

## Research Article

# Evaluation of the Physicochemical Characteristics of a Blend Fruit Juice Powder Mixed with *Lactiplantibacillus plantarum*: A Comparison of Spray Drying and Freeze Drying

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Received 12 March 2023; Revised 8 June 2023; Accepted 22 June 2023; Published 26 July 2023

Academic Editor: Anand Babu Perumal

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The physicochemical properties and the survival of *Lactiplantibacillus plantarum* mixed with blend of fruit juice powders dried by spray and freeze drying methods were investigated. A formula containing maltodextrin 14% and malt extract 6% as a carrier and juices of red grape 20.4%, mulberry 75.6%, and strawberry 4.0% along with the bacterium (*Lpb. plantarum*) with  $3 \times 10^9$  CFU/mL were dried by spray drying and freeze drying methods, separately. The physicochemical properties including color attribute, nutritional properties of the powders such as antioxidant, and phenolic content were measured. Drying efficiency of freeze-dried was 80.48%, which was higher than the spray-dried powders (50.84%). The higher antioxidant (87.95) and total phenolic content (3032.6 mg GAE/L) was achieved by freeze drying technique; however, lower moisture content and flowability as well as higher solubility and bulk density were obtained for spray-dried powders. The survival of probiotics in freeze-dried powders was higher than the spray-dried powders which can be related to mild conditions. The study indicated that the juice powders are suitable carriers for *Lpb. plantarum* and constitute good alternatives to highly acidic fruit juices.

## 1. Introduction

Fruit juice powders have many advantages over their liquid counterparts including decrease in volume, mass, packaging, suitable handling, and transportation as well as long shelf life [1, 2]. Moreover, they are stable and natural ingredients which can be used as “clean label” natural flavoring and coloring agents in food and pharmaceutical industries [1]. Fruit juices containing anthocyanin and antioxidants, due to high sensitivity to the environmental conditions, have increased their research interest in the protection from harsh conditions. Epidemiological research revealed that red juices such as pomegranates and red grapes and different berries, i.e., strawberries and black berries, encompass high amounts of anthocyanin and phenolic compounds,

which have benefits against cancer and cardiovascular diseases [1, 2]. In recent years, there is a growing research trend in the blend of fruit juices to develop new sensations and functional properties [1, 2].

Spray drying is widely used in commercial production of powders, due to high feasibility and protection from oxidation of desirable ingredients through their entrapping in a polymeric matrix [2, 3]. However, the low molecular weight sugars such as fructose, glucose and sucrose as well as organic acids existed in fruit juices are effective factors on the production of sticky and caking powders in the drying chamber, due to their low glass transition temperature ( $T_g$ ). Therefore, it is required to utilize high molecular weight additives such as maltodextrin, Arabic gum, crystallized starch, and cellulose as carrier to increase  $T_g$ , when

introduced into the feed solution [4]. Among the carriers, maltodextrin due to its high solubility, low viscosity, and high  $T_g$  can be used as an excipient into fruit juice and developed hygroscopic juice powders [1, 2, 5].

Freeze drying is another procedure in the juice powder processing which has some advantages over spray drying such as low thermal processing, less color and antioxidants loss as well as higher functional properties. Furthermore, it has been used as general method in the preparations of dried lactic acid bacteria [6, 7]. The nondairy probiotic product is a challenge in food industry, and many researches have been carried out on the application of natural resources such as fruit juices to provide functional foods [6]. Fruit juices due to high amounts of sugars, vitamins, and mineral can enhance the survival of probiotics and lactic acid bacteria during storage [8]; the high content of dietary fiber and protein has also a favorable effect on the survival of bacteria during storage in juices such as orange, apple, grapefruit, blackcurrant, pineapple, and lemon [8–11]. Furthermore, the dietary fiber such as  $\beta$ -glucan into fruit juices along with probiotics has shown more stability of the protected *L. rhamnosus* during storage [9]. The concentration and type of fruit juices are also effective on the survival rate of probiotics [12]. For example, high survivability of probiotics was observed during storage by addition of orange and pineapple juices [12]. In contrast, the high phenolic content and low pH of pomegranate, strawberry, and cranberry juices are responsible for the low survival of the probiotic flora [7, 12]. Recently, production of mixed fruit beverages has been attended because of more interesting sensory acceptance, masking unfavorable properties of some fruits and improving beneficial health properties [1, 2]. However, the effect of mixed fruit juice powders with high content of the total phenolic contents and antioxidant activity on the survivability of lactic acid bacteria has not been described, yet.

Although there are various researches on the processing and physicochemical properties of fruit juice powders such as acai powder [5], amla juice [13], jamun juice powder [14], pomegranate juice powder [2, 4], and black mulberry powder [1], none of them did not investigate the application of blend fruit juice in the processing of powder mixed with lactic acid bacteria. Therefore, the aim of the current work was to study the addition of *Lactiplantibacillus plantarum* to the blend fruit juices containing red grape, mulberry, and strawberry. Furthermore, the physicochemical properties as well as antioxidant behavior of the powders were compared in freeze-dried and spray-dried powders. The physicochemical properties including moisture content, water activity, drying efficiency, bulk density, solubility, and microstructure of freeze-dried and spray-dried blend of fruit juice powder were also investigated.

## 2. Materials and Methods

**2.1. Materials.** Ripen red fruits including red grape (*Vitis vinifera* L.), mulberry (*Morus nigra*), and strawberry (*Fragaria paros*) were procured from Tehran, Iran. The fruits were cleaned with tap water, and their juice was extracted

by a home juicer (Tefal Frutelia ZE350, France). Then, the fruit juices were centrifuged at  $3,000 \times g$  for 20 min (Beckman J2-21, Beckman Coulter Inc., CA, USA) to obtain the clarified fruit juice. *Lpb. plantarum* subsp. *plantarum* PTCC 1058, in the form of freeze-dried vial, was purchased from Persian Type Culture Collection (PTCC) of Iranian Research Organization for Science and Technology (IROST). Maltodextrin with dextrose equivalent (DE) 18-20 (Zar Fructose Co., Karaj, Iran) and malt extract powder (Behmalt, Hafshejan, Iran) were also used as carriers. Sodium carbonate, DPPH, Folin-Ciocalteu, gallic acid, and citric acid were all obtained from Merck for the experiments.

**2.2. Bacterial Suspension.** *Lpb. plantarum* were firstly cultured in 200 mL of MRS broth culture medium and were incubated at  $37^\circ\text{C}$  for 18-21 hours. It was then centrifuged at 5,000 rpm for 15 minutes. The culture medium containing bacteria was washed with 50 mL of sterilized distilled water and mixed again with 50 mL of distilled water. For bacterial coating, 5 g of malt extract powder along with 10 g of maltodextrin was directly added to the bacterial suspension and homogenized at 5,000 rpm for 5 min with a homogenizer (Wise Tis, model HG-15D).

**2.3. Preparation of Probiotic Juice.** Firstly, a formula containing maltodextrin (14%  $w/v$ ) and malt extract powder (6%  $w/v$ ) which are edible compounds applied for the drying and preservation of nondairy beverages and probiotics was prepared [15–17]. The percentages were also obtained from our preliminary work which was selected by the design expert to obtain the suitable amount of the carriers. Briefly, a drinking formula containing red grape 20.4%  $w/w$ , mulberry 75.6%  $w/w$ , and strawberry 4.0%  $w/w$  with high sensory acceptance was prepared [18]. Afterward, 25 g malt extract powder and 60 g maltodextrin were mixed with 450 mL fruit juice, and 50 mL of bacterial suspension was then added to the mixture. The final mixture was homogenized (Wise Tis, model HG-15D) with a homogenizer for 5 min and used for freeze drying and spray drying.

**2.4. Dehydration of Probiotic Juice Powder.** Drying the probiotic juice powder was performed by two methods of dehydration including spray drying and freeze drying with some modifications [18, 19]. Spray drying was done using a table top mini spray dryer (Buchi B191, Switzerland) with a 0.7 mm two-fluid nozzle at a constant air pressure of  $2.5 \text{ kg/cm}^2$  and an aspiration rate of 50%. The juice (250 mL) was fed into the drying chamber through a peristaltic pump. The inlet temperature, air flow rate, feed rate, and outlet temperature were  $120^\circ\text{C}$ ,  $8 \text{ m}^3/\text{h}$ ,  $0.12 \text{ kg/h}$ , and  $78^\circ\text{C}$ , respectively. After the spray drying of the mixed juice, the spray dryer was fed with distilled water for 3 minutes, and the spray-dried powder was collected in the collection vessel.

Prior to freeze drying, the mixture (250 mL) was frozen overnight at  $-80^\circ\text{C}$ , and the freeze dryer (Leybold Heraeus, Cologne, Germany) was warmed up for 30 min. The vacuum pressure was controlled at 0.1 mbar. The drying procedure lasted 48 h. All of the mixture was transformed into dry

flakes at this point. The dry product was sieved after being pounded into a fine powder. For further investigation, the resulting powder was packed in a polyethylene bag and maintained in a freezer at  $-20^{\circ}\text{C}$  [19, 20].

**2.5. Physicochemical Analysis.** Drying efficiency, moisture content, solubility index, flowability, and density of the powders were determined in this section. Drying efficiency of the powders was determined as the ratio of the dry matter content in juice powder to the dry matter content of the feed by the following equation [21]:

$$\text{Drying efficiency} = \frac{m_p}{m_f} \times 100, \quad (1)$$

where  $m_p$  and  $m_f$  are the total solid mass in the juice powder and feed (g), respectively.

The moisture content of the juice powder was calculated through a vacuum oven (NUVE, model EV018, London, UK) at  $85^{\circ}\text{C}$ , until the weight reached the constant value and presented in %w/w [10, 21].

The solubility index was measured according to Jafari et al. with slight modifications [22]. Briefly, 1 g of powders was dispersed in 100 mL distilled water and stirred in a magnetic stirrer at 600 rpm for 5 min. Then, it was centrifuged at  $7,500 \times g$  for 10 min, and 25 mL aliquot of the supernatant was dried in a conventional oven at  $105^{\circ}\text{C}$  to a constant weight. The solubility index was determined by the following equation [22, 23]:

$$\text{Solubility index}(\%) = \frac{\text{weight of dried supernatant}}{\text{weight of the sample}} \times 100. \quad (2)$$

Bulk density ( $\rho_b$ ), true density ( $\rho_t$ ), and porosity ( $\epsilon$ ) were measured according to the previous work [23]. Briefly,  $\rho_b$  of the powder ( $\rho_b$ ,  $\text{g}/\text{cm}^3$ ) was determined by gently adding 2 g of the powder to a 10 mL graduated cylinder and keeping it on a vortex vibrator for 1 min. The ratio of the powder mass and the occupied volume indicates the bulk density ( $\rho_b$ ). The sample cylinder was used to determine true density ( $\rho_t$ ,  $\text{g}/\text{cm}^3$ ) by tapping the cylinder on a bench 100 times from a height of 10 cm. True density was calculated by dividing the powder mass to the occupied volume. Porosity of the powders was measured using the relationship between  $\rho_b$  and  $\rho_t$ .

The flowability of the powder as the Carr index (CI) (Table 1) was calculated as follows [24]:

$$\text{Carr index (CI)} = \frac{V_0 - V}{V_0}, \quad (3)$$

where  $V_0$  and  $V$  are the initial and final volume of the powder (mL), respectively.

The cohesiveness of the powders in terms of the Hausner ratio (HR) was also measured by dividing the true density to the bulk density [14].

TABLE 1: The limits of the Carr index and Hausner ratio on the flowability of the powders.

Flowability	Carr index (CI) (%)	Hausner ratio (HR)
Excellent	0-10	1.00-1.11
Good	11-15	1.12-1.18
Fair	16-20	1.19-1.25
Passable	21-25	1.26-1.34
Poor	26-31	1.35-1.45
Very poor	32-37	1.46-1.59
So poor	>38	>1.60

**2.6. Color Attributes.** Color analysis was carried out using a colorimeter (CS-2000-Konica Minolta, USA) with  $10^{\circ}$  angle and illumination D65. The powder was filled in a plate (1 cm height and 6 cm diameter), and the RGB parameters were converted to  $L^*$  (lightness),  $a^*$  (redness, greenness), and  $b^*$  (yellowness, blueness) values. The differences between color values of the dried samples were also determined. The browning index (BI) which indicates the purity of brown color was determined as follows [2, 25]:

$$\text{BI} = \frac{100(x - 0.31)}{0.17}, x = \frac{(a^* + 1.75L^*)}{(5.65L^* + a^* - 3.01b^*)}. \quad (4)$$

Chroma, the color intensity, and hue angle ( $H^0$ ) were measured by the following equations:

$$\begin{aligned} \text{Chroma} &= \sqrt{a^{*2} + b^{*2}}, \\ H^0 &= \arctan\left(\frac{b^*}{a^*}\right). \end{aligned} \quad (5)$$

The hue angle values vary from  $0^{\circ}$  (pure red color),  $90^{\circ}$  (pure yellow color), and  $180^{\circ}$  (pure green color) to  $270^{\circ}$  (pure blue color).

**2.7. Phenolic Content and Antioxidant Activity.** Total phenolic content (TPC) were quantified using the Folin-Ciocalteu assay [26]. Briefly, 1 g of the powder was homogenized in 2.5 mL of water and centrifuged at 15,000 rpm for 20 min. Then, aliquot of 1 mL of the fruit juice was mixed with 5 mL of Folin-Ciocalteu reagent and thoroughly mixed for 30 s. Then, 300 mL of 20% sodium carbonate solution was added in which the color changed from yellow to blue. The samples were kept at room temperature for 1 hour in a dark condition, and the absorbance was determined at 765 nm using a 1 cm cuvette by a spectrophotometer (Unicam 8620, Thermo Spectronic, UK). The TPC results were expressed as milligram of gallic acid per gram of dry weight [26, 27].

The scavenging activity for DPPH radicals was measured to evaluate the antioxidant. Antioxidant activity was measured based on the DPPH radical scavenging activity according to the previous works [26, 28]. Briefly, diluted sample solution in methanol (200  $\mu\text{L}$ ) was mixed with 800  $\mu\text{L}$  methanolic DPPH solution (0.004 g/100 mL) and placed in a

cabinet in the dark for 60 min. Solution of 200  $\mu\text{L}$  methanol and 800  $\mu\text{L}$  DPPH was treated in the same situation as a control. A solution of 800  $\mu\text{L}$  methanol and 200  $\mu\text{L}$  diluted fruit juice was considered as blank. Finally, free radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \frac{1 - A_s - A_c}{A_c - A_m} \times 100, \quad (6)$$

where the  $A_s$ ,  $A_c$ , and  $A_m$  are the absorbance of the sample, the control, and the methanol, respectively. The concentrate of fruit juices which inhibited 50% DPPH free radicals was considered as  $\text{IC}_{50}$  of fruit juices [26, 28].

**2.8. Probiotic Viability during Storage.** In order to determine the storage ability of the probiotic fruit juice powder, 10 g of samples was stored in dark conditions at 4°C. The viability of *Lpb. plantarum* cells in the powder was measured at 0, 15, 30, 60, and 90 days. Appropriate dilutions for 1 g of the sample were prepared in sterile distilled water. The cells were then permitted to grow on the MRS agar plate. Finally, the cultured cells were incubated at 37°C and anaerobic conditions for 48 h, and the standard plate count procedure was followed to find the viability of the *Lpb. plantarum* in CFU/mL [29, 30].

**2.9. SEM Images.** Microstructural analysis of the spray-dried and freeze-dried powders was carried out using a scanning electron microscopy (SEM, Pemtron PS-230, South Korea) at an accelerated voltage of 15 kV and magnification of  $\times 5000$  [18, 29].

**2.10. Statistical Analysis.** All the experiments were carried out in triplicate, and one-way analysis of variance (ANOVA) was used. The results were given as mean  $\pm$  SD, and the means were compared by the Duncan's multiple range tests at the 5% level ( $P < 0.05$ ) through Minitab version 22 (IBM, USA).

### 3. Results and Discussion

**3.1. Drying Efficiency of Freeze-Dried and Spray-Dried Powders.** Drying efficiency of freeze-dried fruit juice powders was 80.48%, which was significantly higher than that of the spray-dried powders (50.84%) ( $P < 0.05$ ). It has been shown that the drying efficiency of black mulberry spray-dried with 8% maltodextrin 20DE was about 65% [1]. The low value of our drying efficiency (50.84%) can be attributed to the higher maltodextrin concentration (14%) as well as lower inlet temperature (120°C); the viscosity increased and therefore the drying efficiency decreased, which are all in agreement with the previous works [1, 5]. However, various drying efficiencies in spray drying of pomegranate (86%) [22], sohiong fruit (50-65%) [29], coconut water (37.9-8.9%) [26], and orange (18-35%) [10] have been reported; most of them confirmed higher efficiency at higher temperatures which could be attributed to the more heat and mass transfer as well as the more carrier agent concentration. Increasing the later factor, carrier agent, leads to increasing  $T_g$  of the amorphous fractions.

In comparison with freeze drying, it was found the higher efficiency using this technique can be attributed to the less stickiness of the powder to the main chambers's wall. Similar findings have also been observed for the kuini pulp powder (36%) [25] and blueberry (78.1%) [31], which were more than the spray-dried ones. Other physicochemical properties of the probiotic fruit juice powders which are important in terms of stability and acceptance are provided in the next section.

**3.2. Physicochemical Properties of the Fruit Juice Powders.** The physicochemical characteristics of both freeze-dried and spray-dried powders are provided in Table 2. According to the results, the moisture content of the spray-dried samples was 5.34%, which was significantly lower than the freeze-dried powder (6.66%) ( $P < 0.05$ ). This low moisture content diminishes powder adhesion and enables them to high regeneration due to the more surface contact. According to the previous works, the spray drying of the blackcurrant pomace powders led to a reduction in the water content, while the freeze drying did not reduce its values [11]. The spray-dried pineapple powder has shown less moisture content (2.76%), and after that, the freeze-dried powder showed the less water content [32]. Furthermore, it has been found that maltodextrin can reduce the moisture content through decreasing the free water [2, 6, 9]. It has been stated that the presence of larger maltodextrin molecules made it difficult for water molecules to diffuse.

Solubility of the spray-dried was significantly higher than that of the freeze-dried powders ( $P < 0.05$ ), which can be attributed to the time required for drying. In this way, it was found that rapid drying increases the number of pores in the powder particles and reduces the contact angle between the powder surface and the rehydration medium, thus facilitating and accelerating the rehydration [21]. It has been understood that the solubility of freeze-dried black mulberry powder was lower than that of spray-dried ones [1]. It has also been found the storage temperature and moisture content can significantly affect the solubility of samples [20, 21]. The highest solubility values were obtained in samples with lower moisture content, while the solubility decreased during shelf life by absorption of moisture.

The bulk density ( $\rho_b$ ) is a vital property affecting directly on the function and application of powders [21]. The  $\rho_b$  of freeze-dried and spray-dried of the probiotic juice powders was 0.57 and 0.83  $\text{g}/\text{cm}^3$ , while the true density ( $\rho_t$ ) of the powders was 0.74 and 0.94  $\text{g}/\text{cm}^3$ , respectively. There was a significant difference between the freeze drying and spray drying techniques in the density of the probiotic powders (Table 2), which has been similarly found on the Japanese quince powders [33]. Therefore, as the  $\rho_b$  was high, the entrapped air was more which result in high oxidation and reduction of storage stability. Furthermore, the freeze-dried powders showed a more crystalline structure with smaller volume which led to a porous flat surface with occulted air void. The packaging and transportation costs increase with the reduction of  $\rho_b$  [34]. Porosity is another factor in describing the physical properties of the powder and its flow behavior [35]. Spray-dried powders showed higher porosity



TABLE 2: Some physicochemical properties of spray-dried and freeze-dried probiotic fruit juice powders\*.

Drying	Yield (%)	$M_C$ (%)	Solubility (%)	$\rho_t$ (g/cm <sup>3</sup> )	$\rho_b$ (g/cm <sup>3</sup> )	$\epsilon$ (-)	CI (%)	HR	$L^*$	$a^*$	$b^*$	Color attributes		
												C	Hue	
FD	80.48 ± 0.04 <sup>a</sup>	6.66 ± 0.39 <sup>a</sup>	80.23 ± 0.82 <sup>b</sup>	0.74 ± 0.02 <sup>b</sup>	0.57 ± 0.01 <sup>b</sup>	14.28 ± 0.02 <sup>b</sup>	23.42 ± 0.32 <sup>a</sup>	1.25 ± 0.01 <sup>b</sup>	15.47 ± 1.64 <sup>a</sup>	26.21 ± 0.62 <sup>a</sup>	7.45 ± 1.12 <sup>a</sup>	25.12 ± 1.60 <sup>a</sup>	51.11 ± 0.5 <sup>b</sup>	
SD	50.84 ± 0.02 <sup>b</sup>	5.34 ± 0.03 <sup>b</sup>	87.33 ± 0.83 <sup>a</sup>	0.94 ± 0.02 <sup>a</sup>	0.83 ± 0.02 <sup>a</sup>	22.18 ± 0.02 <sup>a</sup>	19.87 ± 0.85 <sup>b</sup>	1.31 ± 0.01 <sup>a</sup>	12.21 ± 1.63 <sup>a</sup>	15.58 ± 0.44 <sup>b</sup>	3.66 ± 0.71 <sup>b</sup>	15.14 ± 0.71 <sup>b</sup>	79.34 ± 0.4 <sup>a</sup>	

FD: freeze drying; SD: spray drying;  $M_C$ : moisture content;  $\rho_t$ : true density;  $\rho_b$ : bulk density;  $\epsilon$ : porosity; CI: Carr index; HR: Hausner ratio. Statistically significant difference between the FD and SD, within columns, are indicated with different lowercase letters.



FIGURE 1: Images of the probiotic fruit juice powder obtained by (a) spray drying and (b) freeze drying methods.

than that of freeze-dried ones (Table 2), which reveals the higher water absorption in spray-dried powders and compact and lower solubility for freeze-dried probiotic fruit juice powder [20, 33].

The Carr index (CI) is a measure of flowability of the powder. Flowability is related to the particle size, surface area, and chemical nature. In a free-flowing powder, the CI value will be smaller because the  $\rho_b$  and  $\rho_t$  of the powder will be closer together, whereas in a compact powder, the difference between the  $\rho_b$  and  $\rho_t$  will be greater due to more interactions between the particles, leading to higher CI values. Therefore, as CI increased, the flowability becomes poor. CI of freeze-dried and spray-dried powders was 23.42, and 19.87, respectively, which clearly explain the lower flowability of freeze drying techniques due to the compact and crystalline structure. Furthermore, the moisture content of the powder significantly affects the flowability, adhesion, and cake properties. The higher the moisture content, the greater the adhesion forces, which leads to poor flowability [36]. Similar results for the CI value of the spray-dried orange juice powder (12.2-22.7) [10] and spray-dried barberry powder (12.5-14.9) [37] indicated the proper flowability of the produced powder in the present study.

The HR of the freeze-dried and spray-dried powders was 1.31 and  $1.25 \pm 0.01$ , respectively. As the moisture content of the powder was increased, the HR increased, and as the bulk density of the powder is more, the lower the HR was obtained. According to Lebrun et al. (Table 1) [38], however, the HR values of the spray-dried powders was more than that of freeze-dried; both of them are in the limits of passable powders and have proper compressibility. Based on the results of cohesion index, powder particles containing maltodextrin have established strong bonds, and a similar cohesion can be observed. These strong and stable bonds will be effective in maintaining nutrients such as anthocyanin. In garlic powders, the lower the HR, the higher the flowability of the product was achieved. Due to lower moisture content and higher solid content, spray-dried garlic powder has shown good flowability [35].

**3.3. Color Analysis.** Images of the probiotic juice powders obtained by spray and freeze drying techniques are given in Figure 1. As it can be seen, the spray-dried powder is lighter than the freeze-dried powder. It has been found that the moisture content of the cranberry powder was more related to the drying procedure than that of the maltodextrin, and they have related particle size of the powder which has an effect on the color attributes [34]. The effect of the drying method on some of the color attributes was significant ( $P < 0.05$ ) (Table 2). The spray-dried powder was much lighter ( $L^*$ ) than that of freeze-dried ones. In addition, the redness ( $a^*$ ) of the freeze-dried powder was significantly higher than the  $a^*$  value of the spray-dried powder. However, drying procedure did not show any significant effect on yellowness ( $b^*$ ). A higher chroma index (C) and a lower color angle were also observed in the powder obtained by the freeze dryer, which can also be related to the moisture level of the powders. The high  $L^*$  value of the spray-dried powder is due to the high drying temperature, which leads to the degradation of the pigment, resulting in a light-colored powder [37, 39], while the low drying temperature in freeze drying can preserve color pigments and produce a slightly darker powder than spray drying [39]. It can be seen that the  $a^*$  value of the powders decreased, and the lowest reduction was achieved by freeze drying due to the drying temperature and low vacuum environment [37]. In spray drying, the value of  $a^*$  is the lowest due to the high inlet temperature that led to the severe destruction of red pigments. Redness reduction has been reported recently by increasing inlet temperature of the spray dryer [2]. The similar phenomenon has also been observed for orange carrot [10], pomegranate [2], and amla powders [13] in spray drying technique, which can be attributed to the destruction of heat-sensitive components such as carotenes and anthocyanin pigments. In the present study, it was attempted that by applying moderately low inlet temperature ( $120^\circ\text{C}$ ) as well as carrier, the active ingredients are protected against thermal degradation. However, high brightness ( $L^*$ ) and hue as well as low  $a^*$  for the spray-dried powder discovered the better color quality in the freeze-dried probiotic fruit juice powders.

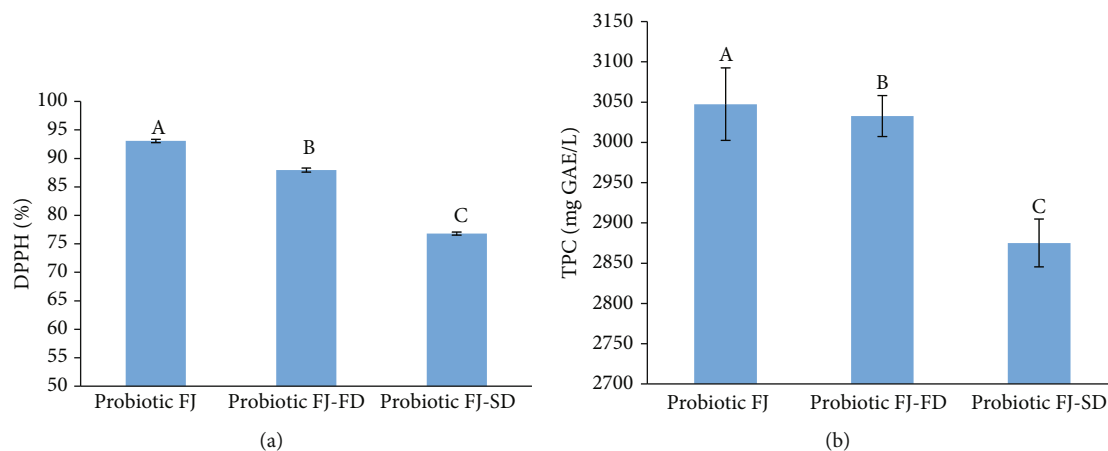


FIGURE 2: The antioxidant activity (DPPH, %) (a) and total phenolic content (TPC) (b) of the probiotic fruit juice (FJ), probiotic fruit juice-freeze dried (FJ-FD), and probiotic fruit juice-spray dried (FJ-SD) powders (statistically significant differences are indicated with different uppercase letters).

**3.4. Antioxidant Properties.** The results of the DPPH of the spray-dried and freeze-dried powders showed that the antioxidant power was significantly higher ( $P < 0.05$ ) in the freeze-dried powder than that of spray-dried samples (Figure 2(a)). It can be accurately said that the spray drying technique causes a reduction of 16.29% in the antioxidant properties compared to fresh fruit juice, while the same reduction was 13.5% in freeze drying method. A multi-objective optimization for the production of leaves aqueous extract powder of artichoke (*Cynara Scolymus L.*) using a spray drying method has shown that at higher input temperatures, the antioxidant capacity decreased which could be due to degradation and thermal decomposition of compounds [40]. On the other hand, when the vacuum pressure decreases, the antioxidant capacity increases due to the reduction of drying time and oxidation reaction. The effect of drying with a freeze dryer on the stability of total phenolic compounds and antioxidant activity of mango extract was also investigated, and they found that it did not have a significant effect on the antioxidant activity of mango extract powder. It can be attributed to the browning process which is intensified and accelerated by the drying process and attributed to the release of enzymes such as polyphenol oxidases and the loss of some phenolic compounds [41]. The effect of drying mango powder with a spray dryer has been studied and found that the antioxidant and free radical scavenging power decreased after spray drying [41]. The results of the present study show that the freeze drying process is preferable than the spray drying in terms of maintaining the antioxidant capacity, which can be due to the elimination of thermal damage in the freeze dryer.

According to the results presented in Figure 3(a), the formulated juice during the shelf life in the refrigerator suffered a 39.69% reduction in antioxidant content which could be due to spoilage, and the fruit juice powder obtained from the spray dryer decreased by 6.86%. The juice powder obtained from the freeze dryer had also a 5.98% drop in antioxidant content during storage. The anthocyanin stability and antioxidant activity of the spray dried açai juice powder

have shown a decrease in the antioxidant activity of the powders during 45 to 65 days [5]. It has been evaluated the physicochemical properties of dried Bael fruit powder during storage for 8 weeks and found a decrease in antioxidant activity from zero time  $1.84 \pm 0.02$  ( $\mu\text{mol TEAC/g}$ ) to  $1.21 \pm 0.05$  in the eighth week at  $35^\circ\text{C}$  [42]. Temperature and storage time usually have a significant effect ( $P < 0.05$ ) on DPPH inhibition activities. The higher the storage temperature, the more effects on the antioxidant compounds [13].

The total phenolic content of the powders obtained from spray dryer and freeze dryer is shown in Figure 2(b). It can be seen that the amount of total phenolic content of the powder samples obtained from freeze dryer is significantly ( $P < 0.05$ ) higher than the powder samples obtained from spray dryer. The high retention of phenolic content by freeze drying is due to the absence of heat and the formation of ice crystals. Ice crystals break the cell structure and facilitate the extraction of phenolic compounds [43]. High temperature in spray drying leads to severe degradation of phenolic compounds. A similar trend was obtained in the literature, where freeze drying resulted in the preservation of the highest phenolic content [44]. The antioxidant properties of barberry juice and its spray-dried powder at different concentrations of maltodextrin were between 98.76 and 111.23 (mg GAE/100 g), which have shown that the maltodextrin concentration as well as the inlet temperature of the spray dryer had a significant effect on the total phenolic content of the powder [45]. According to the obtained results and the effect of drying temperature on phenolic compounds, it seems that the freeze drying process is better than spray drying for preserving phenolic compounds in fruit juice powder.

According to the results obtained in Figure 3(b), during the shelf life in the refrigerator, the total phenolic content of the probiotic fruit juice decreased by 16.42%, and the fruit juice powder obtained from the spray dryer also decreased by 3.08%. In contrast, it was shown that the juice powder

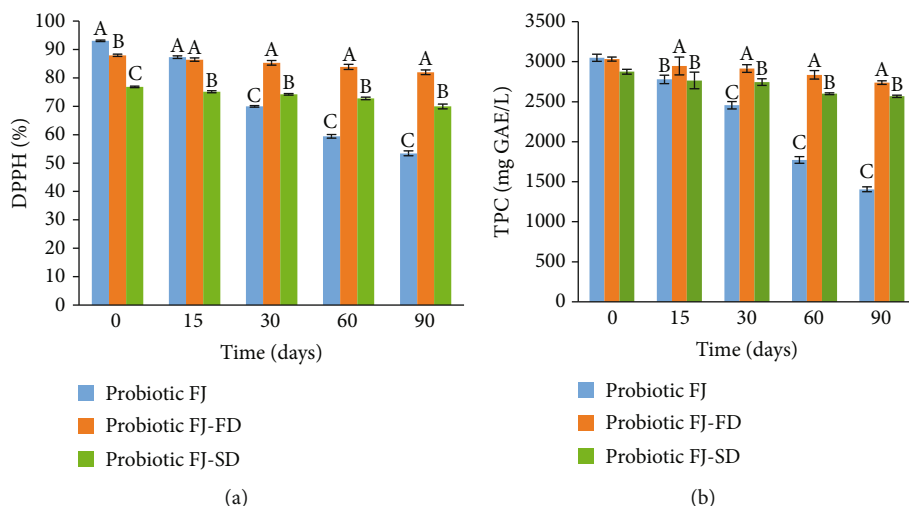


FIGURE 3: Antioxidant activity (DPPH, %) (a) and total phenolic content (TPC) (b) of probiotic fruit juice (FJ) and powders obtained from spray drying (FJ-SD) and freeze drying (FJ-FD) methods during 90 days of storage at 4°C (statistically significant differences of each time are indicated with different uppercase letters).

obtained from the freeze dryer had a 9.32% drop in total phenolic content. The investigation of the storage stability of phenolic compounds of jambolan water powder obtained by drying mat foam stated that after powdering with a spray dryer, the concentration of phenolic compounds of the powder sample was strongly stable during storage [46]. According to a study on the effect of spray drying on mango powder, it was found that the total phenolic content of mangoes decreased after spray drying. It showed that this decrease in phenolic and antioxidant compounds is due to their sensitivity to iodine heat [44]. The effect of spray dryer and shelf life of bayberry juice powder was also studied; they have found that during 6 months of shelf life, the powder samples had a 20% drop in the total phenolic content [47].

**3.5. Effect of Storage on Probiotic Survival.** Drying with a freeze dryer compared to a spray dryer resulted in more survival of probiotic bacteria and less mortality even during storage time at 4°C (Figure 4). A significant difference of *Lpb. plantarum* survivability was observed between freeze-dried (9.44 log<sub>10</sub> CFU/g) and spray-dried powders (8.85 log<sub>10</sub> CFU/g) immediately after drying which could be due to the harsh conditions in spray drying method in comparison with freeze drying. However, the high survivability in both freeze-dried and spray-dried powders could be due to applying protective carriers, especially malt extract which has already been indicated that is a powerful protective agent for the *Lpb. plantarum* viability in acidic fruit beverages [16, 27]. The results also showed less reduction in probiotic survival during storage of the freeze-dried powder. Although the moisture content plays a major role on the survival of *Lpb. plantarum* during storage and spray-dried powders have low water content (5.34%), the survival of probiotics in freeze-dried powders was more. The cause of which can be attributed to extreme changes in temperature and humidity. However, there is obvious differences between the shape of freeze-dried and spray-dried powders (Figure 5) which affect the physicochemical properties of the powders and may reduce the viability of the lactic acid bac-

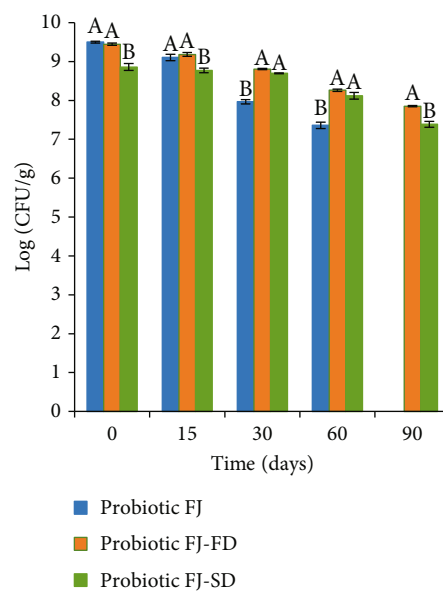


FIGURE 4: The survival of the *Lpb. plantarum* in probiotic fruit juice (FJ), freeze-dried (FJ-FD), and spray-dried (FJ-SD) powders during 90 days of storage at 4°C (statistically significant differences of each time are indicated with different uppercase letters).

teria. Rehydration time is also an important factor, as studies have shown that rapid (2 min) or slow (30 min) rehydration can affect *L. bulgaricus* viability. Slow rehydration (immersion) results in higher cell viability, possibly because the immersion method limits osmotic shock. It has been reported that increasing water activity ( $a_w$ ) increases the rate of death of probiotics during storage [16, 30]. They have suggested that  $a_w < 0.3$  is necessary for the survival of probiotics during storage and the ideal  $a_w$  for many *Lactobacillus* species should be between 0.11 and 0.23. [7, 16] In addition, water activity and the presence of oxygen are important factors that affect the survival of probiotics during storage.



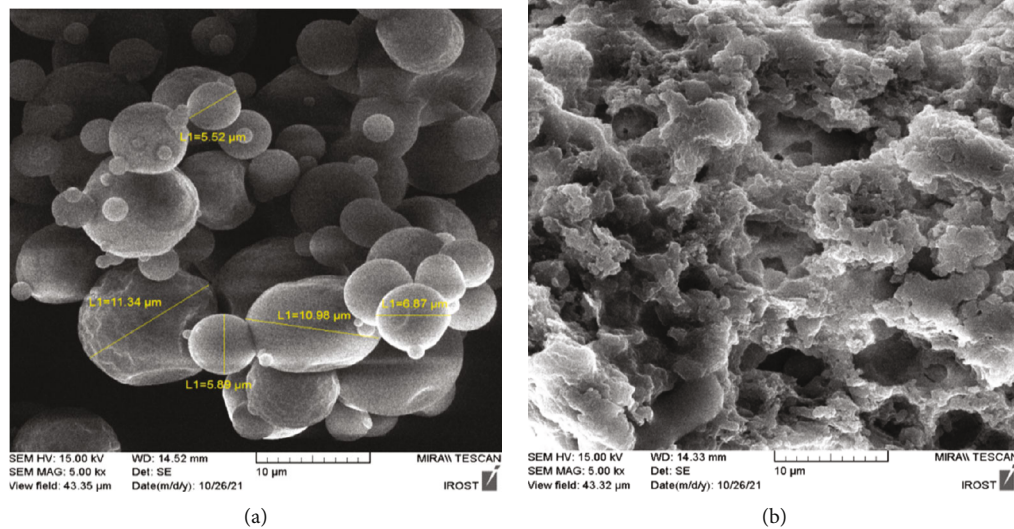


FIGURE 5: SEM images of the probiotic fruit juice powder obtained by (a) spray drying and (b) freeze drying methods.

**3.6. SEM Morphology.** SEM micrograph of the probiotic fruit juice powders prepared by freeze drying and spray drying techniques is provided in Figure 5. As it can be seen, the spray-dried powder particles have a spherical and regular shape and are separate from each other, which indicates less stickiness of its particles, while the freeze-dried powder particles have an irregular and sticky shape. The average particle size of spray-dried powders ranges from 5 to 11  $\mu\text{m}$  which was similar to the spray-dried black mulberry powders [1]. Similar findings for maltodextrin carriers in spray drying of powders with uniform spherical, smooth particles have been also reported [1, 2, 5, 13, 21, 45]. The morphology of sohiong probiotic powder showed that the powder consisted of small-sized spherical particles without fractures or cracks on their surface. No free cells of *Lpb. plantarum* were seen in the micrographs. Encapsulated cells appeared as a raised mass on the spherical surface, which confirmed the proper encapsulation [16]. The microstructural study showed that the spray-dried mango and garlic powders have been also indicated smooth spherical surface for spray drying method in comparison with other techniques such as freeze drying that has an uneven surface and an irregular shape [35, 41, 44].

#### 4. Conclusion

The freeze drying showed lower harmful effects on the total phenolic content, antioxidant activity, color, and survivability of *Lpb. plantarum* in the produced mixed fruit juice powder in comparison with the spray drying method. In contrast, lower moisture content and flowability as well as higher solubility and bulk density were obtained for spray-dried powders. Although the moisture content plays a major role on the survival of *Lpb. plantarum* during storage and spray-dried powders have low water content, the survival of probiotics in freeze-dried powders was more which may be attributed to the harsh condition in spray drying method. However, both freeze drying and spray drying methods are

efficient for the production of mixed fruit juice powder, while for the production of probiotic mixed fruit juice powders, the preferable method is freeze drying. Overall, due to the evaporative cooling that occurs during the process, the products dried by the freeze dryer have higher quality and efficiency.

#### Data Availability

All data are presented in the manuscript.

#### Conflicts of Interest

The authors declare that they have no conflict of interest.

#### Acknowledgments

This research was supported by the personal expenses, and funds and grants were not used for it.

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