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Original Article

Improving Stability of Bioactive Components and Folate and Survival of *Bifidobacterium Bifidum* and *Bifidobacterium Lactis* in Probiotic Ice Creams Containing Japanese Loquat Pulps

Elham Sadat Entezari Sareshkeh[1], Marjan Nouri[2*]

1- MSc of Food Science and Technology, Roudehen Branch, Islamic Azad University, Roudehen, Iran
2- Department of Food Science and Technology, Roudehen Branch, Islamic Azad University, Roudehen, Iran

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ABSTRACT

Background and Objectives: Ice cream is a probiotic carrier and *Bifidobacterium* spp. are used to promote health benefits such as vitamin improvement. These bacteria are commonly known as probiotic bacteria. The objective of the present study was to add Japanese loquats, *Bifidobacterium lactis* and *Bifidobacterium bifidum* to improve beneficial and nutritional characteristics of ice creams.

Materials and Methods: Bacteria were used in various ice cream samples supplemented with Japanese loquat pulps (10, 20 and 30% concentrations). Then, physicochemical (pH, acidity, protein, fat, ash and dry matter), melting, colorimetric (L^*, a^* and b^*), overrun and sensory characteristics were assessed. Bioactive parameters of the ice creams, including phenolic component, antioxidant activity, probiotic bacterial survival and folate value, were investigated within eight weeks.

Results: Ash, dry matter, melting rate and overrun significantly increased by adding Japanese loquat pulps ($p < 0.05$). However, no significant effects on pH, acidity, protein and fat of various probiotic ice creams were seen by increasing pulps ($p > 0.05$). Probiotic ice creams significantly included further phenolic compounds (98.63 mg GAE/100g), antioxidant activity (105.12 mg/100g) and bacterial viability (8.23 log CFU/g) by increasing Japanese loquat pulps. However, these parameters decreased by extending the storage time ($p < 0.05$). Increases in pulp and storage time significantly increased folates of various samples ($p < 0.05$).

Conclusions: The highest functional capacities of the probiotic ice creams belonged to a mixture of *Bifidobacterium bifidum*, *Bifidobacterium lactis* and 30% of Japanese loquat pulps.

Keywords: Probiotic, Folat, Phenolic compound, Survival rate

Introduction

Ice cream is a nutritious dairy dessert that can be used as an ideal probiotic carrier. Probiotic bacteria are incorporated in ice cream formulation, which are extremely beneficial for the production of functional food products. This dessert includes advantageous components such as vitamins, minerals and raw substances (1). Inevitable decreases in survival of probiotic strains is observed during production, processing, shelf-life and thawing of the ice creams (2). *Bifidobacterium lactis* and *B. bifidum* are used predominantly as the most important strains of probiotic bacteria in fermented and dairy products because of their several advantageous characteristics. These bacteria play critical roles in maintaining gut microflora and stimulating immune responses (3). Folate is highly produced in dairy products due to the presence of *B. bifidum* and *B. lactis*. Bacteria are protected against external agents via stabilization on fruits; therefore, their survival is improved (4). To stabilize target bacteria, Japanese loquat pulps were selected as appropriate substitutions in the present study. Bioactive components included polyphenols, carotenoids, phytosterols, biogenic amines and proteins, which are useful for health promotion in deseases such as diabetes, cancers and cardiovascular diseases (CVDs), especially for the people with neurodegenerative changes and problems in their immune responses and guts (5,6).

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical fruit, which its tree is always green and found in various countries such as China, India, Japan, Italy, Brazil and

***Address for correspondence:** Marjan Nouri, Assistant Professor, Department of Food Science and Technology, Roudehen Branch, Islamic Azad University, Roudehen, Iran. E-mail address: M.nouri@riau.ac.ir

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Spain. Extensive acidity is addressed as a major factor to decrease quality and nutrient status in commercial loquat production (7). The major phenolic components detected in loquat fruits include chlorogenic acid, neochlorogenic acid, ferulic acid, 4-O-caffeoylquinic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, ellagic acid and o-coumaric acid. This fruit is used as an anticancer, anti-inflammation and antioxidant fruit with hypoglycemic effects in medicine and pharmaceutical industries (8). Fruits with bioactive characteristics such as *Butia odorata* as protective agents and *B. lactis* as probiotic bacteria have been used to develop nutritional and functional statuses of ice creams (4). Dark blue and white *Myrtus communis* pulps have been used in formulation of probiotic ice creams including *Lactobacillus casei* bacteria from goat milks (1). Polyols of xylitol, erythritol, maltitol and isomalt have positively affected survival of *Bifidobacterium* subsp. *lactis* DSM15954 (BB-12) and improved nutritional levels of probiotic ice creams (9). The aim of the present study was to investigate effects of Japanese loquat nectar on survival of *B. bifidum* and *B. lactis* in probiotic ice creams and effects of the fruit pulps on physicochemical, sensory, antioxidant and color characteristics of the ice creams during storage.

Materials and Methods

Fresh milk of bovine was purchased from the Agricultural Research Farm of University of Tehran, Iran. *Bifidobacterium bifidum* and *B. lactis* were provided by CHR-Hansen, Denmark. The ∝-monoglyceride emulsifier E471 was purchased from Sigma-Aldrich, USA, and vanillin was purchased from Kalleh Dairy Company, Iran. All solvents and chemicals included analytical grades, purchased from Merck, Germany. Pulps were collected from the Japanese loquat fruits in Tehran, Iran. Fruits were rinsed appropriately and sanitized for 15 min using 150 mg L^{-1} sodium hypochlorite. Pulps were removed using horizontal depulper and then sieved through 3.5 and 0.5-mm meshes. Pulps were heated for 95 °C at 15 min and then cooled down to room temperature. Then, these were stored at -18 °C using sealed polypropylene bags until use. Overall, pulps included total soluble solids (8.35 g/100 g^{-1}), malic acidity (1.5%), β-carotene (29.07 mg/100 g^{-1}), gallic acid (48.9 mg/100 g^{-1}), ascorbic acid (8.20 mg^{-1} L/100 g^{-1}) and DPPH radical inhibition capacity (85.1%).

Preparation of cultured cells

To prepare *B. bifidum* and *B. lactis*, these bacteria were cultured individually in 500 ml of MRS (de Man, Rogosa and Sharpe) broth at 37 °C for 18 h and allowed to reach the logarithmic phase. Then, suspensions of probiotic bacteria were concentrated by centrifugation at 15,000 g for 30 min at 4 °C to ensure 10^8 CFU/mL bacteria followed by washing the bacteria with 25 ml sterile phosphate buffer (10).

Optimization of probiotic ice cream formulations

Skimmed milk was thoroughly mixed with 17% of sugar, 20% of cream and 0.5% of stabilizer to produce probiotic ice cream based on 1 kg. The mixture was stored at 4 °C for 24 h (maturation process). After complete blending of ice cream ingredients, Japanese loquat pulps (10, 20 and 30%) and 1% of emulsifier were added to the mixture and pasteurized at 85 °C for 30 s. *Bifidobacterium bifidum*, *B. lactis* and a mixture of these bacteria were added to the ice creams at a level of nearly 10^8 CFU/mL after cooling down the ice creams. Then, overrun and ice cream production were carried out using home ice cream maker (Table 1). Samples were stored at -18 ºC using 100-ml containers until use (4).

Physicochemical assessments of the ice creams

Acidity and pH were assessed using titration method and pH-meter (Metrohm, Switzerland), respectively. Protein, fat contents, ash and overall dry matter were assessed using Kjeldahl method, Gerber procedure, heating in furnace at 600 °C and oven. Colorimetry characteristics of L^*, a^* and b^* were calculated using Hunterlab Model D25 DP9000 (Hunter Associates Laboratory, USA) (11).

Table 1. Formulated components for the ice cream mixtures

PL_1IBB	Probiotic loquat (10% pulp) ice cream containing *Bifidobacterium bifidum*
PL_1IBL	Probiotic loquat (10% pulp) ice cream containing *Bifidobacterium lactis*
PL_1IC	Probiotic loquat (10% pulp) ice cream containing complex of *Bifidobacterium bifidum* and *Bifidobacterium lactis*
PL_2IBB	Probiotic loquat (20% pulp) ice cream containing *Bifidobacterium bifidum*
PL_2IBL	Probiotic loquat (20% pulp) ice cream containing *Bifidobacterium lactis*
PL_2IC	Probiotic loquat (20% pulp) ice cream containing complex of *Bifidobacterium bifidum* and *Bifidobacterium lactis*
PL_3IBB	Probiotic loquat (30% pulp) ice cream containing *Bifidobacterium bifidum*
PL_3IBL	Probiotic loquat (30% pulp) ice cream containing *Bifidobacterium lactis*
PL_3IC	Probiotic loquat (30% pulp) ice cream containing complex of *Bifidobacterium bifidum* and *Bifidobacterium lactis*

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Overrun and melting characteristics

Overrun and melting characteristics were assessed by comparison mixture weight and constant volume for ice creams as represented in Equation 1:

Eq 1:

$$\text{Overrun} = \frac{\text{weight of unit mix} - \text{weight of equal volume of frozen yogurt} \times 100}{\text{weight of equal volume of frozen yogurt}}$$

These factors with various substitutions were assessed. A 30-g sample was transferred on a stainless steel mesh with 1 × 1 mm openings at the top of a beaker. Ice cream was collected and its weight was measured using beaker (20 °C, 45 min) and then melting rate was assessed (12) as expressed in Equation 2:

Eq 2:

$$\text{Meltdown rate (\%)} = \frac{\text{wt of melted sample}}{\text{wt of scoop}} \times 100$$

Sensory assessments

Sensory characteristics were assessed by 15 males and females as panelists, who were trained with experiences. Several characteristics such as appearance, smoothness, mouthfeel, flavour, texture and total acceptance were assessed by panelists. A hedonic scale was represented from 1 to 5; from which, 1 was for dislike and 5 for like extremely, respectively. Sensory assessment was reapeated for ice creams on various days under similar conditions as described previously (5).

Total phenolic levels

Samples were achieved by mixing with 25 mL of methanol (50%, v/v) for a day and then filtered using Whatman filters no. 4. The compounds were assessed using Folin-Ciocalteu reagent. Briefly, 0.2 mL of the extract, 1.8 mL of distillated water (DW), 1 mL of Folin-Ciocalteu reagent and 2 mL of sodium carbonate (20%, w/v) were mixed and then stored at 25 °C for nearly 20 min and absorbance was measured at 725 nm using spectrophotometer (Jenway Model 6700, Jenway, UK). Total phenolic composition was reported as equivalent mg gallic acid/100 g^{-1} using gallic acid standard (Sigma-Aldrich, USA) and calibration curve (10).

1,1-Diphenyl-2-picrylhydrazyl radical scavenging potentials

Antioxidant capacity was assessed to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Concisely, 1 mL of the probiotic samples and 1 mL of the DPPH solution (0.2 mM in 95% methanol) were mixed and then incubated at 37 °C for 30 min in dark. Absorbance was measured at 517 nm using spectrophotometry (Thermo Scientific, USA) (13). Absorbance decreased as a result of radical scavenging assay for composition, which expressed in Equation 3:

Eq 3:

$$\text{DPPH scavenging rate (\%)} = (A_0 - A_1) / A_0 \times 100\%$$

A_0 and A_1 represented the absorption levels for blank and treated groups, respectively.

Probiotic bacteria survivals

Based on the standard plate counting method, cell viability counts were assessed using MRS broth. Treated ice creams with *B. bifidum* and *B. lactis* were inoculated on MRS broth. Bacteria were counted after incubation at 37 °C for 72 h (1).

Folate level assessments

Folate content was assessed using HPLC with a C18 column. Wavelength included 295 nm with a 0.4 ml/min current intensity. After filtering samples using 0.45-nm filters, samples were injected into HPLC device using special syringe. Folate standard concentrations of 25, 50 and 100 ppm were injected into the device followed by sample extraction. Folate content was assessed by detecting area below the diagram (4).

Statistical analyses

in this study, normality analysis was used and factorial test was carried out in a complete randomized pattern using three replications with mean and standard deviations (SD). Therefore, 10, 20, 30% of the Japanese loquat pulps, probiotic bacterial strains and zero, four and eight weeks of storage were expressed as the parameters. All the analyses were carried out in triplicate. Duncan's multiple ranges were carried out at 0.05 levels and statistical tests were carried out using Minitab Software v.15.

Results

Physicochemical components

Results of the physicochemical assessments (pH, acidity, protein, fat, ash and dry matter) are shown in Table 2 for probiotic ice creams including *B. bifidum*, *B. lactis* enriched with Japanese loquat pulp at the highlighted levels. Based on the results, pH, acidity, fat and protein of the samples were not statistically affected by adding target pulps and probiotic bacteria strains ($p > 0.05$). Dry matter and ash content of the samples were significantly linked to Japanese loquat pulps ($p < 0.05$); however, no significant effects of bacteria type on these factors were detected ($p > 0.05$). Apparent characteristics (overrun, color and melting) were investigated in probiotic ice cream samples, which included the target probiotic bacteria and Japanese loquat pulps at various levels (Table 3).

Table 2. Physicochemical characteristics of the ice cream samples including Japanese loquat pulps and probiotic bacteria of *Bifidobacterium bifidum* and *Bifidobacterium lactis* (mean ±standard error)

Samples	pH	Acidity (D°)	Protein (%)	Fat (%)	Ash (%)	Dry matter (%)
PL_1IBB	6.71 ± 0.02^a	0.16 ± 0.01^a	5.25 ± 0.07^a	7.53 ± 0.05^a	1.05 ± 0.01^a	42.54 ± 0.22^a
PL_1IBL	6.72 ± 0.01^a	0.15 ± 0.02^a	5.22 ± 0.03^a	7.55 ± 0.03^a	1.06 ± 0.02^a	42.61 ± 0.46^a
PL_1IC	6.70 ± 0.03^a	0.15 ± 0.03^a	5.20 ± 0.05^a	7.54 ± 0.05^a	1.04 ± 0.03^a	42.58 ± 0.38^a
PL_2IBB	6.71 ± 0.04^a	0.16 ± 0.01^a	5.21 ± 0.02^a	7.52 ± 0.02^a	1.12 ± 0.01^b	44.82 ± 0.38^b
PL_2IBL	6.72 ± 0.02^a	0.15 ± 0.02^a	5.23 ± 0.04^a	7.54 ± 0.07^a	1.15 ± 0.06^b	44.79 ± 0.52^b
PL_2IC	6.73 ± 0.03^a	0.17 ± 0.01^a	5.22 ± 0.05^a	7.50 ± 0.04^a	1.13 ± 0.02^b	44.85 ± 0.41^b
PL_3IBB	6.72 ± 0.02^a	0.15 ± 0.04^a	5.24 ± 0.02^a	7.52 ± 0.02^a	1.22 ± 0.04^c	48.87 ± 0.39^c
PL_3IBL	6.73 ± 0.04^a	0.16 ± 0.02^a	5.26 ± 0.03^a	7.54 ± 0.06^a	1.20 ± 0.03^c	48.90 ± 0.58^c
PL_3IC	6.70 ± 0.03^a	0.17 ± 0.03^a	5.21 ± 0.04^a	7.55 ± 0.01^a	1.23 ± 0.02^c	48.85 ± 0.45^c

*Different lowercase letters indicate a significant difference (p <0.05) in each column.

Table 3. Changes in overrun, color parameters (L^*, a^* and b^*) and melting rates of the ice cream samples including Japanese loquat pulps and probiotic bacteria of *Bifidobacterium bifidum* and *Bifidobacterium lactis* (mean ±standard error)

Samples	Overrun (%)	L^*	a^*	b^*	Melting rate (%)
PL_1IBB	43.45 ± 0.27^a	84.01 ± 0.69^a	2.45 ± 0.18^a	35.79 ± 0.56^a	65.13 ± 1.23^a
PL_1IBL	43.38 ± 0.33^a	83.55 ± 0.58^a	2.31 ± 0.20^a	35.85 ± 0.45^a	64.47 ± 1.06^a
PL_1IC	43.40 ± 0.25^a	83.71 ± 0.73^a	2.56 ± 0.33^a	36.01 ± 0.67^a	64.29 ± 1.42^a
PL_2IBB	45.57 ± 0.59^b	81.59 ± 0.68^b	5.75 ± 0.18^b	42.45 ± 0.72^b	65.34 ± 1.34^a
PL_2IBL	45.62 ± 0.50^b	82.03 ± 0.14^b	5.78 ± 0.11^b	42.61 ± 0.54^b	66.98 ± 1.17^{ab}
PL_2IC	45.64 ± 0.48^b	81.75 ± 0.30^b	5.83 ± 0.13^b	43.10 ± 0.66^b	65.21 ± 1.26^a
PL_3IBB	47.74 ± 0.14^c	78.28 ± 0.42^c	8.67 ± 0.20^c	48.97 ± 0.23^c	67.82 ± 1.09^b
PL_3IBL	47.69 ± 0.44^c	78.36 ± 0.58^c	8.75 ± 0.39^c	48.72 ± 0.15^c	68.32 ± 1.24^b
PL_3IC	47.78 ± 0.52^c	78.40 ± 0.29^c	8.81 ± 0.15^c	48.69 ± 0.30^c	68.09 ± 1.06^b

*Different lowercase letters indicate a significant difference (p <0.05) in each column.

Addition of pulps significantly increased overrun in ice creams ($p < 0.05$). The PL_3IBB, PL_3IBL and PL_3IC samples included the lowest overrun; however, the bacterial strain showed significant effects on increasing overrun ($p > 0.05$). Based on the results of variance analysis, changes in color parameters (L^*, a^* and b^*) were significantly ($p < 0.05$) correlated to pulps (Table 3). However, probiotic bacteria species in ice cream formulations included no significant effects on changes in parameters ($p > 0.05$). Therefore, whiteness index (L^*) of the probiotic ice creams significantly decreased by using Japanese loquat pulps ($p < 0.05$); hence, PL_1IBB, PL_1IBL and PL_1IC samples included least whiteness. Nevertheless, higher pulps significantly led to increases in redness (a^*) and yellowness (b^*) for ice creams ($p < 0.05$). In Table 3, melting rate was 29.64–32% and addition of pulps in formulations significantly improved melting rate of the ice cream samples ($p < 0.05$). However, no significant effects on melting rates by the bacteria type were detected ($p > 0.05$).

Sensory acceptability

Results of the sensory assessment (texture, odor, flavor, mouthfeel, color and general acceptance) are shown in Table 4. Based on the texture and flavor assessments, ice creams supplemented with 30% of pulps (PL_3IBB, PL_3IBL and PL_3IC) included better texture and flavor with significant differences ($p < 0.05$). A lower score was attributed to odor factor with significant differences ($p < 0.05$), when a complex of target bacteria was used in samples (PL_1IC, PL_2IC and PL_3IC). Of all samples, the highest mouthfeel score belonged to PL_3IBB and PL_3IBL since the lowest value belonged to PL_1IC. Results of this factor indicated that pulp addition improved mouthfeel and samples with the two bacteria included lower levels. Regarding colorimetric assay, more scores were observed by lower pulp proportions (PL_1IC, PL_2IC and PL_3IC), compared to other samples. In overall acceptance, PL_3IBB and PL_1IC included the highest and the lowest scores, respectively. Furthermore, ice cream samples containing 30% of pulps and *B. bifidum* showed the most desirable score because pulps resulted in better texture, flavor and mouthfeel; however, these samples did not receive the best score in terms of odor and color.

Biological assessment

Since the aim of the present study was to investigate preservation of biological functions, these factors were monitored within eight weeks of storage in ice cream samples. As represented in Table 5, total phenolic composition and antioxidant status of the probiotic ice creams enriched with Japanese loquat pulps and the target bacteria were assessed. Results of variance analysis demonstrated that changes in phenolic components were

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significantly corresponded to storage, pulp and probiotic bacterial strain in ice cream formulations ($p < 0.05$). Therefore, phenolic compositions were enhanced significantly by extending the storage time ($p < 0.05$) and pulp addition improved total phenolic content in the samples ($p < 0.05$). Furthermore, ice creams produced with a mixture of these bacteria included the lowest phenolic compounds, compared to other treatments. Based on the results of variance analysis, changes in antioxidant capacity (IC_{50}) of the probiotic ice creams were significantly ($p < 0.05$) linked to the storage time, Japanese loquat pulps and probiotic bacterial species in the formulations (Table 5). Results outlined that antioxidant capacity (lower IC_{50}) increased significantly by adding pulps ($p < 0.05$); however, this factor (higher IC_{50}) significantly decreased by over storage until the end of Week 8 ($p < 0.05$). Lower rates were seen in antioxidant activity (higher IC_{50}) of the probiotic ice creams including the target bacteria, compared with other samples ($p < 0.05$).

Bacterial viability assessments

Probiotic bacterial survival of *B. bifidum* and *B. lactis* in ice cream samples during eight weeks of storage are represented in Table 6. Regarding results of the variance analysis, bacteria species, pulp concentration and storage time affected bacterial viability ($p < 0.05$). Use of Japanese loquat pulps in ice cream formulations and their higher levels significantly improved viability of the target bacteria; however, number of the probiotic bacteria in various ice creams significantly decreased by extending the storage time from Day 1 to the end of Week 8 ($p < 0.05$), which decreases in *B.lactis* bacteria were more than those in *B. bifidum* and their complexes.

Table 4. Sensory assessment of the ice cream samples including Japanese loquat pulps and probiotic bacteria of *Bifidobacterium bifidum* and *Bifidobacterium lactis* (mean ±standard error)

Samples	Texture	Odor	Flavor	Mouthfeel	color	Overall acceptance
PL_1IBB	4.12 ± 0.04^{a}	5.00 ± 0.01^{b}	4.31 ± 0.04^{a}	4.04 ± 0.02^{ab}	5.00 ± 0.00^{a}	4.49 ± 0.03^{d}
PL_1IBL	4.01 ± 0.02^{a}	5.00 ± 0.02^{b}	4.42 ± 0.01^{a}	4.02 ± 0.03^{ab}	4.91 ± 0.01^{a}	4.47 ± 0.02^{d}
PL_1IC	3.92 ± 0.03^{a}	4.71 ± 0.04^{a}	4.55 ± 0.03^{a}	3.90 ± 0.04^{a}	4.93 ± 0.05^{a}	4.40 ± 0.04^{e}
PL_2IBB	4.50 ± 0.07^{b}	5.00 ± 0.03^{b}	4.81 ± 0.01^{ab}	4.65 ± 0.05^{c}	4.74 ± 0.03^{b}	4.74 ± 0.15^{b}
PL_2IBL	4.41 ± 0.01^{b}	4.95 ± 0.02^{b}	4.78 ± 0.03^{ab}	4.60 ± 0.03^{c}	4.71 ± 0.01^{b}	4.69 ± 0.06^{b}
PL_2IC	4.72 ± 0.02^{b}	4.51 ± 0.01^{a}	4.72 ± 0.05^{ab}	4.12 ± 0.01^{b}	4.73 ± 0.02^{b}	4.55 ± 0.04^{c}
PL_3IBB	4.91 ± 0.05^{c}	5.00 ± 0.00^{b}	4.94 ± 0.07^{b}	4.98 ± 0.04^{d}	4.40 ± 0.03^{c}	4.91 ± 0.05^{a}
PL_3IBL	4.83 ± 0.02^{c}	4.93 ± 0.02^{b}	5.00 ± 0.02^{b}	4.94 ± 0.05^{d}	4.35 ± 0.04^{c}	4.81 ± 0.07^{b}
PL_3IC	5.00 ± 0.04^{c}	4.32 ± 0.03^{a}	4.98 ± 0.04^{b}	4.20 ± 0.03^{bc}	4.38 ± 0.05^{c}	4.57 ± 0.04^{c}

*Different lowercase letters indicate a significant difference ($p < 0.05$) in each column.

Table 5. Changes in biological compositions of the probiotic ice cream samples including Japanese loquat pulps and probiotic bacteria of *Bifidobacterium bifidum* and *Bifidobacterium lactis* during storage (mean ±standard error)

Phenolic compounds (mg GAE / 100g)					
Samples	1st day	2nd week	4th week	6th week	8th week
PL_1IBB	36.95 ± 0.34^{eA}	35.77 ± 0.32^{fB}	33.49 ± 0.18^{fC}	31.28 ± 0.10^{fD}	28.42 ± 0.27^{fE}
PL_1IBL	36.71 ± 0.27^{eA}	35.26 ± 0.19^{fB}	33.34 ± 0.34^{fC}	31.30 ± 0.17^{fD}	28.37 ± 0.11^{fE}
PL_1IC	35.83 ± 0.41^{fA}	34.40 ± 0.27^{gB}	31.29 ± 0.14^{gC}	29.36 ± 0.22^{gD}	27.52 ± 0.23^{gE}
PL_2IBB	67.21 ± 0.15^{cA}	65.45 ± 0.37^{dB}	63.12 ± 0.17^{dC}	61.38 ± 0.20^{dD}	58.40 ± 0.14^{cE}
PL_2IBL	66.98 ± 0.45^{cA}	65.39 ± 0.33^{dB}	62.45 ± 0.11^{eC}	61.29 ± 0.22^{dD}	57.76 ± 0.28^{dE}
PL_2IC	65.73 ± 0.19^{dA}	64.43 ± 0.25^{eB}	62.10 ± 0.37^{eC}	60.23 ± 0.17^{eD}	56.19 ± 0.15^{eE}
PL_3IBB	98.63 ± 0.27^{aA}	97.54 ± 0.36^{aB}	95.44 ± 0.19^{aC}	92.63 ± 0.23^{aD}	89.12 ± 0.41^{aE}
PL_3IBL	98.42 ± 0.41^{aA}	96.69 ± 0.20^{bB}	94.51 ± 0.12^{bC}	91.78 ± 0.29^{bD}	88.76 ± 0.21^{aE}
PL_3IC	97.68 ± 0.16^{bA}	96.01 ± 0.25^{cB}	93.60 ± 0.28^{cC}	90.20 ± 0.13^{cD}	86.12 ± 0.38^{bE}

IC_{50} (mg/100g)					
Samples	1st day	2nd week	4th week	6th week	8th week
PL_1IBB	185.38 ± 0.19^{bA}	182.28 ± 0.28^{bB}	179.98 ± 0.44^{bC}	176.47 ± 0.33^{bD}	172.61 ± 0.29^{bE}
PL_1IBL	185.52 ± 0.27^{bA}	182.10 ± 0.41^{bB}	180.06 ± 0.38^{bC}	176.51 ± 0.27^{bD}	172.54 ± 0.35^{bE}
PL_1IC	183.63 ± 0.36^{cA}	180.39 ± 0.20^{cB}	177.43 ± 0.56^{cC}	174.12 ± 0.52^{cD}	169.41 ± 0.41^{cE}
PL_2IBB	139.86 ± 0.30^{dA}	138.14 ± 0.28^{dB}	137.02 ± 0.24^{dC}	132.56 ± 0.47^{dD}	128.79 ± 0.19^{dE}
PL_2IBL	140.00 ± 0.25^{dA}	138.25 ± 0.44^{dB}	136.42 ± 0.39^{dC}	132.67 ± 0.36^{dD}	128.63 ± 0.21^{dE}
PL_2IC	137.59 ± 0.12^{eA}	135.42 ± 0.32^{eB}	131.19 ± 0.17^{eC}	128.24 ± 0.19^{eD}	124.52 ± 0.68^{eE}
PL_3IBB	105.12 ± 0.41^{fA}	102.38 ± 0.38^{fB}	99.10 ± 0.28^{fC}	97.29 ± 0.42^{fD}	93.41 ± 0.34^{fE}
PL_3IBL	104.89 ± 0.27^{fA}	102.46 ± 0.27^{fB}	98.42 ± 0.33^{gC}	97.44 ± 0.38^{fD}	93.12 ± 0.29^{fE}
PL_3IC	100.47 ± 0.32^{gA}	97.14 ± 0.52^{gB}	95.23 ± 0.41^{hC}	91.58 ± 0.19^{gD}	87.37 ± 0.44^{gE}

*Different lowercase (each column) and uppercase letters (each row) indicate a significant difference ($p < 0.05$)

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Table 6. Probiotic bacteria viability (log CFU/g) in the ice cream samples including Japanese loquat pulps and probiotic bacteria of *Bifidobacterium bifidum* and *Bifidobacterium lactis* during storage (mean ±standard error)

Probiotic bacteria viability (log CFU / g)					
Samples	1st day	2nd week	4th week	6th week	8th week
PL_1IBB	8.23 ± 0.12^{aA}	7.36 ± 0.09^{eB}	6.64 ± 0.14^{eC}	5.12 ± 0.13^{eD}	3.98 ± 0.14^{fE}
PL_1IBL	8.18 ± 0.15^{aA}	7.30 ± 0.11^{efB}	6.69 ± 0.13^{eC}	5.09 ± 0.14^{eD}	3.95 ± 0.16^{fE}
PL_1IC	8.20 ± 0.16^{aA}	7.39 ± 0.10^{deB}	6.94 ± 0.16^{dC}	6.25 ± 0.12^{dD}	4.63 ± 0.15^{eE}
PL_2IBB	8.19 ± 0.10^{aA}	7.33 ± 0.12^{eB}	6.97 ± 0.14^{dC}	6.15 ± 0.10^{dD}	4.98 ± 0.09^{dE}
PL_2IBL	8.22 ± 0.09^{aA}	7.28 ± 0.14^{fB}	6.95 ± 0.12^{dC}	6.21 ± 0.06^{dD}	4.94 ± 0.11^{dE}
PL_2IC	8.20 ± 0.12^{aA}	7.51 ± 0.08^{cB}	7.17 ± 0.10^{cC}	6.89 ± 0.09^{cD}	5.46 ± 0.12^{cE}
PL_3IBB	8.21 ± 0.11^{aA}	7.72 ± 0.13^{bB}	7.38 ± 0.08^{bC}	7.01 ± 0.11^{bcD}	6.56 ± 0.14^{bcE}
PL_3IBL	8.23 ± 0.13^{aA}	7.68 ± 0.06^{bB}	7.35 ± 0.06^{bC}	7.06 ± 0.08^{bcD}	6.61 ± 0.15^{bcE}
PL_3IC	8.22 ± 0.14^{aA}	7.89 ± 0.11^{aB}	7.62 ± 0.05^{aC}	7.45 ± 0.10^{aD}	7.06 ± 0.14^{aE}
Folate content (µg / g)					
Samples	1st day	2nd week	4th week	6th week	8th week
PL_1IBB	85.44 ± 1.23^{fE}	100.22 ± 1.36^{fD}	116.91 ± 2.47^{fC}	140.82 ± 2.72^{fB}	194.36 ± 1.43^{fA}
PL_1IBL	84.67 ± 1.19^{fE}	98.85 ± 2.47^{fD}	117.42 ± 1.68^{fC}	142.13 ± 1.46^{fB}	192.80 ± 2.39^{fA}
PL_1IC	87.52 ± 1.38^{eE}	109.56 ± 2.23^{eD}	128.67 ± 1.14^{eC}	148.54 ± 1.28^{eB}	202.43 ± 1.54^{eA}
PL_2IBB	90.75 ± 1.51^{dE}	119.69 ± 1.59^{dD}	140.16 ± 1.14^{dC}	172.76 ± 2.36^{dB}	212.27 ± 1.58^{dA}
PL_2IBL	91.36 ± 1.24^{dE}	120.23 ± 1.09^{dD}	138.75 ± 2.12^{dC}	174.32 ± 1.85^{dB}	210.97 ± 2.21^{dA}
PL_2IC	94.49 ± 1.02^{cE}	125.48 ± 1.24^{cD}	157.54 ± 2.10^{cC}	196.62 ± 1.65^{cB}	259.22 ± 1.65^{cA}
PL_3IBB	97.25 ± 0.56^{bE}	144.78 ± 1.41^{bD}	189.30 ± 1.08^{bC}	225.09 ± 1.54^{bB}	314.82 ± 3.11^{bA}
PL_3IBL	96.87 ± 1.43^{bE}	145.19 ± 1.62^{bD}	187.86 ± 2.06^{bC}	223.74 ± 2.43^{bB}	316.24 ± 1.36^{bA}
PL_3IC	99.88 ± 0.64^{aE}	164.66 ± 1.38^{aD}	215.32 ± 1.05^{aC}	286.54 ± 2.41^{aB}	397.62 ± 2.08^{aA}

*Different lowercase (each column) and uppercase letters (each row) indicate a significant difference ($p < 0.05$)

Folate status responses

Results of folate changes in probiotic ice creams including pulps during eight weeks of storage are demonstrated in Table 6. Of the ice cream samples, PL_1IBL (84.67 µg/g) and PL_3IC (397.62 µg/g) included the lowest and the highest folate levels in Weeks 1 and 8, respectively. Results of the variance analysis indicated that folate was affected by the storage time, pulp level and bacterial strain in the formulations ($p < 0.05$). Simultaneous consumption of the probiotic bacteria (*B. bifidum* and *B. lactis*) significantly increased folate levels, compared to individual target bacteria. Moreover, folate was improved significantly using pulp and over storage in probiotic ice creams ($p < 0.05$).

Discussion

No effects of pulp and time on pH, acidity, protein and fat were seen. Dry matter and ash were significantly higher in PL_3IBB, PL_3IBL and PL_3IC samples due to higher quantities of pulps. Further sugars, fibers and minerals were detected in Japanese loquat pulps (7); thus, increases of pulps resulted in higher components in the probiotic ice creams. Overrun was assessed using fibers of pulps because these components were capable to form gels and decrease ice crystallization due to water retention (14). These results were similar to the results of other studies, where addition of phenolic extracts and apple peels in formulation of probiotic yogurt ice cream led to an enhancement in overrun, which attributed to ability for forming gels, retaining and binding to dietary fiber in extract (15). Color changes were due to the presence of flavonoid pigments in Japanese loquat pulps (7), which their yellow color decreased whiteness index and enhanced redness and yellowness in probiotic ice creams. These findings were similar to other findings, which represented that decreases in whiteness index and enhancement in redness and yellowness indices were observed by adding grape pulps to ice cream formulations (16). Overrun, emulsifying capacity and lipid and protein levels affected melting rates (4). Similar results indicated effects of bioactive fruits such as green-kiwi ice cream (17) and *B. odorata* (5) on melting rates of the probiotic ice creams. Addition of apple peel extracts included a significant sensory quality of probiotic yogurts (14); however, negative effects were seen by high supplementation with L. acidophilus on sensory evaluation of probiotic cheeses (18).

In general, phenolic components in ice creams are attributed to this levels produced by Maillard mechanism during milk pasteurization, which caused reactions of reducing sugar and amino groups (10, 19). All probiotic ice creams included Japanese loquat pulps and phenolic levels, which were linked to improved antioxidant statuses. Studies of this fruit revealed phenolic compounds such as hydroxybenzoic acids, hydroxycinnamic acids and their derivatives as well as flavonoids (7, 8). However, phenolic components decreased over storage time (20). Results of

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the present study were similar to those of the pervious studies for phenolic contents. Similarly, these values were higher by adding further apple peel extracts with *L. acidophilus* and *B. lac*tis in probiotic yogurt ice creams (15) and gum Arabic and ginger extract with *B. bifidum* in synbiotic dooghs (21). These researchers stated that phenolic compounds could act as prebiotics and substrates for probiotic bacteria in apple peels or ginger extracts; similar to the present study. Antioxidant status was detected using phenolic compounds because of their chemical structures. These compounds act as hydrogen and reducing agents in food systems and are capable to inhibit free radical activities and oxidation chain reactions. Therefore, there are direct correlations between the phenolic contents and antioxidant characteristics (5). These contents were enhanced by adding target pulps; hence, it was expected that antioxidant activity was higher in probiotic ice creams with further pulps. As an antioxidant and prebiotic agent, ginger extract was effective in improving probiotic bacterial growth and survival (22). However, antioxidant decreased in probiotic ice creams during storage; similar to results of other studies (5, 23).

The most number of the probiotic bacteria was seen in PL_3IC samples at the end of shelf life. Effectiveness of health promotion for probiotic bacteria was linked to the microorganism presence with a recommended range 10^6 CFU/g in diets (24). Based on the results, all probiotic samples with 30% of pulps (PL_3IBB, PL_3IBL and PL_3IC) included greater viability counts than 10^6 CFU/g. Studies illustrated that fruit and vegetable extracts included phenolic, antioxidant and valuable components such as dietary fibers, which acted as prebiotic agents for the probiotic microorganisms (19, 25). Therefore, phenolic components were enhanced by increasing pulps in the formulations, which could help promote bacterial survival. However, probiotic bacteria decreased in other samples due to freezing process and lack of pulps as substrates for the bacterial stabilization. Thus, samples containing 10% of pulps did not include desired levels of probiotic bacteria after Week 4. Formation of ice crystals led to mechanical damages in bacterial cells during freezing and decreased viability of the microorganisms during storage (26, 27). Probiotic bacterial viability increased by further inulin levels in production of synbiotic ice creams including *L. acidophilus* and *B. lactis* (14). Addition of *Siraitia grosvenorii* (a native fruit of China and Thailand) juice was effective on the survival of *L. bulgaricus*, *L. casei* and *Streptococcus thermophilus* in probiotic yogurts (28). These researchers reported that inulin levels in synobiotic ice creams and *S. grosvenorii* in juices acted as bioactive components such as phenolic and anthocyanin; similar to roles of pulps in the present study. However, results revealed that when bacteria complexes were used to produce ice creams, survival potential was significant, which could indicate synergistic interactions of the two bacteria on each other. These interactions were previously established for *B. lactis* and *B. bifidum* (29).

Naturally, folate intake affects DNA replication, repair and methylation as well as fast proliferating cells such as erythrocytes, enterocytes and leucocytes. Moreover, high folate is suggested in breast cancer, inflammatory bowel disease and regulation of rectal cell turnover (29). Results exhibited that the sufficient necessary substrate was provided to the microorganisms by pulp addition because of its fiber content and components, which caused high folate production by the probiotic bacteria. Folic acid with thiamine were significantly higher in probiotic ice creams including *L. acidophilus* and *L. casei* during 60 days of storage. They reported that nutrients and substrates were used by these bacteria in probiotic ice creams to produce folic acid and thiamine during storage, which were then enhanced these components in probiotic ice creams (30). Folate production increased by probiotic bacteria in soy milks using passion fruit by-products and fructooligosaccharides as growth stimulants. They stated that the highlighted compounds acted as prebiotic agents for the target bacteria, which resulted in further folates for the products (4); similar to the present study. When a combination of the two bacteria was used to produce probiotic ice creams, higher folate levels were detected, addressed as a critical reason for the synergistic effects of the target bacteria on each other. These effects have been reported in *B. lactis* and *B. bifidum* (29).

Conclusion

In this study, a novel approach for the use of probiotic foods was developed, including use of folate-producing bacterial species. In the present study, Japanese loquat fruits were used as prebiotic agents for *B. bifidum* and *B. lactis* in ice cream formulation. Stability of the physicochemical statuses was associated to pulps of these fruits, which helped acceptation of the assessors, indicating its capability as a novel functional product. High phenolic content and antioxidant potential were linked to further pulps in the ice creams. In conclusion, this study recommends production of probiotic ice creams from Japanese loquat pulps with acceptable quality and extended survival rates of bacteria, including *B. bifidum* and *B. lactis*.

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