Production of Functional Fermented Milk Beverages Supplemented with Pomegranate Peel Extract and Probiotic Lactic Acid Bacteria

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Fermented milk beverages supplemented with pomegranate peel extract and inoculated with Lactobacillus plantarum and Bifidobacterium longum subsp longum were produced. The antioxidant activity of fermented milk beverages supplemented with pomegranate peel 150 mg/L (FMPO 150) and 300 mg/L (FMPO 300) was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). In addition, the polyphenolic profile and sugars content were determined by HPLC analysis, and the volatile compounds were identified using GC-MS analysis. The effects of FMPO 150 and FMPO 300 (10 g/day) on the lipid profile and antioxidant/biochemical status of rats were also evaluated after 4 weeks of oral intake. Antioxidant activity of the fermented milk beverage FMPO 300 was higher than that of FMPO 150. GC-MS analysis of the volatile compounds revealed that diacetyl, acetoin, and acetaldehyde were the major constituents. FMPO 150 and FMPO 300 were efficient in reducing the LDL cholesterol and triacylglycerol and increased the HDL cholesterol in serum. Liver function bio-markers were not affected by the end of treatment (p < 0.05). Also, the thiobarbituric acid-reactive substances (TBARS) were decreased, while the activity of antioxidant enzymes in the liver (GSH, CAT, SOD, and GPx) were increased. Hence, the combination of pomegranate peel extract and probiotic lactic acid bacteria in a fermented milk beverage provides not only probiotic benefits but also bioactive phenolic compounds that could be functional and possess therapeutic effects.

1. Introduction

Functional food has been driven by both scientific curiosity and public demand [1]. Functional food products are manufactured through the addition of exogenous natural compounds or probiotics or other microorganisms that produce biogenic compounds. Currently, fermented milk is the most common matrix for commercial functional beverages [2]. The thick, reddish skin of pomegranate fruits usually becomes as waste [3]. This pomegranate peel waste is a rich source for polyphenols, which are used as natural antioxidants and biopreservatives. Punicalagin, ellagic acid, and ellagitannins are the main polyphenols identified in this material [4]. These compounds have antioxidant, antimicrobial, anti-inflammatory, antimutagenic, anticancer, and other beneficial effects on health [5]. The low cost of pomegranate peel waste makes it potentially applicable in the food industry. Dairy products contain various antioxidant molecules, such as milk caseins and whey proteins [6]. In addition, milk includes a variety of antioxidant molecules, i.e., thiols with low molecular weight [7], tocopherol, retinol, and carotenoids [8], and ascorbate [9] at trace levels.

Probiotic microorganisms have been used as ingredients in the large-scale production of functional foods due to the numerous health benefits associated with their consumption [10]. Probiotic products have numerous health benefits, including antimicrobial, antimutagenic, anticarcinogenic, and antihypertensive effects, as well as reduces serum cholesterol, alleviates lactose intolerance, reduces allergic symptoms, reduces diarrhoea, and stimulates the immune system.
2. Materials and Methods

2.1. Materials. Pasteurized cow’s milk was purchased from Astra, a local market in Alexandria, Egypt. A standard starter culture (YoFlex–YC-X11) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was obtained from CHR HANSEN’s Lab., Inc. (Milwaukee, WI). Functional strains, *Lactobacillus plantarum* DSMZ 20079 and *Bifidobacterium longum* subsp. *longum* DSMZ 200707, were procured from the Faculty of Agriculture’s culture collection, Ain Shams University, Cairo, Egypt. The MRS medium was used for the routine propagation of the two starter and the two probiotic strains at 30 and 37°C for 24 h, respectively. Before use, the starter and the probiotic cultures were grown in sterile skim milk at 37°C for 8 h and then inoculated into the pasteurized cow’s milk at ca. 1% (v/v) and ca. 4% (v/v), respectively.

Pomegranate peels were purchased from a local market in Alexandria, Egypt, in 2018. Peels were air-dried at 40°C for 48 h in a ventilated oven and ground into a fine powder, as described by Van Acker et al. [13].

2.2. Extraction of the Polyphenols from Pomegranate Peels. The extraction was carried out by dissolving 1:10 (w/v) powdered sample into water for 6 h using the Soxhlet method. The mixture was filtered through Whatman No. 2 filter paper and then lyophilized. The resulted dry extract was stored at 4°C in dark bottles until use.

2.3. Characterization of the Pomegranate Peel Extract. Total soluble phenol and flavonoid content was determined according to Dewanto et al. [14] and Sakanaka et al. [15], respectively. The antioxidant activity of the extract was determined using the DPPH free radical-scavenging assay according to the method [16]. To determine ABTS radical-scavenging assay, the method of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was adopted [17].

2.4. Manufacture of the Fermented Milk Beverages. Pasteurized cow’s milk was reduced in volume by heating at 90°C for 20 min, resulting in an improved viscosity of fermented milk beverages supplemented with pomegranate peel (FMPO). Pomegranate peel extract (POPE) was added to the milk at 150 mg/L (FMPO 150) or 300 mg/L (FMPO 300), expressed as the total phenol concentration (gallic acid equivalents) [14]. Inoculation was carried out of starter cultures *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (ca. 1%, v/v, corresponding to ca. log 9.0 CFU/ml) and functional strains *Lactobacillus plantarum* DSMZ 20079 and *Bifidobacterium longum* subsp. *longum* DSMZ 200707 (ca. 5%, v/v, corresponding to ca. log 9.0 CFU/ml) and incubated at 30°C for 24 h. The control fermented milk beverage (FM) was produced according to the same procedure but without POPE addition. The fermented beverages (FMPO 150, FMPO 300, and FM) were stored at 4°C until further analyses.

2.5. Physical Analysis of the Fermented Beverages. The pH of samples was measured during fermentation by using a pH meter. The viscosity of each sample was measured by a dynamic viscometer (Brookfield Model RVDI, USA). The titratable acidity of each sample was determined as the lactic acid percentage by titrating with 0.1 NaOH using a phenolphthalein indicator [18].

2.6. Determination of the Antioxidant Activities of the Fermented Beverages. The antioxidant activities of the fermented milk beverages were evaluated by DPPH and ABTS methods during 30 days storage (as previously mentioned).

2.7. Microbiological Analyses. Samples of the FMPOs or FM (10 g) were inoculated in sterile 0.1% (w/v) peptone water solution and then homogenized for 2 min at room temperature. Counting of LAB was estimated by plating onto MRS (for lactobacilli), BD (for bifidobacteria), or M17 (for streptococci) agar media (Oxoid, England). Isolated colonies were then cultivated in MRS broth at 30°C for 24 h [19].

2.8. HPLC Analysis of the Sugars. Aqueous extracts from FMPO 150, FMPO 300, and FM beverages were used to determine the sugars. Ten grams of each extract was completed to 50 mL with phosphate buffer (50 mM, pH 7.0). The mixture was incubated at 40°C for 1 h under gentle stirring and then centrifuged at 3000 × g for 15 min at 4°C. The supernatants were filtered through Whatman No. 2 filter paper and adjusted the pH to 4.6, and the suspension was centrifuged at 10,000 × g for 10 min. Finally, the upper solution was filtered through 0.22 μm syringe filters. The sugar contents were determined using an HPLC instrument (Agilent 1260 infinity HPLC Series, USA) according to Siragusa et al. [20] and Xu et al. [21].

2.9. HPLC Analysis of the Polyphenolic Components. The phenols were extracted from 50 g of each sample mixed with 200 mL of 80% methanol for 5 min and then centrifuged at 5000 × g for 10 min. The phenolic compounds were analysed by RP-HPLC using an Agilent 1260 instrument (Santa Clara, CA, United States) according to Tomaino et al. [22].

2.10. GC-MS Analysis. For measuring volatile compounds, 10 grams from each sample were mixed with saturated NaCl solution to complete the volume to 50 mL. Three millilitres...
from each sample were analysed by using gas chromatography-mass spectrometry (GC-MS). The composition of the supernatant was analysed by GC-MS using an Agilent 7890 gas chromatograph (Agilent Technologies, Santa Clara, CA) controlled by Agilent GC/MS Mass Hunter Acquisition software according to Servili et al. [23].

2.11. Animals and Protocols. Twenty-four albino male rats were obtained from Alexandria University Animal House, Alexandria, Egypt, for the experiments. Test animals were divided into four groups (n = 6 per group) and fed certain oral combinations for 30 consecutive days, as follows: (a) control, only diet; (b) control milk beverage (10 g/day) + diet; (c) FMPO 150 (10 g/day) + diet; and (d) FMPO 300 (10 g/day) + diet. All animals were maintained under standard conditions (temperature 22°C, humidity 55%, and reverse 12-hour light/dark cycle) in individual growth cages with access to commercial feed and water ad libitum, until the end of the experiment. The functional milk beverage was given to each rat orally to ensure the same content to each animal. This study adhered to the animal care standards, and the protocol was approved by the Ethics Committee of the Alexandria University on Animal Experimentation.

2.12. Biochemical Blood Analysis. Animals were sacrificed by the end of the experiment for collection of blood samples. Blood samples were centrifuged at 3000 × g (4°C, 12 min) to obtain sera for biochemical assessment. Total protein, albumin, total cholesterol, high-density lipoprotein (HDL) cholesterol, total protein, triacylglycerols, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and cholesterol spectrophotometric determinations were performed using Biodiagnostic kits (Giza, Egypt).

2.13. Antioxidant Biomarker Analysis. Liver thiobarbituric acid-reactive substances (TBARS) were measured at 532 nm by using 2-thiobarbituric(2,6-dihydroxy primidine-2-thio) (TBA). An extinction coefficient of 156,000 M⁻¹·cm⁻¹ was used for calculation [24]. Liver glutathione reduced (GSH) was determined according to the method of Jollow et al. [25]. Catalase (CAT; EC 1.11.1.6) activity was determined by hydrogen peroxide [26]. Superoxide dismutase (SOD, EC 1.15.1.11) activity was measured according to Mishra and Fridovich [27]. The activity of glutathione peroxidase (GPX, EC 1.11.1.9) was assayed by the methods of Chiu et al. [28].

2.14. Sensory Evaluation. Samples and controls were subjected to sensory evaluation. Ten trained panelists evaluated the samples on a hedonic scale consisting of 9 points from 1 (extremely dislike) to 9 (extremely like), following the procedure described by Hooda and Jood [29]. The colour, texture, taste, consistency, and overall acceptability of each sample were evaluated.

2.15. Statistical Analysis. All assays were carried out in triplicate, and the results are expressed as the mean with the standard deviation (mean ± SD) using SPSS 16 software. Values were compared by ANOVA with a general linear model followed by Duncan’s post hoc test, and p values less than 0.05 were considered significant. The dependent variables (y) were all experiments carried out, while the fixed effects (x) were control (FM) and treatments (FMPO 150 and FMPO 300).

3. Results and Discussion

3.1. Characteristics of the Pomegranate Extract. The phenolic compounds are the primary components responsible for the antioxidant activity of fruits. The total phenolic and flavonoid contents of the pomegranate peel extract were 404.13 ± 2.49 μg GAE equivalent/g and 56.83 ± 1.54 μg catechol equivalent/g, respectively (Table 1). The contents obtained herein for the pomegranate peel extract are higher than those reported in the literature; for example, the values are higher than those reported by Shibani et al. [30], who reported total phenolic content (TPC) of pomegranate peel ranging from 80.5 to 274 μg GAE equivalent/g. However, Shehata et al. [31] reported that the TPC in pomegranate peel was 709.66 ± 0.76 μg GAE equivalent/100 g, and the total flavonoid was 132.06 ± 1.1 μg catechol/g. These variations may be due to the different extraction methodologies or solvents employed in these studies. Flavonoids are important for human health because of their high nutraceutical activities as free-radical scavengers [32]. The antioxidant activities of pomegranate peel extract, determined using the DPPH and ABTS methods, are shown in Table 1. The pomegranate peel extract showed a DPPH free radical-scavenging activity of IC₅₀ (63.82 ± 0.61 μg/ml) and an ABTS free radical-scavenging activity of IC₅₀ (54.63 ± 0.82 μg/ml). Thus, the findings obtained in this study demonstrate that pomegranate peel extract possesses antioxidant/free radical-scavenging properties, which are very likely due to its high content of phenolic compounds.

3.2. Survival of Probiotic Strains. Functional milk beverages (FM, FMPO 150, and FMPO 300) were manufactured using starter cultures (Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus) and the potential probiotic strains Lactobacillus plantarum and Bifidobacterium longum subsp. Longum, as described above. Pasteurization was ignored. Based on a comparison of FMPO 150 and FMPO 300 to FM, the addition of the POPE significantly influenced (p < 0.05) the survival of the potential probiotic strain (Figure 1). All the strains could be detected after 0, 7, 14, 21, and 30 days of storage. In particular, the population of Lactobacillus plantarum DMSZ 20079 slightly decreased from log 9.33 ± 0.28 CFU/ml to log 8.02 ± 0.22 CFU/ml for FMPO 300. The cell density of Bifidobacterium longum subsp. longum decreased from log 9.40 ± 0.14 CFU/ml to log 7.58 ± 0.14 CFU/ml. Overall, numerous environmental and technological factors affect the survival of the probiotic strains in the fermented dairy products [11]. Nuvalkaelul and Charalamopoulos [33] reported that, at the end of its shelf life, the probiotics must be at least 1 log 7.00 CFU/mL.
concentration in the product by the end of the storage period in order to resist to the adverse conditions in the gastrointestinal tract and become in the intestine in a sufficient quantity to exhibit a health benefit.

From the results obtained in this study, the addition of polyphenols extracted from pomegranate peel to fermented milk beverages can maintain the probiotic bacterial count within the functional limits required for exerting health benefits. Hence, both polyphenols and probiotics have beneficial effects on the human body, so it might be beneficial to develop new functional-enhanced food products containing both components [34].

### 3.3. Physical Properties

Table 2 shows the effects of functional milk beverage fortification with POPE during storage on the pH and beverage acidity. The pH levels of the functional milk beverages with and without POPE decreased.
significantly ($p < 0.05$) with increasing storage time. This is in agreement with other studies that produce fermented milk beverages supplemented with phenolic compounds extracted from olive vegetation in water and fermented with functional lactic acid bacteria, which recorded lowering of the pH during storage [23]. The mean pH levels of FM ranged from 4.51 to 4.16. The mean pH levels of the functional milk beverages fortified with POPE ranged from 4.41 to 4.03. The higher counts of viable bacteria at the end of the storage period resulted in lower pH levels in the functional milk beverages fortified with and without POPE. In addition, the beverages acidity increased markedly ($p < 0.05$) with increasing storage time regardless of the concentration of POPE. The mean values of acidity obtained for functional milk beverages fortified with and without POPE ranged from 0.20 to 0.35.

Viscosity is an important parameter because it determines the appearance and density of the product as it will be applied in the food industry. Table 2 shows the effects of POPE on the viscosity of the functional milk beverages. After 30 days of fermentation, the viscosity of FMPO 300 was significantly higher (12.00 ± 2.0 cP) than it was prior to storage (day one, 5.63 ± 0.50 cP). This may be related to PPE impact on the aggregation of casein network in yoghurt via electrostatic interaction and on the resistance of the yoghurt matrix to flow [35, 36]. Ramaswamy and Basak [37] reviewed that the addition of plant extracts mainly lowers the viscosity of products by decreasing the water-binding capacity of the milk proteins.

### 3.4. Antioxidant Activity of the Functional Milk Beverages

The antioxidant activities by DPPH and ABTS measured during fermentation are presented in Table 3. In the DPPH assay, the highest antioxidant activity (expressed as IC$_{50}$ μg/ml) after 30 days of storage was observed for FMPO 300 IC$_{50}$ (62.85 ± 1.14 μg/ml), followed by FMPO 150 IC$_{50}$ (75.82 ± 3.62 μg/ml), compared to FM IC$_{50}$ (143.4 ± 5.44 μg/ml), and also in ABTS radical-scavenging activity of FMPO 300 reaching IC$_{50}$ (37.87 ± 0.34 μg/ml) after 30 days of storage. However, FMPO 150 was IC$_{50}$ (67.60 ± 1.04 μg/ml) compared to FM which was IC$_{50}$ (120.41 ± 7.74 μg/ml) at the same point during storage. In general, these antioxidant activity values of fermented milk are greater than whole milk, as reported by Niero et al. [38], due to the addition of pomegranate peel extract in fermented milk. Mousavi et al. [39] also reported that fermentation by *L. plantarum* and *L. acidophilus* reduced the concentration of phenolic compounds and increased the antioxidant activity of pomegranate juice because some LAB strains can degrade certain phenolic compounds into other metabolites with higher antioxidant activities [40].

### 3.5. HPLC Analysis of the Sugars

Lactose concentration in milk was ca. 106.5 mM. The concentration decreased dramatically during storage (Table 4), and the contents of lactose in FM, FMPO 150, and FMPO 300 were 86.5, 90.2 and 95.3 mM, respectively. At the end of the storage period, each beverage had a low concentration of glucose. In addition, the level of galactose was found to be slightly increased by storage and was found at concentrations of 38.1, 40.2, and 41.8 in FM, FMPO 150, and FMPO 300, respectively. Usually, the milk sugar lactose splits to glucose which fermented to lactic acid and galactose which accumulated in yoghurt [11].

### 3.6. HPLC Analysis of the Polyphenolic Profile

Phenolics in FMPO 150 and FMPO 300 was measured at zero and after 30 days storage at 4°C (Table 5). Punicalagin, the major polyphenol, was present at 320.70 mg/L, and this compound has been reported to have anti-inflammatory, anticancer, and antiatherosclerotic properties [41–43]. In addition, gallic acid was the second most abundant polyphenol, present at 235.32 mg/L in the milk beverage, and this compound possesses antioxidant, antifungal, antiviral, and anticancer activities [44–46]. On the contrary, ferulic acid has been reported to have antioxidant, antimicrobial, anti-inflammatory, antithrombosis, and anticancer activities [44]. HPLC showed that other polyphenols, such as p-hydroxybenzoic acid, punicalin, p-coumaric acid, and ellagic acid, were also present in the POPE. Many other polyphenols were detected at trace levels. Pomegranate polyphenols are thus considered to be capable of limiting the effects of reactive oxygen species (ROS) on the body [42, 47]. At the end of the storage period, the contents of the phenolic compounds were lower than they were prior to storage due to hydrolysis. Ciafardini et al. [48] and Servili et al. [49] reviewed that hydrolysis was favourable at acidic pH and due to LAB esterase activities. The consumption of 100 mL of a fermented milk fortified with POPE has an effect similar to that of the consumption of 100 g of pomegranate peel including 500 mg/kg total phenols. This level of consumption is the optimal intake to get the maximum health benefits [50].

### 3.7. GC-MS Analysis of the Volatile Compounds

As expected, acetaldehyde, diacetyl, and acetoin were the major species.
150 and FMPO 300 during storage. From these results, the implementation. In general, ketones can provide several positive sensory/aroma attributes [56]. From these results, the decrease in the ketone concentration during storage period (Table 6). F—_his decrease in the contents of acetaldehyde were 17.7, 23.91, and 15.7 ppm in FM, FMPO 150, and FMPO 300, respectively. In addition, the concentrations of diacetyl in FM, FMPO 150, and FMPO 300 decreased during the storage period (Table 6). This decrease in the aldehyde content with increasing storage time was very interesting because it has been noted that aldehydes cause off flavours and are undesirable compounds in pomegranate beverages [53, 54]. In addition, high contents of aldehydes in pomegranate beverages are not accepted by consumers [55]. GC-MS analysis showed that the ketone concentration decreases during storage with pomegranate extract supplementation. In general, ketones can provide several positive sensory/aroma attributes [56]. From these results, the addition of pomegranate extract to the fermented milk beverage could enhance the aromatic profile of the beverages through the production of desirable volatile compounds. 3.8. In Vivo Study. Rats fed with a functional milk beverage for 30 days exhibited a significant reduction in total cholesterol, LDL cholesterol, and triacylglycerol levels. Also, it had increased levels of HDL cholesterol (Table 7). These results demonstrate the bioefficacy of the functional milk beverage containing Lactobacillus plantarum DSMZ 20079 and Bifidobacterium longum subsp. longum DSMZ 200707 in improving the plasma lipid profile when compared with the control group (without POPE and probiotics bacteria). These results are in agreement with results obtained by Stancu et al. [57], who reported the plasma lipid reduction of rats after the consumption of probiotics for 21 days (L. acidophilus and B. animalis).

These results indicate that probiotic could significantly decrease the risk of cardiovascular disease, especially atherosclerosis. Also, AST and ALT are two important indicators of liver damage [58]. Thus, if the probiotics cause any toxic effect, this could raise the liver ALT and AST [58]. Concerning, the serum total proteins and albumin had no significant change after beverages oral intake. In addition, measuring TBARS, SOD, GST, GPx, CAT, and TAC (Table 8) showed significant decrease in the levels of TBARS and increase in the activities of antioxidant enzymes (SOD, GST, GPx, CAT, and TAC) and the level of GSH compared to the control group (p < 0.05).

### Table 3: Radical-scavenging activity (DPPH and ABTS) of FMPO 150, FMPO 300, and FM during storage.

<table>
<thead>
<tr>
<th>IC_{50} (μg/ml)</th>
<th>FM</th>
<th>FMPO 150</th>
<th>FMPO 300</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH</td>
<td>155.85 ± 7.80(^b)</td>
<td>149.64 ± 4.69(^b)</td>
<td>143.4 ± 5.44(^b)</td>
</tr>
<tr>
<td>ABTS</td>
<td>128.89 ± 10.86(^b)</td>
<td>124.37 ± 6.16(^b)</td>
<td>120.41 ± 7.74(^b)</td>
</tr>
</tbody>
</table>

FM: the control fermented milk beverage; FMPO 150: fermented milk beverages supplemented with pomegranate peel extract (150 mg/L); FMPO 300: fermented milk beverages supplemented with pomegranate peel extract (300 mg/L). Values are mean ± SE (n = 3). Values with different superscripts in the same row differ significantly (p < 0.05).

### Table 4: Concentrations (mM) of sugars of FMPO 150, FMPO 300, and FM during storage.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>FM</th>
<th>FMPO 150</th>
<th>FMPO 300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose (mM)</td>
<td>104.4</td>
<td>86.5</td>
<td>104.5</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>14.1</td>
<td>10.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Galactose (mM)</td>
<td>36.5</td>
<td>38.1</td>
<td>37.4</td>
</tr>
</tbody>
</table>

FM: the control fermented milk beverage; FMPO 150: fermented milk beverages supplemented with pomegranate peel extract (150 mg/L); FMPO 300: fermented milk beverages supplemented with pomegranate peel extract (300 mg/L).

### Table 5: HPLC analysis of phenolic compounds (μg/g) for FMPO 150 and FMPO 300 during storage.

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>FMPO 150</th>
<th>FMPO 300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>3.91</td>
<td>0.78</td>
</tr>
<tr>
<td>Caffeine</td>
<td>20.5</td>
<td>14.33</td>
</tr>
<tr>
<td>Catechol</td>
<td>6.5</td>
<td>4.33</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>1.62</td>
<td>1.05</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>15.4</td>
<td>9.62</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>40.5</td>
<td>31.08</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>167</td>
<td>152.4</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>22.4</td>
<td>11.7</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>90.6</td>
<td>82.4</td>
</tr>
<tr>
<td>Punicalin</td>
<td>162.5</td>
<td>111.2</td>
</tr>
<tr>
<td>Punicolin</td>
<td>19.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Rutin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>3.04</td>
<td>1.66</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>9.14</td>
<td>5.8</td>
</tr>
<tr>
<td>Vanillin</td>
<td>1.96</td>
<td>0.81</td>
</tr>
</tbody>
</table>

ND: not detected; FM PO 150: fermented milk beverages supplemented with pomegranate peel extract (150 mg/L); FMPO 300: fermented milk beverages supplemented with pomegranate peel extract (300 mg/L).

### Table 6: Volatile compounds (ppm) of FMPO 150, FMPO 300, and FM during storage.

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>FM</th>
<th>FMPO 150</th>
<th>FMPO 300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>22.5</td>
<td>17.7</td>
<td>28.71</td>
</tr>
<tr>
<td>Acetoin</td>
<td>9.8</td>
<td>6.61</td>
<td>15.1</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>6.6</td>
<td>4.11</td>
<td>5.66</td>
</tr>
<tr>
<td>2-Pentanone</td>
<td>2.2</td>
<td>0.87</td>
<td>0.70</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>0.04</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>2-Nonanone</td>
<td>0.12</td>
<td>0.087</td>
<td>0.3</td>
</tr>
<tr>
<td>2-Butanone, 3-hydroxy</td>
<td>—</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Butanoic acid</td>
<td>0.31</td>
<td>—</td>
<td>0.013</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>0.62</td>
<td>0.21</td>
<td>0.56</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.081</td>
<td>0.012</td>
<td>0.06</td>
</tr>
</tbody>
</table>

FM: the control fermented milk beverage; FMPO 150: fermented milk beverages supplemented with pomegranate peel extract (150 mg/L); FMPO 300: fermented milk beverages supplemented with pomegranate peel extract (300 mg/L).
3.9. Sensory Evaluation. There were significant differences between any of the estimated sensory parameters in the control milk beverage and the corresponding parameters in the functional milk beverages fortified with FMPO 150 or FMPO 300 (Figure 2). The addition of POPE (150 and 300 mg/L) significantly improved sensory results such as odour, flavor, and overall acceptance. However, all the beverages were of overall acceptable (7.8–8) (Figure 2). Based on these data, some fruit-derived ingredients can be added to beverages to obtain higher sensory acceptability.

4. Conclusions

This is the first study to use pomegranate peel by-product usually discarded by juice factories in order to produce a novel functional fermented beverage with probiotic bacteria L. plantarum DSMZ 20079 and B. longum subsp. longum DSMZ 200707 supplementation. After 24 h of fermentation, the fermented milk supplemented with pomegranate peel extract (150 mg/L and 300 mg/L) contained not only antioxidant compounds but also the potential probiotics L. plantarum DSMZ 20079 and B. longum subsp. longum DSMZ 200707, producing antioxidant and antimicrobial compounds that add a considerable value to the pomegranate peel extract and allow the production of an inexpensive, novel functional fermented beverage with various health effects without any toxic activity observed.

Data Availability

The results and data used to support the findings of this study are included within the article (Tables 1–8 and Figures 1 and 2).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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