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Insights into the Microbiological and Physicochemical Properties of Bio-Frozen Yoghurt Made with Probiotic Strains in Combination with Jerusalem Artichoke Tubers Powder

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Abstract: Frozen yoghurt is a refreshing and nutritious dessert, with or without the flavour that combines the texture of ice cream and yoghurt. Several previous studies have been conducted on Jerusalem artichoke tubers due to their components, which contain inulin compounds and other nutrients with beneficial properties of fresh yoghurt. However, limited studies explored the potential benefits of the addition of Jerusalem artichoke tuber powder as a fat replacer on the physicochemical properties and survival of probiotics in frozen yoghurt. In this respect, the aim of this study was to determine the effect of Jerusalem artichoke tuber powder (JATP) (0, 5, 10, 15, and 20% *w*/*w*) of the fat source used in the mix as a fat, and sugar replacer in frozen yoghurt production. The microbiological, physicochemical, textural, and sensory properties of frozen yoghurt were investigated. Samples with JATP contained viable counts of *bifidobacterium bifidum* BGN4 and *Lactobacillus casei* Lc-01 of 7 log cfu/g during 90 days of storage, as compared to the control sample. The highest viability of probiotics was obtained in the sample formulated with 10% JATP. The formulation of frozen yoghurt with JATP increased the acidity and enhanced the overrun. Compared with the control sample, the incorporation of JATP into frozen yoghurt increased the melting resistance, overrun, and viscosity of the frozen yoghurt. The addition of JATP up to 10% significantly increased sensory attributes. Collectively, the study concluded that the enrichment of frozen yoghurt with JATP up to 20% will provide consumers with health benefits and could be introduced to markets as functional frozen yoghurt.

Keywords: frozen yoghurt; fat replacer; viability; formulated; Jerusalem artichoke tuber powder

1. Introduction

Functional foods, a concept that combines nutrition and health, and is one of the key trends in the food industry, notably in the dairy industry, which has received considerable scientific attention in recent decades [1–9]. Food products comprised of probiotic bacteria have therapeutic benefits, such as response improvement of the immune system, reduction in cholesterol, reduction in lactose intolerance, faster treatment of diarrhoea, and a lower risk of colon cancer [10]. In order to achieve beneficial therapeutic effects, the lowest level of probiotic live microbes at the time of consumption has been recommended to be 10^6–10^7 cfu/mL[1]. Extensive experimental studies report that prebiotics help to maintain the functionality and viability of probiotics throughout food processing [11,12]. Yoghurt ice cream is defined as a milk product that physically resembles ice cream but has an acidic yoghurt flavour [13]. Frozen yoghurt has evolved into a delectable ice cream substitute that combines the nutritious benefits of yoghurt with the refreshing flavour of ice cream. Ice cream is an aerated, highly nutritive, complex food, containing proteins, fats, sugars, minerals, and different flavours, and is created from air cells in the shape of foam wrapped in a partially frozen emulsion [13]. The texture and structure of ice cream are a total dry substance content, milk fat composition, and the distribution and size of the ice crystal/fat particles all influence the quality of these foams [14]. Jerusalem artichoke (*helianthus tuberosus* L.) is a root vegetable that grows widely in cool to warm climates, and its tubers can be globally produced in regions located in Europe, North America, Asia, and Egypt. The chemical composition of the Jerusalem artichoke tuber was found to be 6.8–7.32%, 74.57–84.6%, 2.60–7.60%, 3.81–4.4%, and 5.12–5.20% for moisture, total carbohydrate, crude protein, crude fibre, and ash, respectively [15–17]. As a food, JATP contains a high amount of dietary fibre, namely inulin (63–78.2% dry weight) [18]. Inulin is a prebiotic compound and a good source of a low-calorie ingredient [19]. In addition, recent studies have reported that inulin has both nutritional and functional attributes, particularly beneficial to individuals with Type 2 diabetes, obesity, and reduces the risk of colorectal cancer. Furthermore, inulin facilitates the digestion of high-protein diets, retards fat absorption, and provides roughage, preventing constipation. It also remains in the digestive tract, providing satiety without carrying extra calories, lowers blood cholesterol and triglycerides, helps with blood glucose control for diabetics, and decreases the incidence of colon cancer [20]. In addition, inulin increases the activity of beneficial live active cultures and inhibits harmful bacteria in the digestive tract. Studies on the development of new dairy products from Jerusalem artichoke tubers are limited [15]. Although milk fat has an important role in the texture, flavour and colour development of dairy products, nowadays, consumers are becoming increasingly aware of the health risks associated with a high-fat and high-calorie diet. As a result, there is a greater demand for low-fat and low-calorie foods, including dairy products, owing primarily to dietary concerns about reducing overall fat and sweetener intake [21]. The development of new types of fermented milk depends largely on the ingredients or formulations employed. Fat reduction can cause some defects in yoghurt and nonfat ice creams, such as lack of flavour, weak body, and poor texture [22,23]. Although the manufacture of low or nonfat dairy products has been possible for many years, the use of fat replacers in the manufacture of dairy products is still novel. Jerusalem artichoke tubers can also be used to replace fat and sugar content while also acting as a texturing and water-binding agent [24,25]. The combination of probiotics and prebiotics will produce synbiotic products that will offer health benefits to consumers. Synbiotic formulations containing food products are widely used for therapeutic food production. *Lactobacillus casei* Lc-01 *(L. casei* Lc-01) is a probiotic strain known for its gastrointestinal and digestive health benefits as well as therapeutic properties [26,27]. Similarly, *bifidobacterium bifidum* BGN4 (*B. bifidum* BGN4) was used as a food ingredient because of its ability to produce beneficial compounds. Several in vivo and in vitro studies have demonstrated several biofunctional effects and therapeutic applications of *B. bifidum* BGN4 [28]. Revising the available literature, limited information is available about the potential benefits of the addition of different concentrations of Jerusalem artichoke tuber powder as a fat replacer on the physicochemical properties and survival of probiotics in frozen yoghurt. Consequently, the main objectives of this study were to evaluate the incorporation of JATP, as an ingredient and a fat replacer, on the probiotic viability, melting rate, textural parameters, physicochemical properties, and sensory acceptability of frozen yoghurt during storage.

2. Materials and Methods

2.1. Materials and Reagents

Fresh buffalo milk (15% T.S. and 5.5% fat) was collected from the dairy unit of the Dairy Science and Technology Department, Faculty of Agriculture & Natural Resources, Aswan University, Egypt. The milk was immediately refrigerated until used. Whipped cream (26% T.S. and 20% fat), commercial grade sugar, and skimmed milk powder (97% TS) were bought from a local market. Stabiliser (C.M.C) and emulsifier (mono- and di-glycerides) were obtained from Misr Food Additives-MIFAD (Cairo, Egypt). The Jerusalem artichoke tubers (*helianthus tuberosus*) were obtained from the Experimental Station, Agricultural Research Center, Egypt.

2.2. Starter Culture

Yoghurt culture YC-x11 that consists of *streptococcus thermophilus* and *lactobacillus delbreucki* ssp. *bulgaricus, bifidobacterium bifidum* BGN4 and *lactobacillus casei* Lc-01 (freeze-dried red-set) was obtained from Chr. Hansen Laboratories, Copenhagen, Denmark. The working culture was prepared by adding a few milligrammes of the freeze-dried culture to 100 mL of w/v sterile reconstituted skim milk (10%TS), then incubated at 42 °C until the onset of gelation. Two millilitres of the mother culture from this passage were transferred into 100 mL of sterile skim milk at 42 °C and incubated until a gel had just formed. This second culture was used for the propagation of a bulk culture (1 L) for inoculation of the different treatments. Bulk cultures were prepared 1 day before the production of yoghurt [29].

2.3. Methods

2.3.1. Preparation of Jerusalem Artichoke Tuber Powder

Jerusalem artichoke tubers were prepared according to the method described elsewhere [16]. Tubers of Jerusalem artichoke were washed in tap water and any degraded pieces were removed before being sliced using a standard food-slicing machine. To inhibit polyphenoloxidases activity, the sliced tubers were soaked in boiling water for 5 min, then immediately dipped in (1%) cold citric acid solution. The tuber slices were dried at 54–55 °C in an electronic air oven until they reached a consistent weight, then ground in a hammer mill and sieved through a 40-mesh sieve. The powdered Jerusalem artichoke tubers were then stored at −18 °C until needed.

2.3.2. Preparation of Probiotic Frozen Yoghurt

Different treatments of frozen yoghurt were prepared in duplicate batches in the laboratory of the Department of Dairy Science & Technology, Faculty of Agriculture & Natural Resources, Aswan University, Aswan, Egypt. The formula for frozen yoghurt mixes is shown in Table 1. The control mix was standardised to 8% fat and 30% total solids. The experiment was divided into five equal parts. In each part, JATP was added as a substitute for milk fat at 5%, 10%, 15%, and 20% of fat source used in the mix (T_1, T_2, T_3, and T_4), respectively. One part of the mixture without JATP was used as a control (CN). Then, it was pasteurised at 80 °C for 20 min, homogenised, and quickly cooled to 42 °C. Half of each treatment was used separately to manufacture probiotic yoghurt by adding 1% yoghurt starter (1:1) + 1% probiotic cultures of *lactobacillus casei* Lc-01 and *bifidobacterium bifidum* BGN4 (1:1) and incubating at 42 °C until pH reached 4.60. The remaining half part was remixed with probiotic yoghurt and 0.5% stabiliser (C.M.C) and emulsifier (mono-and di-glycerides) were added. All samples were stored at 4 °C for ageing up to 24 h and then froze and whipped in the ice cream maker (Taylormate^{TM} Model 152, Taylor Company, Blackhawk Blvd, Rockton, IL, USA), and packed in sterilised plastic cups (100 mL), then hardened at −20 °C separately. The frozen yoghurt was stored frozen for further analysis for up to 90 days. The frozen yoghurt was manufactured according to [30], with some modifications. Frozen yoghurt samples were analysed at 1, 30, 60, and 90 days of storage for physicochemical, sensory properties, and survival bacteria. For every treatment, triplication was performed.

Table 1. Formulation of frozen yoghurt mixes (% *w*/*w*).

Amount/100 Kg					Ingredients
T_4	T_3	T_2	T_1	CN	
54	53	52	51	50	Whole buffalo milk
17	18.5	21	23	25	Whipped cream
0.60	1.60	2.60	3.50	5	Skim milk powder
10	10	10	10	10	Commercial grade sucrose
0.10	0.10	0.10	0.10	0.10	Vanilla flavor
0.50	0.50	0.50	0.50	0.50	Stabiliser/Emulsifier
7.00	5.25	3.45	1.73	0	JATP
10.80	11.05	10.35	10.17	9.40	Water
100	100	100	100	100	Total

2.3.3. Microbiological Estimation

Total lactic acid bacterial counts, mould and yeasts, and coliforms were determined according to standard procedures [31], frozen yoghurt containers were wiped, from the outside, with 70% ethanol, and their contents were thoroughly mixed with a sterile spatula. A composite subsample was prepared by transferring yoghurt into a sterile 250 mL Erlenmeyer flask, that contained sterile phosphate buffer and blending with warm buffer (40 °C) for 24 h until a homogeneous mixture was obtained. Total bacterial counts and coliforms were determined by the pour plate technique, while mould and yeasts were counted on the spread plates. Total viable bacterial counts were determined using plate count agar with incubation at 32 °C for 48 h. Coliforms were determined on violet-red bile agar after incubation at 37 °C for 24 h. Mould and yeasts were enumerated on plate count agar that contained 0.01% chloramphenicol and 0.01% chlortetracycline hydrochloride with incubation at 25 °C for 5 days and 5 °C for 10 days, respectively. *Bifidobacterium bifidum* was determined in MRS-OG mixture solution of (0.02% Oxgall and 0.03% Gentamince). *Lactobacillus casei* was determined using lactobacillus selective agar plus 0.2 Oxgell (LBSO). Plates were incubated anaerobically at 37 °C for 48 h. All analyses were performed in triplicates [32].

2.4. Physicochemical Properties

2.4.1. Titratable Acidity and pH

For each parameter, samples were analysed for pH and acidity after 1, 30, 60, and 90 days in three replicates, pH value was measured using a Swiss Gallenkamp stick pH metre with glass electrode. Acidity was measured with the titration method using 0.1 N sodium hydroxide and phenolphthalein (0.5% *w*/*v* in ethanol) as an indicator [33].

2.4.2. Vitamins Analysis

Ascorbic acid was measured using the method of Williams, 1984 [33]. Thiamine (B_1), riboflavin (B_2) niacin (B_3) and pyridoxine (B_6) were determined by the method of Rudenko and Kartsova, 2010 [34], and expressed as mg/100g.

2.4.3. Texture Profile Analysis

Texture analysis was carried out using a textural profile analysis (TPA). For this, the ice cream samples were transferred to a freezer at −10 °C for approximately 24 h prior to analysis. The operation parameters were as follows: penetration rate, 15 mm; applied force, 5 g; probe penetration rate, 3.3 mm/s; and probe speed before and after penetration, 3.0 mm/s. The parameters, including hardness, cohesiveness, and gumminess were determined (n = 3). Gumminess is calculated from hardness × cohesiveness [32]. The viscosity was determined as described elsewhere [35]; then, after 20 h, the viscosity of the refrigerated ice cream mixture was measured using a viscometer. Analysis was performed in triplicate after 1, 30, 60, and 90 days.

2.4.4. Overrun

Overrun was estimated using a 100 mL standard cup at 25 ± 1 °C, as the difference in weight between the resultant ice cream and the original mix was as follows:

$$\text{Overrun\%} = (\text{weight of ice cream mix}) - (\text{weight of ice cream}) \times 100/(\text{weight of ice cream}) - 1. \quad (1)$$

2.4.5. Freezing Point

The freezing point was determined using a special low-temperature thermometer as described elsewhere [36].

2.4.6. Melting Rate

It was measured by placing a 60-g frozen yoghurt sample on a wire mesh (1 mm) at 25 ± 1 °C over a beaker. After 90 min, both the melted and unmelted frozen yoghurt were weighed according to the method adopted elsewhere [32]. The melting rate was calculated as the weight of drip vs. time (minutes). Analysis was performed in triplicate after 1 day.

2.4.7. Sensory Evaluation

All the ice cream samples were sensory evaluated for flavour, body and texture, melting properties, and colour by 15 trained panelists. A hedonic scale from 1 to 100 was used. Scale 1 refers to extreme dislike and scale 100 refers to extreme liking. Overall acceptability was calculated from the total score of the judged attributes.

2.4.8. Statistical Analysis

Significant differences between the samples were detected using analysis of variance (ANOVA) and Duncan's multiple range test. The level of $p < 0.05$ was used to define the significant differences. The SPSS programme was used to conduct all of the analyses (version 20 SPSS Inc).

3. Results and Discussion

3.1. Physicochemical Characteristics

3.1.1. Acidity and pH of The Frozen Yoghurt

Table 2 shows the changes in pH and acidity of frozen yoghurt during 90 days of frozen storage at −20 °C. In general, the pH was lower in frozen yoghurt formulated with JATP compared with the control sample. The titratable acidity results also showed that fortification with JATP decreased pH and increased the acidity of the samples compared with the control sample significantly ($p < 0.05$). In general, the treatment T_4 had the highest acidity value, while the control sample had the lowest one. This decrease in pH values with increasing JATP addition could be attributed to increasing the inulin content of treatments promoted by the growth of cultured micro-organisms and the fermentation of lactose into lactic acid. This result agreed with a previous study [37], which stated that inulin promoted the growth of all strains of LAB with Jerusalem artichoke. The acidity levels in both the control and experimental frozen yoghurts increased during storage. The addition of JATP and probiotics to frozen yoghurt caused a significant decrease in pH and an increase in acidity. A similar observation was reported by Aichayawanich and Wongsa [38] in functional ice cream and in bio-yoghurt [16]. The results herein were in accordance with a previous study [39], which produced a probiotic ice cream containing *Bifidobacterium longum* and showed a lower pH than the unfermented ice cream.

Table 2. Changes in pH and acidity values of different frozen yoghurt affected by adding substantial ratios of JATP during cold storage.

Treatments	Time of Storage (Days)			
	1	30	60	90
		pH		
CN	4.80 ± 0.01 ^{aA}	4.71 ± 0.005 ^{aB}	4.52 ± 0.17 ^{bC}	4.48 ± 0.011 ^{aD}
T_1	4.74 ± 0.00 ^{bA}	4.60 ± 0.00 ^{bA}	4.57 ± 0.01 ^{aA}	3.99 ± 0.34 ^{aB}
T_2	4.65 ± 0.00 ^{cA}	4.61 ± 0.00 ^{bB}	4.50 ± 0.00 ^{bC}	4.34 ± 0.011 ^{aD}
T_3	4.60 ± 0.00 ^{dA}	4.52 ± 0.00 ^{cAB}	4.42 ± 0.01 ^{cAB}	3.95 ± 0.33 ^{aC}
T_4	4.60 ± 0.00 ^{dA}	4.50 ± 0.01 ^{dB}	4.42 ± 0.00 ^{cC}	4.28 ± 0.00 ^{aD}
		Titratable acidity (%)		
CN	0.70 ± 0.00 ^{eC}	0.73 ± 0.00 ^{cB}	0.75 ± 0.00 ^{dA}	0.76 ± 0.01 ^{eA}
T_1	0.76 ± 0.00 ^{dD}	0.79 ± 0.00 ^{bC}	0.82 ± 0.00 ^{cB}	0.85 ± 0.00 ^{dA}
T_2	0.78 ± 0.00 ^{cD}	0.81 ± 0.00 ^{bC}	0.85 ± 0.00 ^{bB}	0.92 ± 0.00 ^{cA}
T_3	0.83 ± 0.00 ^{bD}	0.88 ± 0.00 ^{aC}	0.92 ± 0.00 ^{aB}	0.98 ± 0.00 ^{bA}
T_4	0.85 ± 0.00 ^{aD}	0.90 ± 0.01 ^{aC}	0.93 ± 0.00 ^{aB}	0.99 ± 0.00 ^{aA}

CN: frozen yoghurt without adding JATP. T_1: frozen yoghurt with 5% JATP of the fat source used in the mix. T_2: frozen yoghurt with 10% JATP of the fat source used in the mix. T_3: frozen yoghurt with 15% JATP of the fat source used in the mix. T_4: frozen yoghurt with 20% JATP of the fat source used in the mix. Values represent the mean ± SE; $n = 3$. $^{a–e}$ means in a column with different superscripts and is significantly different between the treatments ($p < 0.05$). $^{A–D}$ means in row with different superscripts and is significantly different between the storage periods ($p < 0.05$).

3.1.2. Textural Profile Analysis and Viscosity of Frozen Yoghurt

Table 3 shows the results of rheological parameters expressed as viscosity, hardness, cohesiveness, and gumminess values of different frozen yoghurts when fresh. A significant difference was observed in the hardness of the samples. The prebiotic contains samples with the highest firmness, while the control sample had the lowest value. These results were in agreement with a previous study [40], which produced synbiotic ice cream using probiotic bacteria and fructooligosaccharides. Generally, polysaccharides increase the firmness of the samples by absorbing water molecules and providing chemical bonds within the molecular structures [27,39]. A previous study [27] investigated the effects of inulin and *L. casei* on the hardness of probiotic and synbiotic ice creams and observed a synergistic effect between the presence of inulin and the growth of *L. casei*. A significant difference was observed in the cohesiveness values of frozen yoghurt samples. Using JATP caused an increase in cohesiveness values compared to the control sample. The adhesiveness values showed a similar pattern to the hardness values. The cohesiveness value increases from 0.13 for control to 0.35 for 20% JATP and corresponding fat reduction. These results are in agreement with those reported by Tiwari et al., 2015 [41]. The frozen yoghurt in the control sample had the lowest gumminess among all samples. The high gumminess observed for the probiotics was probably due to the production of polysaccharide during the fermentation process of JATP, by LAB (including *L. casei* and *B. bifidum)*.

Treatment T_4 had the highest apparent viscosity value ($p < 0.05$), followed by T_3, T_2, T1, and CN. Viscosity is accepted to be among the significant characteristics of the frozen yoghurt mixture. It can be seen that the addition of JATP affected the frozen yoghurt viscosity. Thus, among the several advantages of JATP it can improve the viscosity of frozen yoghurt mix. These results could be due to the increase in total solids with the addition of various amounts of JAT. These results are in agreement with the findings by Bakr et al., 2020 [37].

Table 3. Changes in texture profile and viscosity in fresh frozen yoghurt mixes affected by adding substantial ratios of JATP during cold storage.

		Viscosity (MPas)			
Treatments	**CN**	**T_1**	**T_2**	**T_3**	**T_4**
	328 ± 0.11 ^{e}	329 ± 0.28 ^{d}	332 ± 0.00 ^{c}	334 ± 0.57 ^{b}	336 ± 0.00 ^{a}
		Hardness (g)			
	3.12 ± 0.35 ^{d}	4.27 ± 0.03 ^{c}	4.98 ± 0.13 ^{c}	5.12 ± 0.05 ^{b}	5.50 ± 0.06 ^{a}
		Cohesiveness			
	0.13 ± 0.00 ^{d}	0.19 ± 0.01 ^{c}	0.25 ± 0.01 ^{c}	0.27 ± 0.01 ^{b}	0.35 ± 0.01 ^{a}
		Gumminess (g)			
	0.407 ± 0.00 ^{d}	0.810 ± 0.03 ^{c}	1.247 ± 0.06 ^{c}	1.382 ± 0.07 ^{b}	1.925 ± 0.05 ^{a}

CN: frozen yoghurt without adding JATP. T_1: frozen yoghurt with 5% JATP of the fat source used in the mix. T_2: frozen yoghurt with 10% JATP of the fat source used in the mix. T_3: frozen yoghurt with 15% JATP of the fat source used in the mix. T_4: frozen yoghurt with 20% JATP of the fat source used in the mix. Values represent the mean ± SE; $n = 3$. $^{a–e}$ means in a column with different superscripts and is significantly different between the treatments ($p < 0.05$).

3.1.3. Freezing Point, Melting, and Overrun Properties of Frozen Yoghurt

Generally, the freezing point is depressed as the serum phase concentration is increased or as the solute molecular weight is decreased [42]. Although sugars have a primary effect on the freezing point, the addition of high molecular biopolymers, such as polysaccharides, does not induce a significant freezing point. The type and percentage of the fibres used are affected significantly. It was obvious from Table 4 that the substitution of milk fat with different ratios of JATP decreased the freezing point of frozen yoghurt samples. These results are in agreement with those mentioned by Rubel et al., 2021 [17], who manufactured ice milk with Jerusalem artichoke. Melting resistance increased significantly ($p < 0.05$) for frozen yoghurt with JATP substitution (Table 4). The melting period was 61.20 min for the control frozen yoghurt sample, compared with 62.15–63.20 min for the JATP-fortified frozen yoghurt sample. The effect of the addition of JATP at the levels of 10, 15, and 20% gave the longest melting time compared with other levels. These results show the high resistance of ice cream fortified with JATP against melting. It was suggested by Akın et al. (2007) [11] that the reason for the slower melting of ice cream with added inulin might be the ability of inulin to prevent water molecules from moving freely. The slow melting of ice cream with citrus fibre and green banana flour is in agreement with the findings of this study. Similar trends were observed by Tiwari et al., 2015 [41]. Overrun and melting are associated with the volume of air involved in the manufacturing process. This property can shape the structure of the final product because the air present in the ice cream can provide a light texture and affect some physical properties, such as melting and hardness. The addition of JATP increased the overrun compared with the overrun on the control frozen yoghurt sample; the overrun was at its maximum at 20% JATP fortification (Table 4).

Table 4. Changes in freezing point, melting rate, and overrun of fresh frozen yoghurt affected by adding substantial ratios of JATP during cold storage.

		Freezing Point (°C)			
Treatments	**CN**	**T_1**	**T_2**	**T_3**	**T_4**
	−2.43 ± 0.00 ^{e}	−2.55 ± 0.01 ^{d}	−2.63 ± 0.02 ^{c}	−2.77 ± 0.3 ^{b}	−2.85 ± 0.00 ^{a}
		Complete melting time (Minutes)			
	61.20 ± 0.05 ^{b}	62.15 ± 0.14 ^{b}	63.19 ± 0.19 ^{a}	63.20 ± 0.08 ^{a}	63.15 ± 0.14 ^{a}
		Overrun %			
	38.00 ± 0.29 ^{c}	38.00 ± 0.00 ^{c}	39.00 ± 0.23 ^{b}	39.00 ± 0.11 ^{b}	40.00 ± 0.05 ^{a}

CN: frozen yoghurt without adding JATP. T_1: frozen yoghurt with 5% JATP of the fat source used in the mix. T_2: frozen yoghurt with 10% JATP of the fat source used in the mix. T_3: frozen yoghurt with 15% JATP of the fat source used in the mix. T_4: frozen yoghurt with 20% JATP of the fat source used in the mix. Values represent the mean ± SE; $n = 3$. $^{a–e}$ means in a column with different superscripts and is significantly different between the treatments ($p < 0.05$).

3.2. Vitamins

The vitamin B complex (B_1, B_2, B_3, and B_6 as water-soluble vitamins) of frozen yoghurt formulated with different levels of JATP is shown in Table 5. From the appeared data, the control showed that the control sample contained the lowest content of vitamins B_1, B_2, B_3, and B_6. Furthermore, the data in Table 5 demonstrated that an increase in the vitamin B complex is accompanied by an increase in JATP. Additionally, it could be observed that vitamins B1, B2, B3, and B6 decrease during storage, which may be a result of the consumption of these vitamins by some organisms, such as *L. casei* [42].

Table 5. Changes in vitamin contents in frozen yoghurt affected by adding substantial ratios of JATP during cold storage.

Treatments	Time of Storage (Days)			
	1	30	60	90
	Thiamin (B_1) (mg/100g).			
CN	0.210 ± 0.00^{eA}	0.192 ± 0.00^{eB}	0.185 ± 0.00^{eB}	0.174 ± 0.00^{eC}
T_1	2.150 ± 0.00^{dA}	2.010 ± 0.017^{dB}	1.915 ± 0.00^{dC}	1.832 ± 0.00^{dD}
T_2	2.280 ± 0.011^{cA}	2.110 ± 0.00^{cB}	2.007 ± 0.01^{cC}	1.915 ± 0.00^{cD}
T_3	2.520 ± 0.011^{bA}	2.400 ± 0.011^{bB}	2.305 ± 0.00^{bC}	2.198 ± 0.00^{bD}
T_4	2.850 ± 0.057^{aA}	2.760 ± 0.03^{aA}	2.644 ± 0.00^{aB}	2.510 ± 0.00^{aC}
	Riboflavin (B_2) (mg/100g).			
CN	0.061 ± 0.00^{eA}	0.055 ± 0.00^{eB}	0.050 ± 0.00^{eC}	0.044 ± 0.00^{dD}
T_1	0.450 ± 0.00^{dA}	0.410 ± 0.00^{dB}	0.390 ± 0.00^{dC}	0.372 ± 0.00^{cD}
T_2	0.480 ± 0.00^{cA}	0.450 ± 0.00^{cB}	0.411 ± 0.00^{cC}	0.390 ± 0.00^{bD}
T_3	0.511 ± 0.00^{bA}	0.465 ± 0.00^{bB}	0.424 ± 0.00^{bC}	0.400 ± 0.00^{bD}
T_4	0.520 ± 0.00^{aA}	0.486 ± 0.00^{aB}	0.465 ± 0.00^{aC}	0.445 ± 0.00^{aD}
	Niacin (B_3) (mg/100g).			
CN	0.365 ± 0.00^{eA}	0.341 ± 0.00^{eB}	0.332 ± 0.00^{eC}	0.322 ± 0.00^{eD}
T_1	1.020 ± 0.00^{dA}	0.987 ± 0.00^{dB}	0.921 ± 0.00^{dC}	0.898 ± 0.00^{dD}
T_2	1.204 ± 0.00^{cA}	1.177 ± 0.00^{cB}	1.170 ± 0.00^{cC}	1.158 ± 0.00^{cD}
T_3	1.310 ± 0.00^{bA}	1.280 ± 0.00^{bB}	1.268 ± 0.00^{bC}	1.251 ± 0.00^{bD}
T_4	1.362 ± 0.00^{aA}	1.313 ± 0.00^{aB}	1.291 ± 0.00^{aC}	1.277 ± 0.00^{aD}
	Pyridoxine (B_6) (mg/100g).			
CN	0.122 ± 0.00^{eA}	0.113 ± 0.00^{eB}	0.106 ± 0.00^{dC}	0.101 ± 0.00^{dD}
T_1	0.455 ± 0.00^{dA}	0.449 ± 0.00^{dA}	0.441 ± 0.00^{cB}	0.434 ± 0.00^{cC}
T_2	0.470 ± 0.00^{cA}	0.455 ± 0.00^{cB}	0.444 ± 0.00^{cC}	0.430 ± 0.00^{cD}
T_3	0.510 ± 0.00^{bA}	0.482 ± 0.00^{bB}	0.470 ± 0.00^{bC}	0.455 ± 0.00^{bD}
T_4	0.522 ± 0.03^{aA}	0.507 ± 0.00^{aB}	0.496 ± 0.00^{aC}	0.487 ± 0.00^{aD}
	Vitamin (C) (mg/100g).			
CN	0.241 ± 0.00^{dA}	0.239 ± 0.00^{dA}	0.239 ± 0.00^{dA}	0.239 ± 0.00^{dA}
T_1	3.124 ± 0.00^{cA}	3.121 ± 0.00^{cAB}	3.117 ± 0.00^{cB}	3.116 ± 0.00^{cB}
T_2	3.345 ± 0.00^{bA}	3.342 ± 0.00^{bAB}	3.338 ± 0.00^{bB}	3.337 ± 0.00^{bB}
T_3	3.350 ± 0.00^{bA}	3.343 ± 0.00^{bA}	3.340 ± 0.00^{bA}	3.340 ± 0.00^{bA}
T_4	3.410 ± 0.01^{aA}	3.408 ± 0.00^{aA}	3.407 ± 0.00^{aA}	3.408 ± 0.00^{aA}

CN: frozen yoghurt without adding JATP. T_1: frozen yoghurt with 5% JATP of the fat source used in the mix. T_2: frozen yoghurt with 10% JATP of the fat source used in the mix. T_3: frozen yoghurt with 15% JATP of the fat source used in the mix. T_4: frozen yoghurt with 20% JATP of the fat source used in the mix. Values represent the mean ± SE; $n = 3$. $^{a–e}$ means in a column with different superscripts and is significantly different between the treatments ($p < 0.05$). $^{A–D}$ means in row with different superscripts and is significantly different between the storage periods ($p < 0.05$).

3.3. Survival of Probiotics

Table 6 represents the results of the total viable bacterial counts in the frozen yoghurt samples. The LAB counts ranged between 7.75 for control and 8.65 log cfu/g for 20% JATP after 1 day. The bacterial count in sample T_4 was the highest value. The results showed that the number of LAB decreased during the storage due to the pH reduction and the freezing shock. These results were in accordance with previous works [40]. In addition, Table 6 shows changes in the counts of *B. bifidum* and *L. casei* in frozen yoghurt samples throughout the 90 days of storage. The viable counts of *L. casei* ranged between 7.44 and 9.47 log cfu/g in the samples of control and 20% JATP. Although slight fluctuations were seen throughout the storage, viable counts significantly decreased ($p < 0.05$) in all the samples at the end of storage compared with the at the beginning. This could be due to the bacterial cells being destroyed by the low temperature. A previous study [43] reported that apple fibre increased the viability of *B. lactis* and *L. acidophilus* in yoghurt.

Table 6. Changes in total lactic acid bacteria count, *Bifidobacterium bifidum* and *Lactobacillus casei* in frozen yoghurt affected by adding substantial ratios of JATP during cold storage.

Treatments	Time of Storage (Days)			
	1	30	60	90
	Total lactic acid bacteria count (log cfu/g)			
CN	7.75 ± 0.05 ^{bA}	6.40 ± 0.40 ^{bB}	5.70 ± 0.023 ^{bBC}	5.12 ± 0.13 ^{cC}
T_1	8.85 ± 0.14 ^{aA}	8.20 ± 0.17 ^{aB}	7.30 ± 0.00 ^{aC}	7.05 ± 0.08 ^{bC}
T_2	8.55 ± 0.00 ^{aA}	7.90 ± 0.05 ^{aB}	7.30 ± 0.02 ^{aC}	6.90 ± 0.00 ^{bD}
T_3	8.60 ± 0.14 ^{aA}	8.20 ± 0.05 ^{aB}	7.40 ± 0.11 ^{aC}	7.20 ± 0.14 ^{bC}
T_4	8.65 ± 0.10 ^{aA}	8.34 ± 0.09 ^{aA}	7.63 ± 0.31 ^{aB}	7.54 ± 0.00 ^{aB}
	Bifidobacterium bifidum BGN4 (log cfu/g)			
CN	7.12 ± 0.06 ^{bA}	6.27 ± 0.01 ^{cB}	6.17 ± 0.00 ^{dB}	5.97 ± 0.20 ^{bB}
T_1	8.15 ± 0.08 ^{aA}	7.76 ± 0.00 ^{bB}	7.23 ± 0.00 ^{cC}	7.05 ± 0.05 ^{aD}
T_2	8.30 ± 0.11 ^{aA}	7.83 ± 0.12 ^{bB}	7.35 ± 0.02 ^{bC}	7.12 ± 0.00 ^{aC}
T_3	8.34 ± 0.02 ^{aA}	7.91 ± 0.03 ^{abB}	7.38 ± 0.01 ^{bC}	7.21 ± 0.00 ^{aD}
T_4	8.41 ± 0.06 ^{aA}	8.08 ± 0.01 ^{aB}	7.55 ± 0.05 ^{aC}	7.30 ± 0.00 ^{aD}
	Lactobacillus casei Lc-01 (log cfu/g)			
CN	7.44 ± 0.12 ^{bA}	6.90 ± 0.11 ^{bB}	6.54 ± 0.05 ^{cC}	6.12 ± 0.07 ^{bD}
T_1	9.13 ± 0.07 ^{aA}	8.63 ± 0.00 ^{aB}	8.14 ± 0.00 ^{bC}	7.70 ± 0.11 ^{aD}
T_2	9.42 ± 0.10 ^{aA}	8.70 ± 0.05 ^{aB}	8.32 ± 0.011 ^{aC}	7.80 ± 0.011 ^{aD}
T_3	9.45 ± 0.14 ^{aA}	8.72 ± 0.17 ^{aB}	8.35 ± 0.00 ^{aC}	7.85 ± 0.00 ^{aD}
T_4	9.47 ± 0.07 ^{aA}	8.73 ± 0.02 ^{aB}	8.40 ± 0.05 ^{aC}	7.88 ± 0.02 ^{aD}

CN: frozen yoghurt without adding JATP. T_1: frozen yoghurt with 5% JATP of the fat source used in the mix. T_2: frozen yoghurt with 10% JATP of the fat source used in the mix. T_3: frozen yoghurt with 15% JATP of the fat source used in the mix. T_4: frozen yoghurt with 20% JATP of the fat source used in the mix. Values represent the mean ± SE; $n = 3$. $^{a–d}$ means in a column with different superscripts and is significantly different between the treatments ($p < 0.05$). $^{A–D}$ means in row with different superscripts and is significantly different between the storage periods ($p < 0.05$).

Viable counts of *B. bifidum* in the samples ranged from 7.12 to 8.41 log cfu/g throughout the 90 days of storage (Table 6). The highest counts were in all samples on day one of storage. In addition, frozen yoghurt fortified with JATP generally had significantly higher viable counts of *B. bifidum* than control samples. In the present study, the frozen yoghurt fortified with JATP had the highest viable count of probiotic bacteria, which may have been caused by the presence of inulin as a prebiotic. In addition, *L. casei* survived better than *B. bifidum* in frozen yoghurt over 90 days. However, the minimum effective therapeutic dose, which should exceed 7 log/cfu g1 in a probiotic product, was reached by day 90 in all treatments except control frozen yoghurt. The low counts were enumerated in control samples at the end of storage. The overall viability of *B. bifidum* was low compared to *L. casei*; it may be caused by the high cytoplasmic buffering capacity of *L. casei*. Inulin, vitamins, and organic acids contained in JATP are used as a source of energy by the probiotics; the plant-based soluble dietary fibres have a prebiotic effect [16,44,45]. The total viable count of *B. bifidum* and *L. casei* in frozen yoghurt containing JATP was higher than in the control samples up to the end of frozen storage. These results suggest that the added JATP offered a probiotic effect on the total bacterial count during the storage of frozen yoghurt. These results were in agreement with previous works [27]. Mould, yeasts, and coliforms of the frozen yoghurt were not detected either when fresh or after 90 days of cold storage, these results are in agreement with [7,16]

3.4. Sensory Evaluation

In this study, the flavour, body texture, colour, and melting properties of frozen yoghurt samples were evaluated as sensory characteristics. The obtained scores for the frozen yoghurt samples are given in Table 7. On day 1 and day 60 of freeze storage, scores for flavour, body and texture, melting properties, colour, and overall acceptability were significantly different for treated samples with JATP as compared to control. In terms of flavour, body texture, colour, and melting properties, we found significant differences ($p < 0.05$) between the control and the experimental frozen yoghurt enriched with JATP on day 1 and during storage. The flavour score of frozen yoghurt gradually increased with the addition of JATP. According to the flavour score, T1 and T2 presented the closest results to the control as compared to the other samples. Among all the sensorial evaluation criteria, the statistical difference was found to be important between samples and storage time ($p < 0.05$). According to the evaluations of the total overall acceptance, it is understood that the most liked samples were T1 and T2, which contained 5 and 10% of JATP, respectively. Generally, the addition of JATP at 5 and 10% improved the melting quality of the frozen yoghurt and increased the viable count of LAB.

Table 7. Sensory evaluation of frozen yoghurt affected by using JATP.

Treatments	**Time of Storage (Days)**			
	1	**30**	**60**	**90**
	Flavour (45)			
CN	43 ± 0.28^{aA}	42 ± 0.23^{aA}	41 ± 0.00^{aB}	40 ± 0.57^{aC}
T_1	43 ± 0.11^{aA}	42 ± 0.16^{Aa}	39.66 ± 0.43^{bB}	40 ± 0.00^{aAB}
T_2	43 ± 0.00^{aA}	42 ± 0.57^{Aa}	41 ± 0.57^{aA}	40 ± 0.00^{aA}
T_3	41 ± 0.17^{bA}	41 ± 0.00^{bA}	40 ± 0.28^{abB}	39 ± 0.28^{bB}
T_4	39 ± 0.00^{cB}	37 ± 0.11^{cB}	38 ± 0.00^{cA}	38 ± 0.00^{cA}
	Body and Texture (30)			
CN	27 ± 0.00^{aA}	26 ± 0.57^{aA}	25 ± 0.00^{aAB}	23 ± 0.57^{aB}
T_1	27 ± 0.00^{aA}	26 ± 0.00^{aAB}	25 ± 0.00^{aB}	23 ± 0.00^{aC}
T_2	27 ± 0.17^{aA}	26 ± 0.05^{aA}	25 ± 0.57^{aB}	23 ± 0.00^{aC}
T_3	25 ± 0.00^{bA}	24 ± 0.00^{bB}	23 ± 0.00^{bC}	21 ± 0.57^{bD}
T_4	24 ± 0.05^{cA}	23 ± 0.011^{cB}	22 ± 0.29^{cC}	20 ± 0.57^{bD}
	Melting properties (10)			
CN	8 ± 0.57^{bA}	8 ± 0.00^{aA}	7 ± 0.00^{bB}	7 ± 0.00^{aA}
T_1	8 ± 0.00^{bA}	8 ± 0.57^{aA}	8 ± 0.57^{aA}	7 ± 0.00^{aB}
T_2	9 ± 0.00^{aA}	8 ± 0.28^{aB}	8 ± 0.00^{aB}	8 ± 0.28^{aB}
T_3	9 ± 0.00^{aA}	7 ± 0.00^{bB}	8 ± 0.00^{aB}	8 ± 0.57^{aB}
T_4	9 ± 0.28^{aA}	7 ± 0.05^{bA}	7 ± 0.00^{bA}	7 ± 0.17^{aA}
	Colour (15)			
CN	13 ± 0.28^{aA}	12 ± 0.57^{aA}	11 ± 0.57^{aB}	11 ± 0.57^{aB}
T_1	13 ± 0.05^{aA}	12 ± 0.28^{aB}	11 ± 0.28^{aC}	10 ± 0.86^{bD}
T_2	13 ± 0.14^{aA}	12 ± 0.28^{aB}	10 ± 0.00^{aC}	10 ± 0.57^{bC}
T_3	12 ± 0.00^{bA}	11 ± 0.57^{aA}	10 ± 0.00^{aB}	9 ± 0.23^{cC}
T_4	12 ± 0.00^{bA}	11 ± 0.00^{aAB}	10 ± 0.57^{aB}	9 ± 0.00^{cC}

Table 7. *Cont.*

Treatments	Time of Storage (Days)			
	1	**30**	**60**	**90**
	Overall acceptability (100)			
CN	91 ± 0.00^{bA}	88 ± 0.92^{aA}	84 ± 0.57^{aB}	81 ± 0.57^{aB}
T_1	91 ± 0.17^{bA}	88 ± 0.33^{aA}	84 ± 0.0^{aAB}	80 ± 0.86^{aB}
T_2	92 ± 0.31^{aA}	88 ± 0.05^{aB}	84 ± 1.15^{aC}	81 ± 0.288^{aD}
T_3	87 ± 0.17^{cA}	83 ± 0.57^{bB}	81 ± 0.28^{bC}	77 ± 0.51^{bC}
T_4	84 ± 0.27^{dA}	78 ± 0.28^{cB}	77 ± 0.57^{cC}	74 ± 0.40^{cC}

CN: frozen yoghurt without adding JATP. T_1: frozen yoghurt with 5% JATP of the fat source used in the mix. T_2: frozen yoghurt with 10% JATP of the fat source used in the mix. T_3: frozen yoghurt with 15% JATP of the fat source used in the mix. T_4: frozen yoghurt with 20% JATP of the fat source used in the mix. Values represent the mean ± SE; $n = 3$. $^{a–d}$ means in a column with different superscripts and is significantly different between the treatments ($p < 0.05$). $^{A–D}$ means in row with different superscripts and is significantly different between the storage periods ($p < 0.05$).

4. Conclusions

Given the above information, bio-frozen yoghurt is a functional food with probiotics and prebiotics. The physicochemical, textural, microbiological, and sensory characteristics of the frozen yoghurt generated with four strains of (i.e., *S. thermophilus*, *L. bulgaricus*, *L. casei,* and *B. bifidum*) Jerusalem artichoke tuber powder as a fat replacer were investigated in this study. The results showed that JATP improved the physicochemical, rheological, and sensorial properties of the frozen yoghurt and enhanced the viability of the lactic acid bacteria. In order to improve the functional and nutritional properties of frozen yoghurt, it seems that the supplementation of JATP in the formulation of frozen yoghurt could be a promising strategy. On an industrial scale, when using JATP for the production of bio-frozen yoghurt, it is critical to select the strain and ensure the survival of probiotic bacteria throughout storage. Overall, using JATP as a food ingredient may assist in increasing and improving people's health and well-being.

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