{b} \pm 0,01$	$5,45^{a} \pm 0,10$
Bioactive compound					
TPC (mg GAE/100g)	$170,84^{a} \pm 4,75$	$164,85^{b} \pm 0,45$	$163,97^{b} \pm 0,36$	$163,02^{b} \pm 0,15$	$161,14^{b} \pm 0,34$
TAC (mg/L)	$63,42^{a} \pm 0,95$	$63,80^{a} \pm 0,18$	$63,05^{ab} \pm 0,15$	$62,03^{bc} \pm 0,16$	$61,41^{c} \pm 0,27$
DPPH (mgAAE/100g)	$12,22^{a} \pm 0,52$	$12,07^{a} \pm 0,07$	$11,73^{ab} \pm 0,06$	$11,35^{bc} \pm 0,11$	$10,86^{c} \pm 0,05$
ABTS (mgAAE/100g)	$18,98^{a} \pm 0,01$	$18,66^{b} \pm 0,01$	$18,30^{c} \pm 0,01$	$18,06^{d} \pm 0,01$	$17,78^{e} \pm 0,01$

Values are expressed as mean ± standard deviation. The digits in the same column indicate a statistically significant difference ($p<0.05$).

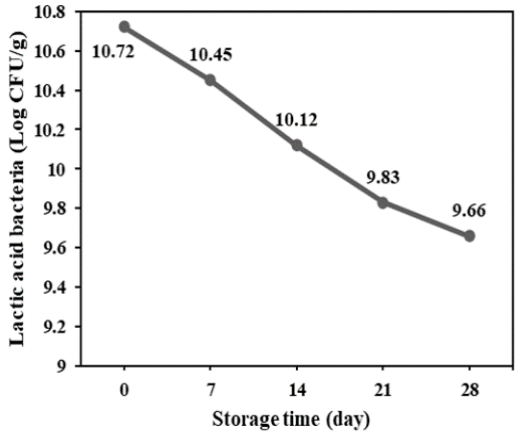

Figure 3. Changes of lactic acid bacteria in freeze-dried mulberry yogurt during storage

4. Conclusions

In conclusion, this study revealed that the addition rate of mulberry syrup and the preservation process are pivotal factors impacting the changes in physicochemical, microbiological, antioxidant, and sensory attributes of freeze-dried mulberry yogurt. Incorporating mulberry syrup at a 9:1 ratio concentration yielded optimal physicochemical properties, such as 3.39% moisture, an aw of 0.3, L* of 59,940, a* of 28,373, TA of 3%, and TSS of 81. Concurrently, total polyphenol content reached 170,84 mg GAE/100 g, while antioxidant activity against DPPH and ABTS free radicals was noted at 12.22 and 18.98 mg AAE/100 g, respectively. This ratio also obtained the highest sensory scores and preference rating, falling between 5.0 and 6.0 out of 7.0 points. Following 28 days of storage at room temperature (25-28℃), humidity, a_w, and ΔE exhibited increases by 3.76%, 0.29, and 5.45, respectively. Microbial density, TA, TSS, TPC, and antioxidant activity indicators, however, remained relatively stable, underscoring the product's overall stability during the storage period.

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