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Functional and Healthy Yogurts Fortified with Probiotics and Fruit Peel Powders

Hafza Fasiha Zahid, Chaminda Senaka Ranadheera, Zhongxiang Fang and Said Ajlouni *

School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Victoria 3010, Australia

* Correspondence: said@unimelb.edu.au; Tel.: +61-03-83448620

Abstract: The application of processing waste by-products along with probiotics is an interesting choice to confer potential functional aspects to food products. This study was designed to investigate the nutritional capacity of freeze-dried mango peel powder (MPP) and banana peel powder (BPP) in the presence of a mixture of three probiotic species (1% of each of three probiotics (*Lacticaseibacillus casei* (431®), *Lacticaseibacillus rhamnosus* (LGG®) and *Bifidobacterium* subsp. *Lactis* (Bb-12®)) as sources of additional nutrients and prebiotics in fresh and rehydrated freeze-dried (RFD) yogurts for 28 days of refrigerated storage. The net count of probiotics in yogurt fortified with MPP and BPP increased by at least 1 log CFU/g after 4 weeks of refrigerated storage. Adding fruit peel powder (FPP) significantly ($p < 0.05$) increased fat, ash, and protein contents in both fresh and RFD yogurts in comparison with the control yogurt. Similarly, the total phenolic contents (TPC) and antioxidant activity (AOA) was enhanced significantly ($p < 0.05$). The TPC reached 2.27 ± 0.18 and 2.73 ± 0.11 mg GAE/g in RFD enriched with BPP and MPP compared to a TPC of 0.31 ± 0.07 mg GAE/g in the control. Additionally, yogurt samples enriched with BPP (Y-5) and MPP (Y-6) demonstrated 12% more sugar contents than non-fortified yogurts (Y-1). Higher titratable acidity and lower pH values were also recorded in the RFD yogurt. Significant differences ($p < 0.05$) in the color parameters were detected in both fresh and RFD yogurts with reduced brightness (L^*) and increased redness (a*) of the product. These findings demonstrated the suitability of MPP and BPP in yogurt formulations to optimize the advantages of such synbiotic products with higher availability of phenolic compounds.

Keywords: synbiotic yogurt; fruit peel powder; lactic acid bacteria; freeze-dried reconstituted yogurts

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1. Introduction

Dairy formulations supplemented with probiotics and prebiotics are gaining increased acceptance around the globe due to their nutritional and therapeutic functionalities [1]. Yogurt is a vigorous source of macro and micronutrients, which contribute towards daily intake of energy, reduced incidence of lactose intolerance, improved gut health and amelioration of lipid and protein digestibility, along with antimicrobial attributions [2]. These could be attributed to the metabolic functions of starter cultures, which can further be improved by co-culturing with probiotics. Probiotics are defined as "live microorganisms that confer positive health impacts on the host's health upon consumption in adequate amounts" [3]. Among various probiotic strains, Lactobacilli and Bifidobacteria are most prevalent, and found in over 90% of probiotic products, which are widespread among health-conscious populations [4]. To confer positive health effects, however, it is necessary that these food matrices sustain the viability of probiotic bacteria to the minimum therapeutic level of 6–7 log CFU/mL, through their passage in the gastrointestinal tract [5]. It should be indicated here that the IPA recommendations regarding the minimum required level of viable cell counts for conferring a probiotic effect is 7 log CFU/g or mL [6].

Various previous studies investigated the potential benefits of co-administration of probiotics in yogurts. Prestes et al. [7] and Alessendri et al. [8] examined *Bifidobacterium* in

relation to its gastrointestinal and immune-modulatory health potential. The authors reported that the genus is associated with anticarcinogenic activity and enhanced lactose tolerance, along with other health benefits. Similarly, strains of the genus *Lactobacillus* incorporated in dairy products were reported to cause a reduction in the levels of serum cholesterol [9]. *Lactobacillus rhamnosus* (LGG®) are great producers of lactic acid, and thus the optimal growth of this species is not affected by the lower pH levels in the gastric tract [10].

Supplementing yogurt with some fruit processing by-products is anticipated to amend some attributes such as bacterial growth kinetics, acidification patterns, sensorial properties, and other physicochemical characteristics. Banana and mango peels have been shown to possess remarkable capability in relation to their antioxidant and prebiotic activities in various fermented dairy products [11–13]. Prebiotics substrates are selectively utilized by the microflora of host, conferring a positive health benefit [14]. Prebiotics such as fructooligosaccharide, galactooligsaccharides [15] and inulin, among others, have been employed in yogurts and fermented milk formulations. Various studies have reported the enrichment of yogurt with FPP, such as green banana flour- and banana peel flour-enhanced growth of probiotics, along with alterations in the textural, rheological and antioxidant profile of yogurts [12,16]. The phytochemical profile of mango peel exhibited this by-product as a promising source of antioxidant substances [17]. The addition of these compounds in diet is a practical tool to prevent the hostile effects prompted by reactive oxygen species (ROS) in the human body [18]. Therefore, total phenol assay and other scavenging activities serve as preliminary assessment tools to measure antioxidant effects of phenolic compounds. Othman et al. [19] demonstrated a significant improvement of nutritional and sensorial attributes of yogurts via the addition of different fruits, such as mango, strawberry, and banana.

Enriching yogurt with both probiotics and prebiotics produces a synbiotic formulation, yet limitations exist with respect to its short shelf life. Various drying techniques have been attempted to make dried yogurt products. Freeze-drying (FD) is one of those drying methods, which can be applied to remove moisture from yogurt while maintaining sufficient levels of beneficial lactic acid bacteria that are alive [20]. Thus, FD could be employed as an efficient method to preserve the quality and functionality of such synbiotic products. A previous study by Carvalho et al. [20] evaluated quality characteristics of freeze-dried yogurts for extended storage period. Jouki et al. [21] investigated the cryoprotective effect of alginate-skim milk microcapsules on the survival of *Lactobacillus plantarum* in freeze-dried yogurt powders. However, no previous study has been performed on the production of freeze-dried yogurt with a combination of three probiotic strains and fruit waste by-products. This current investigation evaluated the effect of MPP and BPP on physicochemical, antioxidant and microbiological characteristics of fresh and freeze-dried yogurts supplemented with *Lacticaseibacillus casei* (LC 431®), *Lacticaseibacillus rhamnosus* (LGG®), *Bifidobacterium* subsp. *Lactis* (Bb-12®) for 28 days of refrigerated storage.

2. Materials and Methods

2.1. Materials

All media used in the current study were purchased from Thermo Fisher Scientific Australia (Scoresby, Australia). Other chemicals, including sodium carbonate (anhydrous), Folin-Ciocalteu reagent, gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), quercetin, bile salts, porcine pepsin, potassium persulfate, α-amylase *aspergillus oryzae,* 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), D-(+)- glucose, D-(-)-fructose, D-galactose, lactose, 1-kestose and raffinose were purchased from Sigma-Aldrich (Castle Hill, Australia). Ethanol was procured from Chem-Supply Pty Ltd (Adelaide, Australia). Pancreatin was bought from Alfa Aesar (Ward Hill, MA, USA). Consumables were purchased from the University of Melbourne specialist Bio21 stores (Parkville campus). Pure probiotic strains of *Lacticaseibacillus casei* 431®, *Bifidobacterium animalis* subsp. *lactis* (BB-12), *Lacticaseibacillus rhamnosus* LGG® and the freeze-dried yogurt starter culture composed of *Streptococcus thermophilus* (St) and *Lactobacillus delbrueckii* subsp. *bulgaricus* were kindly pro-

vided by Chr. Hansen (Bayswater, Australia). Skim milk powder and the fruits of Kensington pride mango (*Mangifera indica*) and Cavendish banana (*Musa acuminta*) were bought from a Coles supermarket (Melbourne, Australia). These fruit peels were chosen because a previous study in our laboratory [21] revealed that these fruit peels had the highest prebiotic capability.

2.2. Methods

2.2.1. Preparation of Fruit Peel Powder

The fruits were thoroughly washed and peeled using a sharp kitchen knife. Thereafter, the peels were cut into small pieces and subjected to overnight freezing (–20 °C) before freeze drying (Dynavac engineering FD3 freeze-drier, Belmont, Australia). The freeze-dried peels (FDP) were powdered using a laboratory grinder (Multigrinder || Coffee grinder EMO405, Sunbeam, Melbourne, Australia) and sifted through 250 µm mesh size to obtain a fine and homogeneous powder [22].

2.2.2. Activation of Starter Cultures and Probiotics

The yogurt starter culture and three probiotic bacteria (*L. casei*, *B. lactis* and *L. rhamnosus*) were grown individually in 14% reconstituted, pasteurized, and cooled to temperature 37 °C milk with two successive transfers [23]. The activated cultures of probiotics were serially diluted using sterilized peptone water and spread plated onto MRS agar and MRS agar supplemented with 0.05% L-cysteine hydrochloride (*w/v*), followed by incubation under anaerobic environment at 38 °C. The activated cultures showed counts of at least 10^7 CFU/mL.

2.2.3. Preparation of Yogurt

Stirred type yogurts with six different formulations were prepared in triplicates [23,24], with minor modifications. Within each replicate, skim milk powder was reconstituted to 14% *w/v* (140g/L) using Milli-Q water (Millipore Milli-Q Ultrapure Water Purification System, Waltham, MA, USA), homogenized using an IKA Ultra-Turrax® T25 homogenizer (Rawang, Malaysia), and distributed into 6 milk bases using sterilized schott bottles (1 L). Freeze-dried MPP and BPP were added into 4 of the milk bases (Y-2, Y-3, Y-5 and Y-6) at a concentration of 2% each (2 milk bases for each FDP) before fermentation. The remaining 2 milk bases (Y-1 and Y-4) were left as controls with no added fruit peels. All 6 milk bases were pasteurized at 85 °C for 30 min and cooled to 42 °C in a water bath. All pasteurized milk bases were aseptically inoculated with 2% of the mixed starter culture (*Streptococcus thermophilus* (St) and *Lacticaseibacillus delbrueckii* subsp. *bulgaricus*). Finally, milk bases Y-4, Y-5 and Y-6 were inoculated also with 1% of each active probiotic culture (*Lacticaseibacillus casei*, *Bifidobacterium animalis* subsp. *lactis* (BB-12) and *Lacticaseibacillus rhamnosus* LGG), as summarized in Table 1. The inoculated milk samples were immediately mixed and incubated at 42 °C until a pH of 4.5 ± 0.5 was reached, followed by refrigerated storage (4–5 °C) for 28 days. The same yogurt preparation procedures were repeated, where produced yogurt samples were immediately freeze dried before storage at refrigeration temperature (4–5 °C) for 28 days.

Table 1. Formulation of yogurt samples.

Sample Codes	Treatments *
Y-1	SC only
Y-2	SC + 2% BPP
Y-3	SC + 2% MPP
Y-4	SC + Bb-12+ LC, LGG (No FPP)
Y-5	SC + Bb-12+ LC, LGG + 2% BPP
Y-6	SC + Bb-12+ LC, LGG + 2% MPP

SC = starter culture, Bb-12 = *Bifidobacterium lactis*, *LC* = *Lactobacillus casei*, *LGG* = *Lactobacillus rhamnosus*, BPP = banana peel powder, and MPP = mango peel powder. * Starter culture was added at 2% and probiotic at 1%.

2.2.4. Freeze-Drying of Yogurt

The fresh yogurt samples were placed in small trays at 0.2 cm thickness, frozen overnight at −20 °C and then transferred to a freeze-drier (FD3 Freeze Drier-Dyanavac Engineering, Australia). Freeze drying was performed at −48 °C under vacuum for 16 h, and the resultant freeze-dried powder was stored in sealed plastic bags at 4 °C [21].

2.2.5. Analysis of Water Holding Capacity

The freeze-dried yogurt (FDY) samples were reconstituted after 28 days of storage before the start of the physical and chemical analyses. The rehydration was performed by mixing 25 g of yogurt powder with 75 mL milli-Q water at 37 °C and stirred up to achieve structural uniformity of the RFD. The water holding capacity (WHC) of fresh and reconstituted freeze-dried yogurts were determined using the modified protocol of Zahid et al. [22]. Briefly, 10g of each yogurt sample (Y) was weighed and centrifuged at 5000× g for 15 min. The separated whey (W) was weighed, and WHC was calculated using below Equation (1):

$$\text{WHC } (\%) = \frac{Y - W}{Y} \times 100 \quad (1)$$

2.2.6. Calculation of Percentage Yield of Freeze Drying

The percentage yield of freeze-dried yogurt powders was calculated based on the ratio of freeze-dried powders to the fresh yogurt before drying [21].

2.2.7. Proximate Analyses of Fresh and Rehydrated Freeze-Dried Yogurt

Proximate analyses of fresh and rehydrated freeze-dried yogurt were conducted after 28 days of refrigerated storage. The total protein contents were determined using the Micro-Kjeldahl method, with the total nitrogen content converted into protein by multiplying with 6.25 as a conversion factor. Total fat, and total ash and moisture contents were analyzed according to AOAC (2005), using methods 996.06, 934.01 and 925.45, respectively [25].

Titratable acidity (TA) was determined according to the AOAC official method 947.05, and reported as % lactic acid content using Equation (2):

$$\% \text{ TA} = V \times N \times \frac{90.08}{W \times 10} \quad (2)$$

where V = volume of NaOH used, N = normality of NaOH and W = weight of sample.

The changes in pH of all the samples were determined using a calibrated pH meter (HI5222, Hanna Instruments Pty Ltd, Melbourne, Australia) at ambient temperature.

2.2.8. Analysis of Soluble Carbohydrates in Fresh and RFD Yogurts Using HPLC-RID

Soluble carbohydrates from yogurt samples were extracted following the method of da Costa et al. [26]. Sugar extraction involved the homogenization of 1 g of each sample with 45 mmol/L H_2SO_4 using IKA Ultra-Turrax® T25 homogenizer for 1 min at room temperature. Then, the solution was stirred for 40 min at 270 rpm using an orbital shaker (Ratek-0M8, Australian Scientific PTY Ltd, Kotara, Australia). The obtained homogenates were subjected to centrifugation at 5500× g, for 15 min at 4 °C. The supernatants were passed through a 0.45µm pore size filter units and injected to HPLC coupled with a refractive index detector (RID) of Agilent 1260 infinity II (Agilent Technologies, CA, USA). The chromatographic separation was achieved at 30 °C with a Euroshere 100-5 NH_2 column (250 × 4.6 mm, 5 µm, Knauer, Berlin, Germany). The elution procedure was isocratic with a mobile phase composition of acetonitrile: water (65:35 v/v), flow rate of 0.9 mL/min and injection volume of 10 µL. The compounds were identified and quantified based on their chromatographic comparisons with authentic standards. The robustness of the method was evaluated by estimating the effect of two parameters: mobile phase composition (acetonitrile: water 80:20, 70/30 and 65:35), flow rate (0.6, 0.7, 0.9 mL/min) and extraction solvent (45 mmol/L H_2SO_4 and milli Q water in boiling water bath). Stock solutions of

standards were prepared as 1 mg/mL in milli-Q water and the working solutions were prepared in 65:35 (*v*/*v*) acetonitrile/water.

2.2.9. Total Phenolic Contents and Antioxidant Properties of Fresh and Rehydrated Freeze-Dried Yogurt

The yogurt samples (fresh and rehydrated) were extracted by homogenizing 1 ± 0.1 g sample in 5 mL of 70% ethanol using an IKA Ultra-Turax (Rawang, Malaysia), followed by overnight shaking at 120 rpm and 4 °C in an incubator shaker (ZWYR-240, Labwit, Ashwood, Australia), and centrifuged using a benchtop centrifuge at 5500× *g*, 4 °C and 20 min (Fixed angle rotor FX6100, Allegra® X-12R Centrifuge, Beckman Coulter, Inc., Brea, CA, USA) and the supernatant was kept at −20 °C until used [17].

Total Phenolic Content (TPC)

The sample extracts and gallic acid standard solutions were analyzed by transferring 25 µL of each into 96 well microplate, mixing with 25 µL Folin-Ciocalteu phenol reagent already diluted in water at 1:3 ratio (*v*/*v*) and incubating for 15 min at room temperature. These incubated mixtures were then mixed with 200 µL water and 25 µL of 10% (*w*/*v*) Na_2CO_3 and incubated for 60 min in the dark to allow color development. Gallic acid solutions were used as standards and total phenolic content was expressed as mg gallic acid equivalents per gram sample (mg GAE/g dw). A standard curve of gallic acid (3.125 µg/mL−200 µg/mL) was plotted (r^2 = 0.999) and the absorbance was measured at 765nm using a Multiskan® Go microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) [27].

Antioxidant Activity

The antioxidant activities in all ethanolic extracts were measured using the DPPH and ABTS methods, as described by Cáceres-Vélez et al. [28] and Ali et al. [29], with a few modifications. The sample extract (10 µL) was mixed with 290 µL of 0.1 mM DPPH solution in methanol. The mixture was incubated at room temperature for 30 min before measuring the absorbance at 517 nm using a 96 well microplate reader. In another assay, the ABTS (7 mM) was dissolved in potassium persulfate solution (140 mM) and incubated (25 °C) in dark for 12–16 h to allow the generation of ABTS radical cation ($ABTS^+$). The prepared $ABTS^+$ was diluted with ethanol to attain an absorbance value of 1.0 ± 0.02 at 734 nm. An aliquot (10 µL) of each yogurt sample extract and 290 µL of the prepared ABTS solution were mixed in 96 well microplates, and the absorbance was read at 734 nm after 6 min at 25 °C, using a microplate reader. The standard curve was plotted using Trolox (0–0.75 mM/mL) and quantified as mM Trolox equivalent (TE) /g sample [24].

2.2.10. Color Measurement

The method of Hernández-Carranza et al. [30] was employed to measure the color parameters of fresh and reconstituted freeze-dried yogurts using a tristimulus portable CR-400 color reader (Minolta chromameter CR-400, Osaka, Japan). The color meter is equipped with a pulsed xenon lamp with an 8mm-diameter measuring area and illuminant *C. The spectral color parameters *L** (lightness), a* (green to red) and b* (blue to yellow) of CIELAB color were employed to calculate the chroma ($C = (a^{*2} + b^{*2})^{½}$) and hue angle ($H^o$).

2.2.11. Microbiological Analysis of Yogurts

Microbial counts in both fresh and freeze-dried yogurts were carried out at 1, 14 and 28 days of storage using selective agar media by serial dilution and spread plating techniques and expressed as log cfu/g as described by Sah et al. [4]. The media used included M17 agar supplemented with amyl media and aerobic incubation at 42 °C for *S. thermophilus* acidified MRS agar (pH = 5.4) for the enumeration of *L. bulgaricus*, and MRS agar supplemented with 0.05% L-cysteine (*w*/*v*) and MRS agar (pH = 6.2 ± 0.2) for *B. lactis* and lactobacilli under anaerobic environment at 38 °C [4,12,31].

2.2.12. Statistical Analysis

The collected data for the physicochemical and antioxidant analysis were replicated on three independent occasions resulting in 6 observations. The microbiological data represented triplicate observations in each independent experiment (n = 3). The obtained results were analyzed by General Linear Model (ANOVA) using Minitab® 19.0 windows version (Minitab, LLC, State College, PA, USA). The means of data were separated using Tukey's test and the level of significance was defined at $p < 0.05$. Results were reported as means ± SD.

3. Results and Discussion

3.1. Physicochemical Properties of Fresh and Freeze-Dried Yogurts Fortified with Fruit Peel Powder

All values in Table 2, except for moisture content, represent the measurements in fresh and RFD yogurts. The values obtained for chemical characteristics revealed non-significant differences between fresh and RFD yogurt samples except moisture content where the differences were significant ($p < 0.05$). The moisture content in fresh yogurts ranged from 69.33 ± 5.51% to 76.33 ± 4.51% in Y-1 and Y-5 treatments, respectively, and declined to 7.74 ± 0.63 and 8.17 ± 0.33% in same treatments of RFD yogurt. These values are compatible to those reported by Tontul et al. [32] for freeze-dried yogurt powders. Both fresh and RFD yogurts fortified with banana peel powder (Y-2 & Y-5) had noticeably higher values of moisture, total ash, total fat, and total protein in relation to other yogurt samples (Table 2). These variations could be attributed to the higher percent of ash, fat, and protein in BPP, as reported in our previous study [22]. The small fat contents (0.01 ± 0.01 to 0.05 ± 0.13%) in the controls (Y-1) of both yogurt samples were clearly related to the fact that skim milk powder was used in milk preparation (Section 2.2.3). However, adding BPP (Y-2 and Y-5) and MPP (Y-3 and Y-6) in both fresh and RFD yogurts caused significant increases in the fat contents (Table 2).

Table 2. Physicochemical properties, production yield and acidification properties of fresh and RFD yogurts.

Yogurt Types	Parameters							
	Fresh yogurts							
	Moisture (%)	**Fat (%)**	**Ash (%)**	**Protein (%)**	**WHC (%)**	**Production Yield (%)**	**pH**	**T. A**
Y-1	69.33 ± 5.51 ^{b}	0.01 ± 0.01 ^{e}	2.70 ± 0.16 ^{h}	1.52 ± 0.16 ^{e}	63.23 ± 5.38 ^{a}	——	4.51 ± 0.05 ^{a}	0.75 ± 0.05 ^{bc}
Y-2	76.01 ± 3.61 ^{a}	2.64 ± 0.29 ^{bc}	6.91 ± 0.52 ^{bc}	2.19 ± 0.14 ^{d}	55.30 ± 5.02 ^{cd}	——	4.50 ± 0.07 ^{a}	0.80 ± 0.05 ^{ab}
Y-3	69.72 ± 3.61 ^{b}	2.07 ± 0.07 ^{d}	4.48 ± 0.33 ^{g}	2.15 ± 0.13 ^{d}	57.58 ± 3.74 ^{bc}	——	4.49 ± 0.10 ^{a}	0.79 ± 0.04 ^{ab}
Y-4	68.02 ± 3.61 ^{b}	0.02 ± 0.01 ^{e}	2.76 ± 0.27 ^{h}	1.23 ± 0.14 ^{e}	62.79 ± 4.33 ^{ab}	——	4.50 ± 0.08 ^{a}	0.76 ± 0.05 ^{bc}
Y-5	76.33 ± 4.51 ^{a}	2.97 ± 0.08 ^{b}	6.39 ± 0.27 ^{cd}	3.57 ± 0.19 ^{b}	57.45 ± 4.03 ^{bc}	——	4.49 ± 0.13 ^{a}	0.89 ± 0.04 ^{a}
Y-6	72.33 ± 4.04 ^{b}	2.09 ± 0.09 ^{d}	4.95 ± 0.29 ^{fg}	3.16 ± 0.06 ^{c}	57.93 ± 2.48 ^{bc}	——	4.46 ± 0.16 ^{a}	0.90 ± 0.03 ^{a}
	RFD yogurts							
Y-1	* 7.74 ± 0.63 ^{c}	0.05 ± 0.13 ^{e}	3.22 ± 0.36 ^{h}	2.10 ± 0.22 ^{d}	52.85 ± 1.67 ^{cd}	16.43 ± 1.66 ^{c}	4.13 ± 0.08 ^{a}	0.87 ± 0.08 ^{bc}
Y-2	* 8.67 ± 1.01 ^{c}	3.77 ± 0.31 ^{a}	8.54 ± 0.44 ^{a}	3.61 ± 0.24 ^{b}	45.33 ± 3.00 ^{e}	18.11 ± 2.26 ^{a}	4.09 ± 0.11 ^{a}	0.91 ± 0.13 ^{b}
Y-3	* 8.02 ± 0.48 ^{c}	2.81 ± 0.11 ^{bc}	6.11 ± 0.31 ^{de}	3.31 ± 0.12 ^{bc}	49.10 ± 2.52 ^{de}	17.75 ± 3.08 ^{b}	4.07 ± 0.11 ^{a}	0.91 ± 0.24 ^{b}
Y-4	* 7.93 ± 0.37 ^{c}	0.06 ± 0.13 ^{e}	3.30 ± 0.24 ^{h}	1.93 ± 0.29 ^{d}	52.61 ± 3.63 ^{cd}	16.89 ± 0.92 ^{c}	4.11 ± 0.15 ^{a}	0.88 ± 0.31 ^{bc}
Y-5	* 8.17 ± 0.33 ^{c}	3.48 ± 0.23 ^{a}	7.56 ± 0.29 ^{b}	4.18 ± 0.16 ^{a}	47.74 ± 4.68 ^{de}	18.81 ± 1.77 ^{a}	4.01 ± 0.14 ^{a}	0.99 ± 0.23 ^{ab}
Y-6	* 7.90 ± 0.52 ^{c}	2.52 ± 0.28 ^{c}	5.50 ± 0.35 ^{ef}	4.11 ± 0.15 ^{a}	47.58 ± 2.15 ^{e}	17.41 ± 2.38 ^{b}	3.98 ± 0.12 ^{a}	1.01±0.19 ^{a}

* Moisture was determined before rehydration of FD yogurts. Values presented the means ± SD (n = 6). Means in a column within each yogurt treatment (fresh and RFD) followed by different superscript letters are significantly different ($p < 0.05$).

The mean values of ash and protein in all RFD yogurt treatments were significantly ($p < 0.05$) higher than those in fresh yogurts. The highest percentages of ash and protein were detected in Y-5 of RFD yogurts (7.56 ± 0.29 and 4.18 ± 0.16%, respectively). Similar observations of increased protein and ash contents in yogurt powders were also reported by Tontul et al. [32] and Ismail et al. [33], when yogurt powders were produced using refractance window drying (infrared heating) and freeze-drying techniques. These data

demonstrated also that adding FPP (banana and mango) contributed to the significant increases in ash and protein contents in yogurt samples.

In contrast to the greater amounts of ash and protein detected in RFD, smaller WHC values were determined in RFD than in fresh yogurt formulations. The reduction in WHC values of the former ranged from 45.33 ± 3.00 to 52.85 ± 1.67%, as compared with 55.30 ± 5.02–63.33 ± 5.38% in fresh yogurt (Table 2). These results indicate that drying and then rehydration of the powder will affect the physical structure and interaction between various yogurt components, leading to lower WHC. Similar reduced WHC values of reconstituted yogurt powder was reported by Jouki et al. [21].

The production yield (PY) of freeze-dried yogurt powders was calculated based on the ratio of freeze-dried powders to the fresh yogurt before drying. The results demonstrated that production yield of FD yogurts increased significantly ($p < 0.05$) upon the addition of FPP (Table 2). For example, a 2.47% increase in PY of yogurt with added banana peel powder (Y-5) was observed with respect to control yogurt (Y-1). The PY was increased from 16.34 ± 1.66% in the Y-1 to 18.81 ± 1.77% in Y-5. These findings corroborate with the results of Jouki et al. [21], where they demonstrated a surge in PY of yogurt powers after the enrichment of yogurts with sorbitol microcapsules. Santos et al. [34] also observed an increase in yogurt production efficiency to 18% after the addition of 4% skim milk. Hence, the total soluble solids are determinants of enhanced PY. Increasing the amounts of total solids in foods before freeze-drying will yield higher production efficiency.

The pH values of fresh and RFD yogurts ranged from 4.46 ± 0.16 to 4.51 ± 0.05 and 3.98 ± 0.12 to 4.13 ± 0.08, respectively (Table 2). All RFD yogurts presented significantly ($p < 0.05$) lower pH values than fresh samples due to the rehydration process. The incorporation of fruit peel powder to the yogurt caused insignificant ($p > 0.05$) variations in pH values. These findings agree with those of Casarotti et al. [35], who reported a noticeable decline of pH in yogurt fortified with guava, orange, and passion fruit by-product, which is due to continual production of lactic acid by the bacteria.

The decline in pH values in RFD yogurt samples was combined with an increase in titratable acidity (TA). Significantly ($p < 0.05$) higher values of TA were recorded in the synbiotic yogurts when compared with the control. Similar results for TA were confirmed by Santo et al. [22] in the yogurts fortified with passion fruit peel.

3.2. Impact of Added Fruit Peel Powder on Yogurt Color

Color of yogurt is one of the major quality characteristics that influence consumer acceptability and attractiveness [21]. The values obtained for the color coordinates of the fresh and RFD yogurt preparations are presented in Table 3. The incorporation of FPP instigated a significant ($p < 0.05$) decrease in the luminosity (L^*) of the BPP and MPP fortified fresh and RFD yogurts. This decline was more prominent in yogurts fortified with BPP (Y-2 and Y-5). The L^* declined significantly ($p < 0.05$) from 88.42 ± 5.2 in Y-1 to 59.07 ± 0.07 in RFD enriched with BPP. Such a significant decline in lightness (L^*) could be attributed to the darker brown color of the banana peel. Similarly, the parameter −a* in yogurt formulations enriched with BPP increased significantly ($p < 0.05$) from −0.24 ± 0.76 (Y-1) to 4.92 ± 1.07 (fresh, Y-2), which reflects greater redness. These results agreed with the lower L^* in Y-2 yogurt (Table 3) since more redness (a*) will lead to lower lightness (L^*). Similar pattern of changes was also noted in RFD yogurt with the largest a* (4.97 ± 1.0) and smallest L^* (56.23 ± 4.08) values reported in Y-5 samples (Table 3). Meanwhile, a significant ($p < 0.05$) loss in red hue color was detected as emphasized by the negative values of Y-1 (−2.10 ± 0.28 and −3.86 ± 0.33) and Y-4 (−1.81 ± 0.16 and −3.59 ± 0.26). These observations in the readings of a* parameter correspond to those reported by Ścibisz et al. [36] and Szoltysik et al. [37] for yogurts fortified with *Rosa spinosissima* fruit extracts and strawberry fruits, respectively.

Table 3. Color characteristics (*L**—lightness, a*—red/green, b*—blue/yellow) of fresh and RFD yogurts.

Types of yogurts	Parameters		
	Fresh yogurts		
	*L**	a*	b*
Y-1	81.32 ± 4.32 ^{ab}	−0.24 ± 0.76 ^{c}	23.55 ± 2.86 ^{ab}
Y-2	54.11 ± 3.55 ^{f}	4.92 ± 1.07 ^{a}	19.46 ± 2.44 ^{c}
Y-3	71.38 ± 2.01 ^{cd}	1.26 ± 0.29 ^{b}	24.91 ± 1.11 ^{ab}
Y-4	79.46 ± 2.65 ^{abc}	−1.08 ± 0.37 ^{b}	24.52 ± 4.24 ^{abc}
Y-5	56.23 ± 4.08 ^{f}	5.78 ± 0.22 ^{d}	19.49 ± 2.48 ^{c}
Y-6	66.85 ± 4.47 ^{de}	2.08 ± 0.74 ^{b}	24.62 ± 0.74 ^{ab}
	RFD yogurts		
Y-1	88.42 ± 5.20 ^{a}	−0.17 ± 1.09 ^{c}	22.83 ± 1.24 ^{abc}
Y-2	59.07 ± 5.29 ^{ef}	4.33 ± 1.97 ^{a}	21.39 ± 2.31 ^{bc}
Y-3	75.65 ± 2.01 ^{bcd}	1.26 ± 0.29 ^{b}	27.14 ± 1.11 ^{a}
Y-4	82.36 ± 4.54 ^{ab}	−1.10 ± 0.37 ^{b}	26.58 ± 1.47 ^{a}
Y-5	59.22 ± 4.08 ^{ef}	4.97 ± 1.00 ^{a}	20.81 ± 1.01 ^{bc}
Y-6	71.01 ± 4.63 ^{cd}	−1.41 ± 0.34 ^{b}	27.62 ± 0.76 ^{a}

Values presented the means ± SD (n = 6). Means in a column within each yogurt treatment (fresh and RFD) followed by different superscript letters for each parameter were significantly different ($p < 0.05$).

The b* color coordinate (+b* = yellower and −b* = bluer) did not demonstrate any tendency towards the yogurt samples with BPP additives. Yet, MPP-fortified samples (Y-3 and Y-6) recorded higher ($p < 0.05$) b* values than all the other samples. However, all reported b* values were positive, which reflected the yellowness of yogurt samples. The largest b* value (27.62 ± 0.76) was recorded in yogurt enriched with mango peel powder (MPP) (Table 3, Y6). These differences in color readings among fortified and non-fortified yogurts could be ascribed to the varying concentrations of phenolic compounds that are known to interact with anthocyanins and contribute towards the fluctuations occurring in color intensities [36]. Furthermore, like the results reported by Jouki et al. [21], no significant changes ($p > 0.05$) in color parameters were observed between the fresh and RFD yogurts.

The reported changes in the physical (color and water holding capacity) and chemical (ash, fat, and protein content) characteristics in yogurt enriched with FPP and probiotics are also expected in the commonly produced yogurt enriched with whole fruits. Consequently, it could be hypothesized that the reported changes in physical and chemical characteristics will not affect the consumers' acceptability of yogurt enriched with FPP. However, the increment in the fat content in yogurt enriched with FPP to about 2% could be objected by consumers who prefer fat-free yogurt. It is recommended that sensory tasting of yogurt enriched with FPP is necessary before the production of such yogurt at a commercial scale.

3.3. Sugar Composition of Fresh and FDR Yogurts

Chromatographic analysis of major carbohydrates showed insignificant differences ($p \geq 0.05$) in the content of individual and total sugar contents between fresh and RFD yogurts (Table 4). The main detected sugars in both types of yogurts (fresh and freeze dried) included lactose, galactose, and glucose. Similar sugars in yogurts were reported in previous studies [38]. However, small quantities of fructose were recorded in both types of yogurts enriched with FPP. The fructose contents ranged from 0.58 ± 0.03 in Y-3 to 0.69 ± 0.04 g/100g in Y-2 of fresh type yogurt. Similar quantities of fructose ranging from 0.59 ± 0.11 (Y-3) to 0.62 ± 0.11 g/100g (Y-2) were also detected in RFD yogurt. However, no fructose was detected in the control yogurt sample (Y-1). Other newly detected sugars in both types of yogurts enriched with FPP were small amounts of 1-kestose (0.15 ± 0.10 to 0.17 ± 0.04 g/100g in fresh yogurt) and raffinose (0.25 ± 0.20 to 0.31 ± 0.12 g/100g in RFD yogurt).

Table 4. Sugar contents (g/100 g) in fresh and RFD yogurts.

Yogurt Types	Detected Sugars	Yogurt Samples					
		Y-1	Y-2	Y-3	Y-4	Y-5	Y-6
Fresh yogurts	Lactose	1.69 ± 0.15 ^{ab}	1.52 ± 0.05 ^{b}	1.68 ± 0.21 ^{ab}	1.61 ± 0.23 ^{b}	1.89 ± 0.16 ^{a}	1.54 ± 0.04 ^{b}
	Galactose	1.02 ± 0.07 ^{a}	1.01 ± 0.09 ^{a}	1.02 ± 0.07 ^{a}	1.01 ± 0.21 ^{a}	0.88 ± 0.06 ^{a}	1.01 ± 0.07 ^{a}
	Glucose	1.42 ± 0.15 ^{a}	0.95 ± 0.20 ^{c}	1.21 ± 0.11 ^{b}	1.47 ± 0.21 ^{a}	1.01 ± 0.06 ^{bc}	1.31 ± 0.04 ^{ab}
	Fructose	N. d	0.69 ± 0.04 ^{a}	0.58 ± 0.03 ^{a}	N. d	0.65 ± 0.07 ^{a}	0.62 ± 0.05 ^{a}
	1-kestose	N. d	0.17 ± 0.04 ^{a}	N. d	N. d	0.15 ± 0.10 ^{a}	N. d
	Raffinose	N. d	N. d	0.15 ± 0.24 ^{a}	N. d	N. d	0.21 ± 0.07 ^{a}
	Total sugars	4.13 ± 0.37 ^{c}	4.34 ± 0.41 ^{b}	4.64 ± 0.67 ^{a}	4.09 ± 0.65 ^{c}	4.58 ± 0.47 ^{a}	4.69 ± 0.27 ^{a}
RFD yogurt	Lactose	1.76 ± 0.13 ^{a}	1.79 ± 0.09 ^{a}	1.82 ± 0.71 ^{a}	1.67 ± 0.18 ^{a}	1.61 ± 0.16 ^{ab}	1.40 ± 0.08 ^{b}
	Galactose	1.08 ± 0.13 ^{a}	0.99 ± 0.07 ^{b}	1.03 ± 0.14 ^{a}	1.07 ± 0.21 ^{a}	1.09 ± 0.06 ^{a}	1.15 ± 0.07 ^{a}
	Glucose	1.53 ± 0.19 ^{a}	1.08 ± 0.18 ^{b}	1.15 ± 0.19 ^{b}	1.55 ± 0.23 ^{a}	1.21 ± 0.11 ^{b}	1.39 ± 0.09 ^{ab}
	Fructose	N. d	0.62 ± 0.11 ^{a}	0.59 ± 0.11 ^{b}	N. d	0.61 ± 0.13 ^{a}	0.65 ± 0.11 ^{a}
	1-kestose	N. d	0.20 ± 0.13 ^{a}	N. d	N. d	0.17 ± 0.15 ^{a}	N. d
	Raffinose	N. d	N. d	0.25 ± 0.20 ^{a}	N. d	N. d	0.31 ± 0.12 ^{a}
	Total sugars	4.37 ± 0.45 ^{c}	4.68 ± 0.58 ^{ab}	4.84 ± 1.35 ^{a}	4.29 ± 0.62 ^{b}	4.69 ± 0.63 ^{ab}	4.90 ± 0.45 ^{a}

Values are represented by means ± SD (n = 6). Means in a row within each yogurt type (fresh and RFD) followed by different superscript letters are significantly different ($p < 0.05$). N. d specifies not detected.

However, it was interesting to note 1-kestose appeared in yogurt samples enriched with BPP (Y-2 & Y-5), raffinose was recorded in yogurt samples enriched with MPP (Y-3 and Y-6) in both types of yogurts (Table 4). An increase of 12% was detected in sugar content of Y-6 in comparison to the control (Y-1), which could be attributed to the presence of raffinose (0.21 ± 0.07 g/100 g) and fructose (0.65 ± 0.11 g/100 g). These observations could be ascribed to the varying sugar contents in each fruit peel. In fact, the detection of 1-kestose in Y-2 & Y-5 treatments that were fortified with BPP were in agreement with the observations of Pereira et al. [39], who reported that 1-kestose was the main fructooligosaccharide (FOS) in banana peel. Similarly, the detected raffinose in Y-3 and Y-6 that were enriched with MPP could indicate that mango peel is a good source of raffinose. Both FOS and raffinose can act as prebiotics and support the growth of probiotics. A similar conclusion was mentioned by Macfarlane [40], who indicated that both FOS and galactooligsaccharides enhanced probiotic growth.

3.4. Changes in the Total Phenolic Content and Antioxidant Properties in Probiotic Yogurts Enriched with FPP

Varied values of total phenolic content (TPC) were obtained in fresh and RFD yogurts (Table 5). As expected, TPC in RFD containing MPP (2.27 ± 0.18 mg GAE/g yogurt) and BPP (2.73 ± 0.11 mg GAE/g yogurt) were greater than TPC in the plain yogurts/Y-1 (0.31 ± 0.07 mg GAE/g yogurt) on day 1 of refrigerated storage. Similar trends were also recorded after 14 and 28 days of refrigerated storage (Table 5). The amounts of detected TPC in the RFD yogurt fortified with BPP (2.27 ± 0.18 mg GAE/g yogurt) and MPP (2.73 ± 0.11 mg GAE/g yogurt) were significantly larger ($p < 0.05$) than those values (0.19 ± 0.01 and 0.23 ± 0.01 mg GAE/ g yogurt) detected in Y-5 and Y-6 treatments of fresh yogurts, respectively. Such differences could be attributed to the faster interactions between polyphenols and protein and the formation of insoluble compounds in the fresh yogurt. Meanwhile, the immediate freeze-drying of yogurt reduced such chemical interactions, and revealed the larger TPC in the RFD yogurts during the 28 days of refrigerated storage (Table 5). Similar chemical interactions between polyphenols and proteins were reported in yogurt enriched with strawberry [15]. Furthermore, the positive effects of added fruit waste on yogurt TPC were reported by Demirkol et al. [41] and Kennas et al. [2], who observed a positive correlation between TPC contents in yogurt and the amounts of added grape pomace and pomegranate peel.

Table 5. Phenolic contents (mg GAE/g sample) and antioxidant activities (mM TE/g sample) in fresh and RFD yogurts during refrigerated storage.

Storage Days	Parameters	Yogurt Treatments					
		Y-1	Y-2	Y-3	Y-4	Y-5	Y-6
				Fresh yogurt			
01	TPC	0.16 ± 0.01^{dA}	0.18 ± 0.02^{bcA}	0.24 ± 0.01^{aA}	0.06 ± 0.01^{dA}	0.19 ± 0.01^{bcA}	0.23 ± 0.01^{aA}
	DPPH	0.11 ± 0.05^{bc}	0.33 ± 0.08^{b}	0.93 ± 0.34^{a}	0.08 ± 0.04^{bc}	0.20 ± 0.06^{bc}	0.89 ± 0.33^{a}
	ABTS	0.05 ± 0.01^{d}	0.44 ± 0.06^{bc}	1.27 ± 0.31^{a}	0.07 ± 0.02^{d}	0.59 ± 0.06^{b}	1.20 ± 0.24^{a}
14	TPC	0.06 ± 0.01^{bA}	0.15 ± 0.05^{abA}	0.19 ± 0.07^{aB}	0.04 ± 0.01^{bA}	0.16 ± 0.04^{aAB}	0.18 ± 0.04^{aB}
	DPPH	0.09 ± 0.05^{cd}	0.24 ± 0.06^{c}	0.83 ± 0.37^{a}	0.14 ± 0.09^{c}	0.14 ± 0.06^{cd}	0.63 ± 0.30^{ab}
	ABTS	0.05 ± 0.02^{d}	0.32 ± 0.08^{bc}	1.18 ± 0.31^{a}	0.05 ± 0.03^{d}	0.48 ± 0.11^{b}	1.05 ± 0.27^{a}
28	TPC	0.04 ± 0.02^{hA}	0.11 ± 0.07^{aB}	0.13 ± 0.07^{aC}	0.03 ± 0.01^{hA}	0.13 ± 0.03^{aB}	0.15 ± 0.07^{aB}
	DPPH	0.04 ± 0.03^{d}	0.15 ± 0.05^{c}	0.69 ± 0.48^{a}	0.04 ± 0.03^{d}	0.10 ± 0.06^{c}	0.50 ± 0.38^{ab}
	ABTS	0.03 ± 0.01^{b}	0.20 ± 0.11^{bc}	1.08 ± 0.31^{a}	0.03 ± 0.02^{b}	0.34 ± 0.19^{b}	0.76 ± 0.42^{ab}
				RFD			
01	TPC	0.31 ± 0.07^{dA}	0.82 ± 0.08^{cA}	2.15 ± 0.05^{bA}	0.68 ± 0.03^{cdA}	2.27 ± 0.18^{bA}	2.73 ± 0.11^{aA}
	DPPH	$1.45 \pm 0.16^{b\ cA}$	2.58 ± 0.24^{bA}	13.67 ± 3.45^{aA}	1.16 ± 0.18^{bcA}	3.00 ± 0.74^{bA}	11.90 ± 3.32^{aA}
	ABTS	2.07 ± 0.07^{cA}	8.13 ± 0.40^{bA}	46.50 ± 3.12^{aA}	1.84 ± 0.21^{cA}	8.85 ± 0.81^{bA}	44.01 ± 3.16^{aA}
14	TPC	0.27 ± 0.01^{cdA}	0.79 ± 0.02^{cA}	1.67 ± 0.16^{bB}	0.56 ± 0.10^{cA}	2.11 ± 0.12^{aA}	2.15 ± 0.50^{aB}
	DPPH	1.20 ± 0.47^{cA}	2.33 ± 0.35^{cA}	12.18 ± 2.93^{aA}	0.71 ± 0.24^{dA}	2.19 ± 0.10^{cB}	5.05 ± 0.36^{bB}
	ABTS	1.82 ± 0.25^{cA}	7.02 ± 0.28^{bA}	39.14 ± 6.56^{aB}	1.70 ± 0.33^{cA}	6.82 ± 0.81^{bA}	39.27 ± 7.66^{aB}
28	TPC	0.21 ± 0.10^{cA}	0.61 ± 0.10^{bB}	1.39 ± 0.46^{aB}	$0.48 \pm 0.39b^{cA}$	1.52 ± 0.46^{aB}	1.66 ± 0.35^{aC}
	DPPH	$0.90 \pm 0.63^{c\ dA}$	1.93 ± 0.46^{cA}	11.84 ± 1.03^{aA}	$0.60 \pm 0.21^{c\ dB}$	1.88 ± 0.26^{cB}	4.64 ± 1.99^{bB}
	ABTS	1.25 ± 0.51^{cA}	5.95 ± 1.15^{bA}	35.63 ± 8.00^{aB}	1.24 ± 0.04^{cA}	5.93 ± 2.29^{bB}	35.00 ± 7.95^{aB}

Values presented the means ± SD (n = 6). Means in a row followed by different lowercase superscript letters for each parameter were significantly different ($p < 0.05$) and means with similar uppercase letters were insignificantly different ($p > 0.05$).

Data in Table 5 also revealed a gradual decline in the % TPC during the refrigerated storage in both fresh and FDR yogurts. Such a decrease in TPC could be attributed to the slow decomposition of phenolic compounds by LAB and the generation of aromatic acids such as phenyl propionic, acetic, and benzoic acids during refrigerated storage. Muniandy et al. [42] reported the hydrolysis of polyphenols by probiotic bacteria present in yogurts. Kabir et al. [16] reported degradation of phenolic compounds and antioxidant activities during refrigerated storage of yogurts fortified with grape seed extracts and banana peel flour, respectively. It was speculated that the metabolic action of probiotic bacteria could produce certain enzymes that assist in the release of phenolic moieties from plant cell wall matrices [43].

The results obtained for ABTS, and DPPH antioxidant activity assays followed a similar trend to that of the TPC. Data in Table 5 clearly demonstrated that yogurts fortified with MPP (Y3 & Y6) exhibited significantly ($p < 0.05$) higher DPPH scavenging activities than other yogurt samples at each point of refrigerated storage. These higher values of DPPH in Y-3 and Y-6 could be attributed to the elevated antioxidant capacity of the mango peels, as mentioned by Peng et al. [17]. Other studies have also demonstrated that the chief components responsible for antioxidant potential of mango are carotenoids, phenolics, and vitamin C, which are reported to be abundant in the cell walls of mango peels [44]. However, the DPPH activities declined from 0.93 ± 0.34 to 0.69 ± 0.48 mg TE/g and 0.89 ± 0.33–0.50 ± 0.38 mg TE/g in Y-3 and Y-6 of fresh yogurts, respectively, after 28 days of storage.

Such a decline in DPPH values could be attributed to the slow probiotic metabolic activities and the breakdown of polyphenols during the refrigerated storage, which were more obvious in fresh yogurt. Similar observations were also noted in $ABTS^{+}$ values (Table 5). However, the losses of DPPH and $ABTS^{+}$ activities in fresh yogurts were greater than those of RFD yogurts throughout cold storage. These findings are in agreement with those noticed by Chouchouli et al. [31], who reported the degradation of antioxidant activity in grape seed extract fortified yogurt after 3 weeks of storage. Lozano et al. [45] reported better retention of antioxidant activity in freeze-dried human milk during storage

at 4 °C. Contrary to this, Trigueros et al. [46] reported increased antioxidant potential of the yogurts fortified with pomegranate juice. According to a study by Helal et al. [47], the acidic nature of the dairy matrices, such as yogurt, encourage interactions between milk proteins and phenolic components. It could be speculated that removing most moisture contents from fresh yogurt during freeze drying would slow or even stop the microbial activities. Consequently, less bacterial activity and slower phenolic hydrolysis would be expected in RFD yogurts.

3.5. Microbial Viability in Yogurts during Refrigerated Storage

Data in Figure S1 represent the counts of *S. thermophilus*, *L. bulgaricus*, lacticaseibacilli and bifidobacteria in log CFU/g in all yogurt treatments during refrigerated storage.

The starter culture (*S. thermophilus*) count in fresh and RFD yogurts on day 1 of storage varied from 8.26 ± 0.09 to 9.23 ± 0.43 log cfu/g in fresh yogurt and from 8.12 ± 0.26 to 8.91 ± 0.60 log cfu/g in FRD sample (Figure S1-A). These variations revealed a significant ($p \leq 0.05$) surge of *S. thermophillus* viability in Y-5 (9.23 ± 0.43 log CFU/g) fresh and Y-6 (8.91 ± 0.60 log CFU/g) of RFD yogurts with their respective controls (8.26 ± 0.09 and 8.23 ± 0.37 in fresh and RFD yogurts, respectively). Previous studies indicated the survival capability of this strain under low temperatures and in milks of various origins [48]. *S. thermophillus* presented increased counts during the first two weeks of refrigerated storage, and it remained approximately stable by the end of 4 weeks of storage in all fresh yogurts, except Y-5. Such declines in counts in fresh yogurt ranged from 0.06 to 0.47 log CFU/g in Y-4 and Y-1, respectively. This trend is aligned with previous studies, which reported increased viability of *S. thermohpillus* for up to 7 days of storage, followed by a steady decline [49]. Alternatively, a significant ($p \leq 0.05$) decline in the viability of *S. thermohpillus* was observed in all RFD yogurts. However, this strain retained maximum viability during storage in yogurts with and without the addition of FPP (Figure S1-A). It should also be noted that the reduction in log CFU/g in both fresh and RFD yogurts were larger in Y-1, Y-2 and Y-3 than in Y-4, Y-5 and Y-6 treatments. These results might indicate a positive correlation among yogurt starters, probiotic strains and MPP during refrigerated storage. This effect could also be attributed to increased phenolic contents of symbiotic yogurts, particularly RFD yogurts. Recently, phenolics have been included in the definition of prebiotics by Gibson et al. [14], and various in-vitro studies evaluated the prebiotic properties of phenolics from fruit extracts [50,51]. The results were in partial agreement with Santo et al. [23], who observed a decline in microbial counts after 28 days of storage in plain yogurts and escalated growth in those fortified with apple and banana peel powders. Results revealed also that yogurt enriched with BPP (Y-5) in both fresh and RFD had the largest counts in all tested starter cultures and probiotics in comparison with all other treatments.

The viable counts of the 2nd starter culture (*L. bulgaricus*) in fresh and RFD yogurts were at their maximum on day 1 of refrigerated storage (Figure S1-B). However, the log counts in RFD yogurt on day one (5.95 ± 0.35 CFU/g) was significantly ($p < 0.05$) smaller than that of in fresh yogurt (7.38 ± 0.23 CFU/g). Such differences of log count in the RFD yogurt could be attributed to the sudden decline in the *L. bulgaricus* counts during freeze drying. This loss of viability during freeze drying could be ascribed to the generation of ice crystals that may cause deformities to the bacterial cell wall proteins, which leads to cellular inactivation [52]. However, RFD yogurts showed lower viability than a fresh batch, yet higher stability during storage with a non-significant ($p > 0.05$) drop in cell counts. Freeze-drying had major negative effects on enumerations of *L. bulgaricus*, which is more evident in control yogurts where a reduction of more than 1 log cfu/g was detected. Meanwhile, the presence of MPP in probiotic yogurts effectively enhanced the survival and stability of *L. bulgaricus*, with population reductions of 0.86 in Y-6. The results demonstrated that supplementing yogurt with MPP and BPP had protective effects on the viability of *L. bulgaricus* during freeze-drying, and these FPP are also reported to have beneficial effects on the growth of probiotics as prebiotic substances [22]. However, changes

in the *L. bulgaricus* counts in fresh and RFD yogurt during refrigerated storage showed a similar pattern to those reported previously on the starter culture *S. thermophilus.* Fortifying yogurt (fresh and RFD) with both FPP and probiotics (Y-5 & Y-6) had maintained steady log counts in yogurts after 28 days of storage. The log counts in yogurt (fresh and RFD) enriched with FPP was greater by about 1 log CFU/g than the log counts in the control (Y-1) throughout the storage period (Figure S1-B). These findings may suggest that added FPP can act as prebiotics and support the growth of LAB in yogurt. Similar findings were reported by [53], in which yogurt supplemented with *Gnaphalium affine* extract stimulated the growth of *L. bulgaricus.*

The probiotic counts were performed in Y-4, Y-5, and Y-6 treatments, since probiotics (*B. lactis*, *L. casei* and *L. rhamnosus*) were incorporated in these treatments only. With respect to fresh yogurt analysis, the comparison between the two synbiotic yogurts (Y-5 and Y-6) and probiotic yogurt (Y-2 and Y-3) detected significant differences ($p < 0.05$) in counts where Y-5 showed a maximum increase, followed by Y-6 and Y-4, respectively. At day 1, the probiotic yogurt without addition of FPP presented *B. lactis* counts ranged from 7.80 to 8.23 cu/g during storage. The incorporation of FPP at concentration of 2% increased viability of *B. lactis*, as Y-5 and Y-6 yogurts presented *B. lactis* counts ranging from 8.15 to 9.13 cfu/g and 8.16 to 9.22 cfu/g, respectively, during storage. The counts of *B. lactis* were highest at the intermediate storage (day 14) period, and thereafter, the cell proliferation dropped in all the treatments irrespective of the composition of yogurts yet was higher in FPP yogurts co-fermented with probiotics. On the other hand, survival of *B. lactis* in RFD yogurts was mainly evaluated after the drying process, and the attenuating effects of freezing are evident from the results. The microbial viability in RFD yogurts was comparatively lower than fresh yogurts, yet the difference was non-significant ($p > 0.05$). The probiotic counts in Y-5 and Y-6 treatments were 7.76 ± 0.67 and 7.50 ± 0.47, respectively, on day 28 of refrigerated storage of freeze-dried yogurt (Figure S1-C). These counts were smaller than those detected in fresh yogurt (8.48 ± 0.21 and 8.25 ± 0.01 log CFU/g). It is evident from the results that the prebiotic function of added banana and MPP was more pronounced and evident in fresh yogurts, where LAB can continue their slow metabolism and growth under refrigeration condition. On the contrary, freeze-drying may slow down such bacterial metabolic activities. Capela et al. [54] investigated the protective effects of various oligosaccharides against damages caused by freeze-drying upon the survivability of lactic acid bacteria (LAB). They found that inulin, fructo-oligosaccharides, and Hi-maize did not improve the viability of LAB in freeze-dried yogurt. By the end of cold storage, however, all fresh and RFD yogurt formulations showed counts higher than 6 log cfu/g, which is the minimum criteria defined by the scientific community to be labelled as probiotic. Therefore, it is a manifestation that added FPP are favoring the viability of *B. lactis* as a function of synbiotic association between probiotic lactobacillus and FPP fibers. Various studies have demonstrated better resilience of *Bifidobacterium spp.* in response to stresses caused by freezing and chilling [54,55]. In addition, with the presence of BPP and MPP in yogurts, a synergistic effect could be detected in relation to the count of *B. lactis*, which reached higher than 9 log CFU/mL after 2 weeks of storage. Similarly, Hayayumi-Valdivia et al. [56] evaluated beneficial effects of microencapsulation of MPP in combination with sodium alginate in symbiotic ice creams. They found that the addition of 2% MPP had significant influence on the survival of *L. acidophillus* and *B. lactis* during 180 days of freezer storage and retained microbial population at > 6 log cfu/g. Likewise, Massounga et al. [57] confirmed the potential of banana powder as a safe tool to preserve the efficiency of *L. acidophillus* and *L. casei*. These boosting effects of FPP on the growth of probiotics such as B. *lactis* has been previously pointed out by other researchers when they enriched yogurt with various concentrations of green banana flour [12].

The initial enumerations of *Lacticaseibacillus* in the Y-1 of both fresh and RFD was 8.48 and 7.94 log CFU/g, and a gradual decline occurred throughout the storage, representing a loss of 0.77 ($p < 0.05$) and 0.73 log CFU/g ($p > 0.05$) in fresh and RFD yogurts. A similar trend of a gradual decrease in Y-5 and Y-6 of fresh and RFD yogurt formulations was

presented, but the loss in viability was less than Y-1. This decline in count over time could be attributed to the accretion of lactic acid, which can overwhelm the growth of bacteria [41]. The results showed that highest count of 8.04 log cfu/g in RFD yogurts was presented by Y-5, which is 0.07% less than its fresh counterpart (8.85 log cfu/g). Conde-Islas et al. [58] and Capela et al. [54] reported that LAB suffered a viability loss of 4.53% and 7% in freeze-dried Mexican kefir grains and yogurt, without the addition of cryoprotectants. Highest initial counts of Lacticaseibacillus in fresh yogurts, at day 1, were presented by Y-5 (8.51 cfu/g) and Y-6 (8.88 log cfu/g). Fruit peels are deemed to contain decent amounts of phenolic compounds, which, along with proteins and dietary fibers, can contribute to the protection of probiotics from harsh acidic environments [9]. With regards to FDR, the loss of viability was lowest in the formulations containing MPP during the whole storage period. After 4 weeks of storage at 4 °C, the Lacticaseibacillus remained viable above 6 log cfu/g, with Y-5 retaining the maximum count of 7.76 log cfu/g (Figure S1-D). This observation is not harmonious with the enumerations observed for *B. lactis* in relation to MPP-fortified yogurts, which indicates possible variation in a synergistic effect between mango peel and probiotics. The findings of this study are contrasting to those reported by El-Batawy et al. [59], where mango peel-supplemented yogurts decreased the viability of *lactobacillus* throughout storage. The retention of higher probiotic counts in yogurts enriched with 2% BPP (Y-5) or 2% MPP (Y-6) is an important outcome from this study. These findings will establish a new approach to commercially utilize such fruit waste products and use them to fortify yogurt and other food products as natural high-fiber prebiotics. These products with high probiotic counts could be more attractive to consumers than the traditional starter culture containing only yogurts. However, a follow-up sensory tasting to assess the consumer acceptance of the products is essential before full commercialization.

Therefore, the utilization of FPP for the enrichment of fermented products proved to be a convenient approach to increase the viability of probiotics during the refrigerated storage of fresh yogurt. This outcome might be accredited to the growth stimulating nutrients in the medium due to the presence of fruit peels. Based on the above results, the yogurt formulations are categorized as a probiotic product, since they presented counts higher than 6 log CFU/mL. This is the least count recognized by the scientific community for a product to be regarded as probiotic in order to confer potential health benefits [60]. The study also made a significant impact in differentiating the effects of processed vs. non-processed yogurts upon chemical and microbial attributes of the final product during storage.

4. Conclusions

The incorporation of fruit processing by-products in food products is desirable to sustain the environment and to develop novel functional foods rich in nutritional constituents. The present study showed that yogurts fortified with FPP exhibited increased values of TPC and antioxidant activities during storage compared to non-fortified yogurts. Furthermore, yogurt enriched with FPP showed the presence of the oligosaccharide raffinose and FOS, which are known as good prebiotics. These synbiotic formulations exhibited improved survival of LAB during refrigerated storage. Therefore, yogurts enriched with fruit peel powders proved to be effective matrices in terms of retaining stability and viability of potential LAB for 28 days of storage of fresh yogurt. *S. thermophillus* and all probiotics presented the highest stability and survival both at the beginning and after 28 days of storage in all fresh synbiotic formulations. Freeze-drying appeared to slow down the metabolic functionalities of LAB and reduce the prebiotic role of FPP. Yogurt fortified with MPP and BPP may be a convenient food format with beneficiary effects of starter cultures, original nutrients and added polyphenols. Research is currently continuing in our laboratory to further examine the possible benefits of combining yogurt with FPP and some selected probiotics on the bioaccessibility of nutrients.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8090469/s1, Figure S1: Viability (CFU/g) of yogurt starters and lactic acid bacteria during refrigerated storage of fresh and RFD yogurts.

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