Article

Valorization of Different Dairy By-Products to Produce a Functional Dairy–Millet Beverage Fermented with *Lactobacillus paracasei* as an Adjunct Culture

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Abstract: Fermented dairy products not only have a long shelf-life but also have beneficial nutritional values. The products are deficient in dietary fiber and certain bioactive compounds. Adding grains to dairy products is a widespread practice to improve the nutritional and economic aspects. In this work, we studied the effect of fermented millet–milk beverages (FMBs) using pearl millet grains and three different dairy by-products (sweet whey, sweet buttermilk, and skimmed milk). A control treatment prepared with water was also manufactured for comparison. Samples were continuously prepared and fermented using a commercial yogurt starter culture (YC-381) containing *L. delbrueckii* subsp. bulgaricus, *Streptococcus thermophilus*, and a pure strain of *L. paracasei* subsp. *Paracasei*. Four FMBs (water based: WB-FMB, whey based: WHB-FMB, buttermilk based: BMB-FMB, and skimmed milk based: SMB-FMB) were analyzed during cold storage at 4 °C for up to 15 days for chemical, microbiological properties, minerals content, antioxidant properties, glycemic index, and glycemic load on days 1, 8, and 15. The sensory characteristics of the FMBs were also evaluated during cold storage (4 °C/15 days). In general, the progression of acidity was slower in SMB-FMB and WHB-FMB samples during fermentation compared to in the BMB-FMB sample. The longest fermentation time was for the SMB-FBM sample (3 h), while the shortest was for the BMB-FMB sample (1.5 h). Reflecting the good manufacturing practices, all samples were free of coliform, mold, and yeast. No bacterial growth was detected in the WB-FMB sample at days 8 and 15 of storage, while the growth of *Lactobacillus spp*. and *S. thermophilus* was significantly higher (9.97 ± 0.01 and 9.48 ± 0.06, respectively) in the BMB-FMB sample compared to in the other three FMBs. The FMBs produced using dairy by-products had more antioxidant properties. All samples were better perceived during sensory evaluation by panelists than the water-based sample, except for the BMB-FMB sample, in which a bitter taste appeared. In the BMB-FMB sample, the proteolytic degree was significantly higher (4.8 ± 0.09) after 3 h of fermentation by about 460% than in the fresh sample. All samples had a low glycemic index and glycemic load. In addition, acidity progression was slower in SMB-FMB and WHB-FMB samples during fermentation and storage compared to the WB-FMB sample. Therefore, it could be recommended that it is more beneficial to prepare fermented millet–milk beverages using dairy by-products and suitable starter cultures under optimal fermentation conditions instead of using water to maximize the nutritional and economic aspects.

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Keywords: dairy by-products; millet–milk; buttermilk; fermentation; in vitro glycemic index; nutrition; food supply

1. Introduction

The primary purpose of food is to provide the essential nutrients required to meet an individual's nutritional needs [1]. However, different countries encounter unique nutritional obstacles, such as malnutrition and imbalanced nutrition, that hinder their citizens from leading healthy lives and enjoying longevity [2]. Scientific studies have increasingly shown that certain foods and components can offer beneficial physiological and psychological effects beyond their basic nutrient provision [3]. Researchers are now looking for physiologically active dietary components that can improve physical and mental health and minimize illness risk. To meet the requirement for healthy foods, natural food additives and health-promoting ingredients are becoming more popular [4]. Innovative intervention in traditional food recipes may help solve local and regional nutritional challenges. It combines traditional dishes with local elements to boost nutrition [2].

Nowadays, consumers are increasingly demanding functional food characterized by its health-promoting activities. Functional foods are part of a healthy diet and contain biologically active ingredients that may improve health or lower the risk of serious diseases. This increasing demand resulted from a deep understanding of the relationship between food, nutrition, and health. Most food consumers require bioactive components such as polyphenols, antioxidants, prebiotics, and probiotics [3]. Those functional properties can improve food's organoleptic, technical, nutritional, and health benefits or microbial safety [5].

Throughout history, humans have used fermentation technology as one of the methods of preserving foods and drinks before reaching the cooling method. The consumption of foods and drinks that have been subjected to fermentation processes is a food habit with great health benefits, as the changes resulting from the fermentation processes in the food or the drink itself act as the transformation of sugars and starch to promote the desirable and beneficial bacteria known as probiotics, which help solve many health problems in the body, especially digestive problems [6]. Fermented dairy products are foods produced using functional lactic acid bacteria (LAB) starters. These starters can express certain functional properties that give an added value to the end product in terms of the production of bacteriocin, exopolysaccharides (EPS), vitamins, conjugated linoleic acid (CLA), and biopeptides [7]. Fermented milk's functionality is due to its high digestibility and bioavailability of protein, calcium, potassium, and vitamin B [4,8]. Probiotic micro-organisms and prebiotic substances in fermented milk stimulate the immune system, improve lactose digestion and blood glucose management, and reduce constipation, diarrhea, colon cancer, inflammatory bowel disease, and allergies [9,10]. Despite all the features and benefits of fermented dairy products containing probiotics, they lack dietary fiber and some microelements. Therefore, grains and milk are mixed to compensate for the lack of nutrients of both of them [11].

Recently, researchers have been interested in the benefits of millet's high nutritive values, comparable to those of other major cereals such as wheat and rice. Millets are a highly nutritious gluten-free food that can help prevent and treat a range of health problems, such as by reducing blood pressure, heart disease, cancer, and tumors. Millet also aids in digestion and provides roughage to the intestine [12].

On the other side of the research interests, efforts in valorizing dairy by-products are increasing. Skimmed milk is the main by-product used to prepare important milk and dairy products. Other dairy by-products such as sweet whey and sweet buttermilk have excellent nutritional properties. The majority of bioactive peptides that support the cardiovascular, gastrointestinal, immunological, and neurological systems come from whey proteins. Milk fat globule membrane (MFGM) includes bioactive molecules with

anticancer and cholesterol-lowering properties. However, functional food production underutilizes buttermilk and whey. Technological options to improve whey or buttermilk food production have been researched, with beverage manufacturing being the most economically and technologically viable [13,14].

This study was designed to combine the benefits of fermented milk and millet grains and provide a product with great health benefits and potential nutritional and low economic values. The development of a millet–milk functional beverage prepared in skim milk, sweet whey, or sweet buttermilk and fermented by yogurt starter culture, along with *L. paracasei* subsp. *Paracasei*, and sweetened by sugar date powder instead of sucrose is the main objective of the current study. The developed product's microbial, physicochemical, and bioactive properties and sensory attributes during storage were assessed.

2. Materials and Methods

2.1. Materials

Raw cow milk was purchased from Al-Zunaidi private farm in Unaizah governorate, Qassim region, KSA, immediately after milking. It was kept under cooling conditions (4 ± 1 °C) and directly transferred to the Food Science and Human Nutrition department lab, College of Agriculture and Veterinary Medicine, Qassim University, KSA, for further preparations. Skimmed milk powder (95% total solids (TS)) was brought from Arla Foods Company (Viby J, Denmark). Pearl millet (*Pennisetum glaucum*) was obtained from a local store in Abu Arish City, Jazan region, Saudi Arabia. Direct Vat Set (DVS) YC-381 commercial starter culture (Chr. Hansen laboratories, Copenhagen, Denmark.) containing *L. delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus* was purchased from Misr Food Additives company (MIFAD, Badr City, Egypt). *L. paracasei* subsp. paracasei (B-1932) strain was procured from the Agriculture Research Service (ARS) Culture Collection, Norwegian Radio Relay League (NRRL). Rennet enzyme (FAR-M sticks, Chr. Hansen laboratories, Copenhagen, Denmark.) suitable for milk coagulation was purchased from Misr Food Additives (MIFAD, Badr City El Roubeky Rd., Area 250 Fadan Plot 154, Badr, Egypt). Pepsin (1:3000) was purchased from Aldon Corporation (221 Rochester Street, Avon, NY 14414, USA). Pancreatin from porcine pancreas (≥3 × USP specifications: Sigma-Aldrich, Merck Group, St. Louis, MO, USA) and Amyloglucosidase from Aspergillus Niger (260 U mL^{-1}, Sigma) were purchased from Bayouni Trading Co., Ltd., Riyadh St., Cross 21 Bayouniya, Alkhobar, KSA. De Man, Rogosa, and Sharpe (MRS) broth and agar, M17 agar, MacConkey agar, malt extract agar, and nutrient agar media were purchased from Condalab, Calle Forja 9, 28850, Torrejón de Ardoz, Madrid, Spain. A glucose determination kit, GOD-PAP (Fortress Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, UK), was also purchased. All chemicals used in the study were of analytical grade. Chemicals for each experimental method are detailed under its method of analysis, described subsequently.

2.2. Preparation Methods

2.2.1. Preparation of Different Milk By-Products

Different dairy by-products, named skimmed milk, sweet buttermilk, and sweet whey, were prepared from fresh cow milk following regular processing steps. Skimmed milk is a by-product of the cream-making process [15]. For sweet whey, raw milk was pasteurized (72 °C 15 s^{-1}) and then cooled down to 37 °C before rennet addition. After coagulation, the sweet whey was drained from the rennet curd. This remaining liquid is the sweet whey [16]. For the sweet buttermilk, which is a by-product of butter manufacturing, the cream was left for aging (4 °C 24 h^{-1}), and then the milk fat globules were destabilized during churning. As a result, sweet buttermilk was separated [15].

2.2.2. Preparation of Starter Culture

The *L. paracasei* subsp. *paracasei* (B-1913) strain was activated in MRS broth at 37 °C overnight five times, followed by a sixth passage in sterilized skimmed milk (9% TS

containing 1% glucose) at 37 °C overnight to prepare a starter culture available to be used in the rest of the study.

2.2.3. Preparation of Different Millet–Milk Beverages

According to Mugocha et al.'s [17] method, pearl millet grains were first processed with some modifications. Briefly, millet grains were cleaned of impurities, washed well, and left to dry in an air-dryer oven at 40 °C for 6–8 h. Then, the grains were roasted (70 °C) in a pan for about 10 min until the color turned golden. According to Sunny et al. [18], the grains were mixed with water in a ratio of 1:6 (grains:water) and boiled (110 °C) for 10 min. Finally, water was discarded, and the cooked grains were retained as they were ready for processing. Then, four types of millet–milk beverages were prepared using four different bases. The first treatment, set as control, was based on water. The other treatments were sweet whey, sweet buttermilk, and skimmed milk. Millet was mixed well using an electric mixer for an estimated 7 min with the suitable base for each treatment in a ratio of 1:6 (cooked millet grains:base) and then filtered with muslin. The four prepared millet–milk samples were then heat treated (85 °C/10 min) and cooled to 40 °C before continuous fermentation by yogurt starter culture (*S. thermophilus* and *L. delbrueckii* subsp. bulgaricus (YC-381; Chr. Hansen Laboratories, Copenhagen, Denmark) at the level of 0.1 g L^{-1} and *L. paracasei* subsp. *Paracasei* (B-1913: ARS Culture Collection, USA) at 1.5%). The samples were then incubated at 40 °C for the time needed for the pH to reach about 4.6. The final products were stored under cooling (4 ± 1 °C for 15 days) for different analyses (Figure S1).

2.2.4. Preparation of Trichloroacetic Acid (TCA) Soluble Extracts

The TCA soluble extracts from different fermented samples were prepared for different antioxidant determinations. The TCA soluble extract was prepared according to Lievore et al. [19]. A 2 ml amount of FMBs was mixed thoroughly with 1 mL of d·H_2O, and then 5 mL of TCA (12%, *w*/*v*) was added and mixed. After 10 min standing, the mixture was centrifuged at 10,000× *g* for 30 min, and the supernatant was collected and frozen at −25 °C until used.

2.3. Methods of Analysis

2.3.1. Chemical Composition

The total protein, TS, and ash contents were determined according to the methods described in the A.O.A.C. methods [20], and carbohydrate content was calculated using the following equation:

$$\text{Carbohydrates \%} = \text{Total solids} - (\text{Fat} + \text{Protein} + \text{Ash}) \quad (1)$$

The fat content was determined by the micro-Folch extraction method [21]. A neutral polar mixture of chloroform:methanol (2:1, *v*:*v*) was prepared, shaken well, and left in an ice bath. A solution of 0.73% NaCl was prepared. About 2 g of the sample was placed in a test tube with a cap, then 20 mL of the mixture of chloroform:methanol and 8 mL of NaCl solution was added, then the tube was tightly closed, shaken well, and then placed in an ice bath for 30 s. Centrifugation was carried out at 2500 rpm for 2 min. As a result of centrifugation, 3 layers were formed in the tube. The upper layer, a methanol–water phase, was carefully removed and disposed of. First, a standard beaker was washed, dried, and weighed before being left inside a desiccator. Then, using a glass pipette, the chloroform–lipid phase was extracted and transferred to the beaker. The beaker was then placed on a heated surface until all the chloroform had evaporated. Finally, the beaker was weighed again, and the remaining fat was calculated. The fat percent was then calculated using the following equation:

$$\text{Total Fat \%} = \frac{\text{W2}}{\text{W1}} \times 100 \quad (2)$$

where W1 = the initial weight (g) of the sample, and W2 = weight (g) of the remaining fat after solvent evaporation.

2.3.2. Titratable Acidity and pH

The titratable acidity of milk (expressed as lactic acid %) was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator [22]. Different samples' pH was measured using a digital pH meter (HANNA HI 8314 Portable).

2.3.3. Minerals Determinations

Minerals were evaluated according to Milani et al. [23]. The evaluation was performed after the acid digestion of the samples. Briefly, to 0.5 g of samples in a Teflon tube, 1.6 mL of HCl (37%) and 1.7 mL of HNO_3 (65%) were added, and the tubes were left for 30 min, and then 1.7% of H_2O_2 (30%) was added to the system. The digestion proceeded with heating in a microwave, with a temperature ramp-up to 170 °C for 20 min, and the temperature was maintained at 170 °C for 15 min. The power ranged from 290 to 1800 W. At the end of the program, the samples were cooled to room temperature, and the solution was transferred to 25.0 mL conical tubes and filled with ultrapure water. The concentration of the elements was determined by a Perkin Elmer Optima 7300DV ICP OES (Inductively Coupled Plasma Optical Emission Spectrometry) (Waltham, MA, USA) under the following conditions: measuring power 1300 W, integration time of signal 1 s, plasma gas flow 15 L min^{-1}, auxiliary gas flow 1.5 L min^{-1}, nebulization gas flow 0.70 L min^{-1}, pumping rate of sample 0.70 mL min^{-1}.

2.3.4. Total Phenolic, Total Flavonoid Contents, and Antioxidant Activity

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent, according to Bettaieb et al. [24]. The total flavonoid content (TFC) was spectrophotometrically measured by forming a flavonoid–aluminum complex [25]. DPPH radical scavenging activity of samples was determined according to the modification mentioned by Mudgil et al. [26]. The decolorization of DPPH free radicals after scavenging was monitored by measuring the absorbance at 517 nm after 30 min of incubation at 37 °C using 96-well microplate reader (Multiskan Sky, Thermo Fisher Scientific, Cambridge, MA, USA). Briefly, 25 µL of each soluble nitrogen extract was mixed with 275 µL DPPH reagent (0.1 mmol L^{-1} in 95% methanol) in a 96-well microplate, left in the dark for 30 min, and measured at 517 nm in a microplate reader (BioTek, Winooski, VT, USA). Results are expressed as µm Trolox equivalent mL^{-1} sample.

2.3.5. Microbial Assays

During storage of the fermented samples, different types of agar mediums such as nutrient agar, MacConkey agar, malt extract Agar, MRS agar, and M17 agar were used to count the viable cells of total microbial, coliform, mold, and yeast, *Lactobacillus* spp. and *S. thermophilus*. The microbial counts (log CFU g^{-1}) were measured in triplicate.

2.3.6. In Vitro Glycemic Index (GI) and Glycemic Load (GL)

The glycemic index was estimated according to the method of [27] with some modifications. Briefly, 0.1 g of each sample was weighed into a 50 mL falcon tube. Then, 2 mL of HCl (0.05 M) containing pepsin (0.117 g mL^{-1}, Sigma, P3000) was added to the tubes, and the tubes were incubated at 37 °C in a shaking water bath for 30 min. Sodium acetate buffer (4 mL, 0.5 M, pH 5.2), 1 mL of enzyme solution containing 0.243 g pancreatin (Sigma, P3000), and 14.45 U (56 µL) Amyloglucosidase from Aspergillus Niger (260 U/mL, Sigma) were added to each tube. The tubes were incubated horizontally at 37 °C in a shaking water bath. Aliquots (100 µL) were taken into Eppendorf tubes at each 20 min interval of time during 160 min before being mixed with 1 mL of absolute ethanol. These solutions were centrifuged at 800× *g* for 10 min, and glucose content was measured with a glucose determination kit, GOD-PAP (Fortress Diagnostics limited, unit 2 °C Antrim Technology

Park, Antrim, UK), and the absorbance was detected using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 500 nm wavelength. After that, the absorbance values were plotted against time to draw the hydrolysis curve. To calculate the GI, the hydrolysis index (HI) of each sample was first calculated by using the following equation:

$$\text{HI} = \frac{\text{The area under the hydrolysis curve of the sample}}{\text{The area under the hydrolysis curve of white bread}} \quad (3)$$

Then, the in vitro GI was determined by using the following equation:

$$\text{GI} = 39.71 + 0.549\ \text{HI} \quad (4)$$

Finally, GL was calculated for each sample from the following equation:

$$\text{GL} = \frac{\text{GI} \times \text{available carbohydrate (g)}}{100} \quad (5)$$

2.3.7. Sensory Evaluation

The sensory evaluation analysis was applied to the different prepared fermented millet beverages during storage on days 1, 8, and 15. The descriptive analysis test described by Sameen et al. [28] was applied by 15 trained staff members of the College of Agriculture and Veterinary Medicine at Qassim University, Qassim region, Saudi Arabia. The distribution of 20 degrees according to color (2 points), appearance (1 point), flavor (10 points), odor (2 points), and sediment (5 points) for each sample was evaluated. A 50 mL sample was provided to each panelist in individual unidentified, transparent, disposable containers, and water at room temperature was offered to mitigate the influence of one sample on another.

2.3.8. Degree of Proteolysis by O-phthaldialdehyde (OPA) Method

The total free amino acids (mmol) of the BMB-FMB sample were determined by the OPA method as previously described [26].

2.3.9. Instrumental Color Analysis

Color changes in the products during storage on days 1, 8, and 15 were performed [29]. The Hunter Lab Minolta colorimeter with a 20 mm aperture set for illumination D65 at 100 standard observer angles was used for instrumental color determinations. In terms of the initial Hunter color, lightness (L*), redness (a*), and yellowness (b*) values were calibrated against white and black standard discs before analysis of the measurement for color change. For each sample, 10 mL was poured into a Petri dish (leading to a liquid layer approximately 1 cm high). Triplicate distinct readings at random positions on the sample surface for the 4 samples were conducted for all analytical determinations.

2.3.10. Statistical Analysis

Data are expressed as the mean ± standard deviation (SD). A randomized complete block design and analysis of variance of factorial methods were carried out using the SPSS program (ver. 22). All data were analyzed in three replications for each parameter except sensory evaluation, which had 15 replications. According to Koop et al. [30], results were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Physicochemical Characteristics of Different Millet–Milk-Based Preparations

3.1.1. Physicochemical Composition of Fresh FMBs

Moisture, TS, pH, and TA of millet–milk samples prepared in water (water-MM), whey (whey-MM), buttermilk (buttermilk-MM), and skimmed milk (skimmed milk-MM) are presented in Table 1. No significant differences were found for moisture and TS except for in the buttermilk-MM sample. The buttermilk-MM sample contained less TS and more water

content than all other samples. Significant differences ($p < 0.05$) in TA% were observed between all samples. The highest titratable acidity was given by the buttermilk-MM sample (0.45%), while the least titratable acidity was for the water-MM sample (0.06%). An inverse relation between TA% and pH values was found for all samples. The pH values were recorded to be 6.61 and 6.49 in water-MM and skimmed milk-MM, respectively, while it was 5.73 and 5.51 for buttermilk-MM and whey-MM, respectively.

Table 1. Chemical composition of prepared different-based millet–milk.

Millet–Milk	Moisture (%)	TS (%)	TA (%)	pH
Water-MM	94.49 ± 2.39^{b}	5.51 ± 2.39^{a}	0.06 ± 0.01^{d}	6.61 ± 0.01^{a}
Whey-MM	95.31 ± 0.21^{b}	4.69 ± 0.21^{a}	0.35 ± 0.01^{b}	5.51 ± 0.01^{d}
Buttermilk-MM	98.29 ± 0.70^{a}	1.71 ± 0.70^{b}	0.45 ± 0.00^{a}	5.73 ± 0.01^{c}
Skimmed milk-MM	95.55 ± 01.47^{b}	4.45 ± 1.47^{a}	0.31 ± 0.01^{c}	6.49 ± 0.01^{b}

Water-MM: water-based milk–millet, Whey-MM: whey-based millet–milk, Buttermilk-MM, buttermilk-based millet–milk, and Skimmed milk-MM: skimmed-milk-based milk–millet, $^{a,\,b,\,c,\,d}$: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters.

3.1.2. Changes in pH and Titratable Acidity (TA%) during Fermentation

It was observed that the pH values gradually decreased, and the TA% increased during fermentation in all samples (Table 2). Regarding the total fermentation time, the decrease in pH and increase in TA% were faster in the BMB-FMB sample than in other samples, reaching 4.7% and 0.50%, respectively, after only 1.5 h of fermentation. WB-FMB and WHB-FMB samples, as they took longer to ferment (2.5 h), in the second stage, had recorded pH 4.90 and 4.50 and TA% 0.15 and 0.54, respectively.

Table 2. Changes in pH and titratable acidity % during fermentation with a yogurt starter culture (YC-381) and *L. paracasei* subsp. Paracasei (mean ± SD).

Milk–Millet Type	Fermentation Time (h)					
	0.5	1.0	1.5	2.0	2.5	3.0
	pH					
WB-FMB	5.57 ± 0.03^{bA}	5.48 ± 0.01^{bA}	5.30 ± 0.02^{bB}	5.10 ± 0.01^{bC}	4.90 ± 0.1^{bD}	-
WHB-FMB	5.37 ± 0.02^{cA}	5.34 ± 0.02^{cAB}	5.30 ± 0.02^{bB}	5.10 ± 0.03^{bC}	4.50 ± 0.03^{cD}	-
BMB-FMB	5.31 ± 0.01^{dA}	5.36 ± 0.04^{cA}	4.70 ± 0.01^{cB}	-	-	-
SMB-FMB	6.26 ± 0.03^{aA}	6.16 ± 0.03^{aA}	6.00 ± 0.06^{aB}	5.80 ± 0.02^{aC}	5.11 ± 0.02^{aD}	5.00 ± 0.07^{aA}
	Titratable acidity %					
WB-FMB	0.07 ± 0.01^{dD}	0.09 ± 0.01^{dC}	0.13 ± 0.01^{dB}	0.15 ± 0.01^{cA}	0.15 ± 0.01^{cA}	-
WHB-FMB	0.37 ± 0.02^{bE}	0.40 ± 0.00^{bD}	0.42 ± 0.02^{bC}	0.46 ± 0.04^{aB}	0.54 ± 0.05^{aA}	-
BMB-FMB	0.47 ± 0.01^{aB}	0.48 ± 0.02^{aB}	0.50 ± 0.01^{aA}	-	-	-
SMB-FMB	0.33 ± 0.00^{cF}	0.36 ± 0.00^{cE}	0.39 ± 0.01^{cD}	0.41 ± 0.01^{bC}	0.44 ± 0.01^{bB}	0.49 ± 0.02^{aA}

^{a,b,c,d}: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters, ^{A,B,C,D,E,F}: there is no significant difference ($p > 0.05$) between any two means within the same row that have the same superscripted letters.

3.1.3. Chemical Composition of Fresh FMBs

Data presented in Table 3 show the chemical composition of different fermented millet–milk samples. The BMB-FMB and SMB-FBM samples contained higher contents of TS% than the others, with no significant differences ($p > 0.05$). The TS% of WHB-FMB was intermediate (8.47%) between both BMB-FMB (10.43%) and SMB-FBM (10.74%) and the millet beverage prepared in water (WB-FMB: 3.50%). The total protein and ash contents were significantly ($p < 0.05$) higher in SMB-FMB than in other samples. The carbohydrate content was highly significant in WHB-FMB compared to in other samples. The fat %

was highly significant in BMB-FMB compared to in other samples, while no significant difference ($p > 0.05$) was found in its percentage between WB-FMB and SMB-FMB.

Table 3. Chemical composition of different fresh fermented millet beverages.

Fermented Millet Beverage	Moisture %	TS %	TP %	Fat %	Ash %	Carbohydrates %
WB-FMB	96.50 ± 0.06 ^{a}	3.50 ± 0.06 ^{c}	1.08 ± 0.08 ^{d}	0.57 ± 0.08 ^{c}	0.56 ± 0.03 ^{c}	1.29 ± 0.08 ^{c}
WHB-FMB	91.53 ± 0.17 ^{b}	8.47 ± 0.17 ^{b}	1.67 ± 0.04 ^{c}	0.97 ± 0.03 ^{b}	1.03 ± 0.01 ^{b}	4.80 ± 0.18 ^{a}
BMB-FMB	89.57 ± 0.38 ^{c}	10.43 ± 0.38 ^{a}	2.88 ± 0.02 ^{b}	2.82 ± 0.03 ^{a}	1.05 ± 0.01 ^{b}	3.68 ± 0.37 ^{b}
SMB-FMB	89.26 ± 0.35 ^{c}	10.74 ± 0.35 ^{a}	4.34 ± 0.12 ^{a}	0.53 ± 0.03 ^{c}	1.28 ± 0.11 ^{a}	4.59 ± 0.50 ^{a}

WB-FMB: water-based fermented millet beverage, WHB-FMB: whey-based fermented millet beverage, BMB-FMB: buttermilk-based fermented millet beverage, SMB-FMB: skimmed-milk-based fermented millet beverage, ^{a,b,c,d}: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters.

3.1.4. pH and TA% of FMBs during Storage for 15 Days at 4 ± 1 °C

The changes in TA% of different fermented FMBs during cold storage (4 ± 1 °C) for 15 days are shown in Table 4. The longer the storage period, the greater the increase in TA% for all samples observed. The TA% was significantly higher after 15 days of cold storage than after 8 days for all samples except the BMB-FMB, which presented no significant difference between days 8 and 15 regarding the TA%. In addition, the pH value for all samples gradually decreased slightly as the storage period increased. The highest pH value after 15 days of cold storage (4 ± 1 °C) was for SMB-FMB (4.59), and the lowest was for WHB-FMB (3.82) samples.

Table 4. Changes in pH and TA% during storage (4 ± 1 °C/15 days) of different fermented millet–milk beverages.

Fermented Millet Beverage	Storage Period (Day)			
	Zero	**1**	**8**	**15**
	pH			
WB-FMB	4.90 ± 0.02 ^{abA}	4.90 ± 0.02 ^{aA}	4.21 ± 0.02 ^{bB}	3.99 ± 0.01 ^{cC}
WHB-FMB	4.50 ± 0.03 ^{cA}	4.50 ± 0.01 ^{cA}	4.22 ± 0.02 ^{bB}	3.82 ± 0.01 ^{dC}
BMB-FMB	4.70 ± 0.02 ^{bcA}	4.68 ± 0.01 ^{bAB}	4.67 ± 0.01 ^{aB}	4.32 ± 0.01 ^{bC}
SMB-FMB	5.00 ± 0.3 ^{aA}	4.70 ± 0.01 ^{bB}	4.64 ± 0.01 ^{aB}	4.59 ± 0.01 ^{aB}
	TA%			
WB-FMB	0.15 ± 0.01 ^{dC}	0.57 ± 0.00 ^{cB}	0.57 ± 0.01 ^{cB}	0.61 ± 0.01 ^{dA}
WHB-FMB	0.54 ± 0.03 ^{aC}	0.61 ± 0.01 ^{bB}	0.62 ± 0.01 ^{bAB}	0.64 ± 0.01 ^{cA}
BMB-FMB	0.50 ± 0.00 ^{bC}	0.73 ± 0.01 ^{aB}	0.82 ± 0.01 ^{aA}	0.80 ± 0.00 ^{bA}
SMB-FMB	0.49 ± 0.01 ^{cD}	0.52 ± 0.01 ^{dC}	0.81 ± 0.01 ^{aB}	1.04 ± 0.01 ^{aA}

^{a,b,c,d}: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters, ^{A,B,C,D}: there is no significant difference ($p > 0.05$) between any two means within the same row that have the same superscripted letters.

3.2. Viable Bacterial Counts (log CFU g^{-1}) of Different Bacterial Groups during Storage for 15 Days at 4 ± 1 °C

Regarding the microbiological determinations in the current study, no coliform and mold or yeast growth was found, indicating good manufacturing practices during the processing. In contrast, colonies on M17 and MRS plate agar were observed in most samples at the end of the storage period (after 15 days) at 4 ± 1 °C. To quantify the bacterial counts on M17 agar and MRS agar for different prepared millet–milk beverages during storage for 15 days at 4 ± 1 °C, the log CFU g^{-1} sample for each medium is presented in Table 5. Data presented in Table 5 indicate that, although there was bacterial growth on TC, M17, and MRS agar media in the water-based fermented millet–milk beverage (WB-FMB)

sample after one day of fermentation, viable bacterial counts after 8 and 15 days of cold storage were not detected.

Table 5. Viable bacterial counts (log CFU g^{-1}) enumerated on nutrient agar, M17 and MRS medium, during storage for 15 days at 4 ± 1 °C.

Fermented Millet Beverage	Total Bacterial Count			*Lactobacillus* spp.			*S. thermophilus*		
	Storage Period (Day)								
	1	8	15	1	8	15	1	8	15
WB-FMB	8.77 ± 0.04 ^{aA}	ND	ND	9.08 ± 0.04 ^{bA}	ND	ND	8.61 ± 0.42 ^{aA}	ND	ND
WHB-FMB	8.31 ± 0.01 ^{bB}	8.41 ± 0.004 ^{aA}	8.46 ± 0.01 ^{bA}	8.39 ± 0.02 ^{dB}	9.35 ± 0.01 ^{aA}	9.42 ± 0.01 ^{bA}	8.31 ± 0.02 ^{bC}	9.41 ± 0.02 ^{bB}	9.61 ± 0.06 ^{bA}
BMB-FMB	8.22 ± 0.02 ^{cC}	8.43 ± 0.01 ^{aB}	9.04 ± 0.02 ^{aA}	9.23 ± 0.01 ^{aC}	9.43 ± 0.02 ^{aB}	9.97 ± 0.01 ^{aA}	8.30 ± 0.03 ^{bC}	9.30 ± 0.17 ^{bB}	9.84 ± 0.06 ^{aA}
SMB-FMB	8.27 ± 0.03 ^{bcA}	7.62 ± 0.13 ^{bB}	7.65 ± 0.13 ^{cB}	8.67 ± 0.54 ^{cB}	9.40 ± 0.02 ^{aA}	9.35 ± 0.03 ^{bA}	8.25 ± 0.02 ^{bC}	9.88 ± 0.07 ^{aA}	9.15 ± 0.09 ^{cB}

$^{a, b, c, d}$: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters, ^{A,B,C}: there is no significant difference ($p > 0.05$) between any two means within the same row that have the same superscripted letters.

3.3. Minerals Content (mg 100 mL^{-1}) of Different Fresh Fermented Millet-Based Beverages

The results of the mineral content of the fermented millet–milk samples are presented in Table 6. Detected macro-elements (Calcium and Magnesium) contents were significantly ($p < 0.05$) higher in samples prepared on the base of whey (WHB-FMB) and skim milk (SMB-FMB) than on water (WB-FMB) and buttermilk (BMB-FMB). The least Calcium and Magnesium content was observed for the BMB-FMB sample prepared on the base of buttermilk, in which they registered 0.71 and 0.53 mg 100 mL^{-1}, respectively. Iron, Zinc, and Manganese, which belong to the micro-elements, were significantly higher in WB-FMB and WHB-FMB samples than in BMB-FMB and SMB-FMB samples.

Table 6. Macro- and micro-elements of different fermented millet–milk beverages.

Fermented Millet–Milk Beverage	Macro-Element (mg 100 mL^{-1})		Micro-Element (µg 100 mL^{-1})		
	Ca	Mg	Fe	Zn	Mn
WB-FMB	1.317 ± 0.016 ^{c}	0.711 ± 0.003 ^{c}	0.111 ± 0.012 ^{b}	0.023 ± 0.001 ^{b}	0.057 ± 0.0001 ^{a}
WHB-FMB	5.198 ± 0.065 ^{b}	1.536 ± 0.043 ^{a}	0.178 ± 0.020 ^{a}	0.043 ± 0.0006 ^{a}	0.005 ± 0.0003 ^{c}
BMB-FMB	0.709 ± 0.051 ^{d}	0.533 ± 0.0006 ^{d}	0.044 ± 0.0006 ^{c}	0.006 ± 0.0001 ^{d}	0.002 ± 0.000 ^{d}
SMB-FMB	5.334 ± 0.042 ^{a}	1.204 ± 0.007 ^{b}	0.027 ± 0.003 ^{c}	0.022 ± 0.0004 ^{c}	0.009 ± 0.0003 ^{b}

^{a,b,c,d}: no significant difference ($p > 0.05$) exists between any two means within the same column with the same superscripted letters.

3.4. Antioxidant Properties of Different Fermented Millet-Based Beverages during Storage for 15 Days at 4 ± 1 °C

3.4.1. DPPH Radical Scavenging Activity (%)

Data presented in Figure 1 show the changes in the DPPH radical scavenging activity % as affected by sample type and the storage period. After one day of cold storage, the highest DPPH radical scavenging activity % was detected for WHB-FMB (51.44 ± 0.31), while the lowest was for WB-FMB (39.69 ± 2.36). For all samples, the DPPH radical scavenging activity % was decreased after 8 and 15 days compared to their activities on the first day of storage. However, on days 8 and 15, the SMB-FMB (38.67 ± 2.00 and 43.65 ± 5.39, respectively) and BMB-FMB (35.94 ± 2.15 and 43.28 ± 8.23, respectively) had higher DPPH radical scavenging activity % than WB-FMB (28.05 ± 1.63 and 31.27 ± 2.59, respectively) and WHB-FMB (31.06 ± 0.38 and 35.88 ± 0.82, respectively) samples.

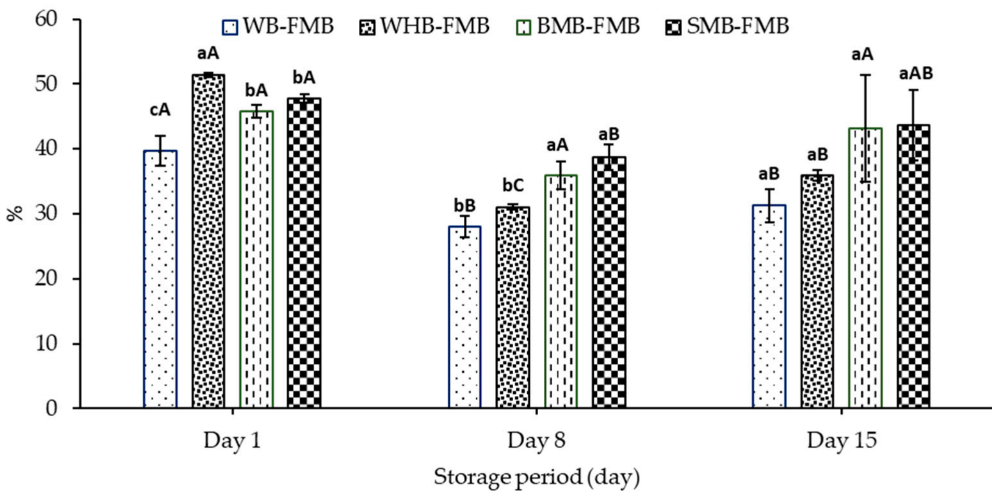

Figure 1. DPPH radical scavenging activity % of fermented millet–milk samples during storage for 15 days at 4 ± 1 °C. ^{a,b,c}: Bars do not share similar letters are differ significantly ($p > 0.05$) among treatments in each storage period. ^{A,B,C}: Bars do not share similar letters are differ significantly ($p > 0.05$) among treatments over storage periods.

3.4.2. DPPH, TPC, and TF of Different Fermented Millet-Based Beverages during Storage for 15 Days at 4 ± 1 °C

The DPPH radical scavenging activity (mM Trolox equivalent mL^{-1}), TFC (mg quercetin equivalent 100 g^{-1} DW), and TFC (mg gallic acid equivalent 100 g^{-1} DW) were determined for all fermented millet–milk beverages during storage for 15 days at 4 ± 1 °C, and the resulting data are presented in Table 7. The DPPH radical scavenging activity was significantly ($p < 0.05$) higher in SMB-FMB (65.79 mM Trolox equivalent mL^{-1}) and BMB-FMB (64.56 mM Trolox equivalent mL^{-1}) at 15 days of cold storage than in samples prepared on the base of water (36.74 mM Trolox equivalent mL^{-1}) and whey (47.56 mM Trolox equivalent mL^{-1}). During storage, the TFC and TPC of WB-FMB and WHB-FMB samples were significantly ($p < 0.05$) decreased by increasing the storage period. An inverse observation was made for BMB-FMB and SMB-FMB samples.

Table 7. DPPH, TFC, and TPC of different fermented millet-based beverages during storage for 15 days at 4 ± 1 °C (mean ± SD).

Fermented Millet Beverage	DPPH (μm TE g^{-1} dw)			TFC (μg QE 100 g^{-1})			TPC (μg GAE 100 g^{-1})		
	Storage Period (Day)								
	1	8	15	1	8	15	1	8	15
WB-FMB	56.50 ± 5.55 ^{cA}	29.20 ± 3.85 ^{bB}	36.74 ± 6.09 ^{bB}	2.11 ± 0.21 ^{aA}	0.53 ± 0.19 ^{aB}	0.097 ± 0.05 ^{bC}	155.94 ± 29.17 ^{aA}	22.87 ± 11.35 ^{bB}	7.12 ± 0.65 ^{bB}
WHB-FMB	84.06 ± 0.74 ^{aA}	36.13 ± 1.04 ^{bC}	47.56 ± 1.93 ^{abB}	1.37 ± 0.09 ^{bA}	0.73 ± 0.63 ^{aB}	0.11 ± 0.49 ^{bC}	22.25 ± 1.75 ^{cA}	7.53 ± 7.29 ^{bB}	2.96 ± 1.07 ^{bB}
BMB-FMB	70.89 ± 2.35 ^{bA}	47.69 ± 5.04 ^{aB}	64.56 ± 19.31 ^{aAB}	0.04 ± 0.03 ^{cB}	0.06 ± 0.06 ^{bB}	0.62 ± 0.33 ^{aA}	75.93 ± 15.72 ^{bA}	68.58 ± 14.98 ^{aA}	70.46 ± 12.92 ^{aA}
SMB-FMB	75.51 ± 10.77 ^{bA}	54.10 ± 4.70 ^{aB}	65.79 ± 12.65 ^{aAB}	0.21 ± 0.12 ^{cB}	0.71 ± 0.25 ^{aA}	0.75 ± 0.16 ^{aA}	17.29 ± 0.68 ^{cB}	46.74 ± 12.26 ^{aA}	69.38 ± 18.00 ^{aA}

$^{a, b, c}$: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters; $^{A, B, C}$: there is no significant difference ($p > 0.05$) between any two means within the same row that have the same superscripted letters.

3.5. In Vitro Glycemic Index (GI) and Hydrolysis Index (HI)

To evaluate the in vitro GI and GL related to different FMBs prepared under the current study conditions, Figure S2 shows the hydrolysis curves of different samples at 20 min time intervals during 160 min of digestion. Additionally, the in vitro GI and GL are presented in Table 8. Plotting glucose levels during 20 min time intervals for 160 min resulted in the hydrolysis curve for each sample (Figure 2). The hydrolysis curve for WHB-FMB and BMB-FMB showed that glucose levels (mg/dL) reached the highest point of the curve after 100 min of in vitro digestion, while in SMB-FMB and WB-FMB, it happened after about 70 and 60 min, respectively. Data presented in Table 8 show the intensity of releasing glucose

in vitro (GI) and the glycemic load (GL). The GI tells us how high blood sugar could rise with certain foods. Still, it does not tell how high our blood sugar will go when we actually eat the food. Thus, the use of GL for representing the quantity and quality of carbohydrates in the overall diet and their interactions in the body is encouraged. This depends on the categories of the GI (low GI: 55 or less, medium GI: 56–69, and high GI: 70 or higher) and the food GL (high GL: 20 or higher, medium GL: 11–19, and low GL:10 or less). So, the prepared fermented millet–milk samples were evaluated for their GI and GL (Table 8).

Table 8. In vitro glycemic index (GI) and glycemic load (GL) (mean ± SD).

Fermented Millet Beverage	GI	GL
WB-FMB	40.01 ± 0.01 ^{b}	0.52 ± 0.02 ^{c}
WHB-FMB	40.07 ± 0.03 ^{b}	1.93 ± 0.04 ^{a}
BMB-FMB	40.21 ± 0.02 ^{a}	1.48 ± 0.09 ^{b}
SMB-FMB	40.28 ± 0.02 ^{a}	1.85 ± 0.12 ^{a}

^{a,b,c}: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters.

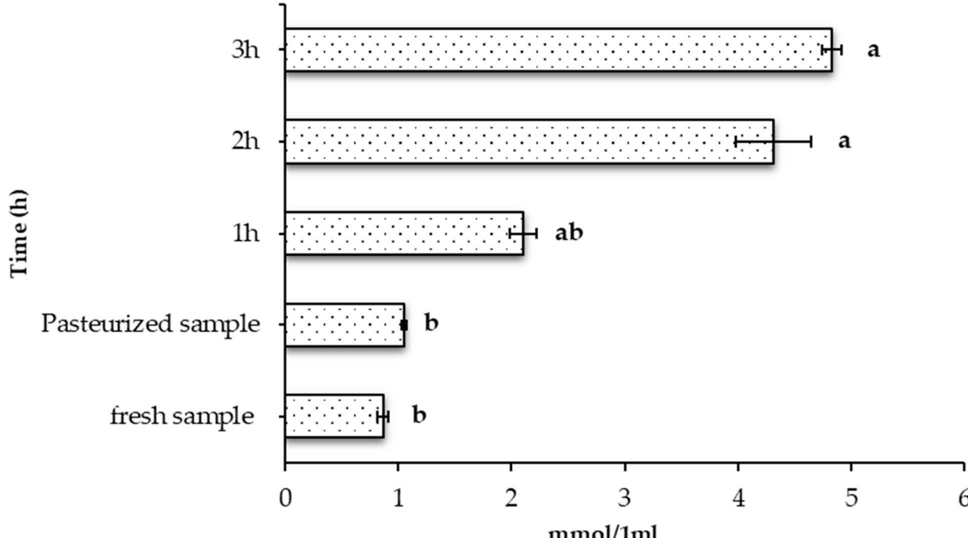

Figure 2. Proteolytic degree of buttermilk-based fermented millet–milk sample during fermentation by yogurt starter culture (YC-381) and *L. paracasei* subsp. *Paracasei.* ^{a,b}: Bars do not share similar letters are differ significantly ($p > 0.05$).

3.6. Sensory Evaluation of Different Fermented Millet-Based Beverages during Storage for 15 Days at 4 ± 1 °C

The descriptive analysis test, with a total of 20 scores depending on different sensory attributes, of FMBs during 15 days of cold storage at 4 ± 1 °C are tabulated (Table 9). The collected data show that, at the end of the storage period, the highest overall scores were recorded for SMB-FMB (13.25), then secondly for WHB-FMB (11.65), then for BMB-FMB (10.05), and, finally, for the WB-FMB sample (9.60). The total score of the BMB-FMB sample, which was processed on the base of buttermilk, gave a strange impression because it took the lowest flavor score among the samples. The reason behind the low scores in the flavor is that it seemed to have a bitter taste, and the bitterness was not significantly decreased by increasing the storage period. Evidence for this is the low scores for this sample in flavor after day 1 (5.15 out of 10), day 8 (5.7 out of 10), and day 15 (5.0 out of 10) of cold storage.

Table 9. Sensory attributes of different products during 15 days of storage at 4 ± 1 °C (mean ± SD).

Fermented Millet Beverage	Sensory Attributes								
	Color (2)			Appearance (1)			Flavor (10)		
	Storage Period (Day)								
	1	8	15	1	8	15	1	8	15
WB-FMB	1.25 ± 0.35 ^{bA}	1.05 ± 0.16 ^{bB}	1.00 ± 0.00 ^{bB}	0.70 ± 0.10 ^{bA}	0.70 ± 0.26 ^{bA}	0.65 ± 0.24 ^{bA}	6.70 ± 1.06 ^{bA}	5.95 ± 0.72 ^{bB}	5.40 ± 0.70 ^{bC}
WHB-FMB	1.25 ± 0.35 ^{bA}	1.10 ± 0.21 ^{bA}	1.10 ± 0.21 ^{bA}	0.71 ± 0.10 ^{bA}	0.75 ± 0.26 ^{bA}	0.70 ± 0.26 ^{bA}	7.75 ± 1.14 ^{aA}	7.40 ± 1.07 ^{aA}	6.80 ± 0.79 ^{aB}
BMB-FMB	1.90 ± 0.32 ^{aA}	1.70 ± 0.48 ^{aB}	1.70 ± 0.48 ^{aB}	0.97 ± 0.03 ^{aA}	1.00 ± 1.00 ^{aA}	1.00 ± 0.00 ^{aA}	5.15 ± 0.58 ^{bA}	5.70 ± 0.92 ^{bA}	5.00 ± 2.16 ^{cA}
SMB-FMB	1.90 ± 0.32 ^{aA}	1.85 ± 0.34 ^{aA}	1.60 ± 0.52 ^{aB}	1.00 ± 0.00 ^{aA}	1.00 ± 0.00 ^{aA}	1.00 ± 0.00 ^{aA}	8.10 ± 1.79 ^{aA}	7.80 ± 0.92 ^{aA}	7.00 ± 0.82 ^{aB}
	Odor (2)			Sedimentation (5)			Total score (20)		
WB-FMB	1.48 ± 0.56 ^{aA}	1.35 ± 0.53 ^{aAB}	1.15 ± 0.47 ^{aB}	2.80 ± 1.23 ^{bA}	1.60 ± 0.66 ^{cB}	1.40 ± 0.52 ^{bB}	12.92 ± 1.81 ^{cA}	10.65 ± 1.27 ^{cB}	9.60 ± 1.33 ^{cC}
WHB-FMB	1.30 ± 0.42 ^{aA}	1.50 ± 0.47 ^{aA}	1.35 ± 0.47 ^{aA}	3.10 ± 1.37 ^{bA}	2.10 ± 0.74 ^{bB}	1.70 ± 0.67 ^{bC}	14.11 ± 2.61 ^{bA}	12.85 ± 1.84 ^{bB}	11.65 ± 1.33 ^{bC}
BMB-FMB	1.23 ± 0.69 ^{aA}	0.55 ± 0.44 ^{bB}	0.40 ± 0.46 ^{bB}	3.90 ± 0.88 ^{aA}	2.10 ± 0.74 ^{bB}	1.80 ± 0.79 ^{bB}	13.00 ± 3.09 ^{cA}	11.05 ± 1.76 ^{cB}	10.05 ± 1.40 ^{cC}
SMB-FMB	1.40 ± 0.52 ^{aA}	1.55 ± 0.43 ^{aA}	1.35 ± 0.47 ^{aA}	3.80 ± 0.91 ^{aA}	2.70 ± 0.48 ^{aB}	2.30 ± 0.67 ^{aC}	16.20 ± 2.64 ^{aA}	14.90 ± 1.60 ^{aB}	13.25 ± 1.14 ^{aC}

^{a,b,c}: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters; $^{A, B, C}$: there is no significant difference ($p > 0.05$) between any two means within the same row that have the same superscripted letters.

3.7. Degree of Proteolysis in BMB-MM Sample at Different Time Intervals during 3 h of Fermentation

As a result of the highly proteolytic ability of starter cultures on milk proteins during fermentation, bitterness that appeared in the BMB-FMB was sensed during the sensory evaluation test. Thus, the proteolytic levels of buttermilk-based millet–milk samples (BMB-FMB) were monitored during fermentation to determine the amount of Tyrosine released over time during fermentation as an indicator of other amino acids released during fermentation, which may be behind the bitterness. The ongoing results are shown in Figure 2. As the fermentation time increased, the proteolytic degree increased, which could have a fetal effect when a bitter taste is observed. *L. paracasei* subsp. accompanied the fermentation of buttermilk-based millet–milk. *Paracasei* is a proteolytic strain [31]. So, to benefit from the action of those bacterial strains in increasing the release of antioxidants and to limit the bitterness, more study is needed to determine the fermentation time suitable for fermentation.

3.8. Color Changes of Different Fermented Millet–Milk Beverages during Storage for 15 Days at 4 ± 1 °C

In order to mask the bitter taste that appeared in the BMB-FMB sample and to sweeten the prepared fermented millet–milk beverages, the addition of sterilized Sukkari date powder solution (14%) was made to reach 4% date powder in each sample before blending. So, the changes in color parameters in samples as affected by the addition of Sukkari date powder were detected. As previously reported, red, green, blue, and yellow are the colors defined by hue (h), lightness (L*) is the parameter for color brightness, and chromaticity (C), or colorfulness, represents the color sensation, which all are measured using instrumental colorimeters [32]. Incorporating Sukkari date powder as a natural sweetener in the fermented millet–milk beverages positively affected their color when fresh or stored (Table 10). A significant decrease in lightening (L*) was observed in all samples containing Sukkari date powder compared to in the same samples with no Sukkari date powder added. However, this effect was reversed by cold storage as the color significantly ($p < 0.05$) turned lighter. A significant ($p < 0.05$) increase was also observed in the redness (a*) and yellowness (b*) in all samples that incorporated Sukkari date power. The relation between redness (a*) and yellowness (b*) was inverse for all samples with increasing storage period. The more the storage period increased, from 8 to 15 days under cold conditions (4 ± 1 °C), the more the significant the increase in redness and decrease in yellowness.

Table 10. Changes in the color of different fermented millet beverages stored for 15 days at 4 ± 1 °C.

Fermented Millet Beverage	L*			a*			b*		
	Storage Period (Day)								
	1	8	15	1	8	15	1	8	15
WB-FMB *	60.17 ± 0.14 ^{aC}	60.63 ± 0.30 ^{aB}	61.05 ± 0.62 ^{aA}	−2.34 ± 0.16 ^{aC}	−2.09 ± 0.03 ^{aB}	−1.82 ± 0.08 ^{aA}	3.89 ± 0.71 ^{bA}	3.52 ± 0.47 ^{bB}	2.95 ± 0.45 ^{bC}
WB-FMB **	49.14 ± 0.1.36 ^{bC}	50.84 ± 0.06 ^{bB}	51.48 ± 0.33 ^{bA}	−0.85 ± 0.68 ^{bC}	−0.81 ± 0.02 ^{bB}	−0.76 ± 0.06 ^{bA}	13.89 ± 1.13 ^{aA}	11.56 ± 0.13 ^{aB}	10.46 ± 0.08 ^{aC}
WHB-FMB *	67.60 ± 0.57 ^{aB}	69.20 ± 1.57 ^{aA}	69.43 ± 1.38 ^{aA}	−3.25 ± 0.16 ^{aC}	−3.18 ± 0.09 ^{aB}	−2.62 ± 0.05 ^{aA}	5.27 ± 0.14 ^{bB}	5.76 ± 0.36 ^{bA}	5.17 ± 0.48 ^{bB}
WHB-FMB **	55.57 ± 0.01 ^{bA}	55.64 ± 0.03 ^{bA}	55.84 ± 0.05 ^{bA}	−2.78 ± 0.06 ^{bA}	−2.82 ± 0.05 ^{bB}	−2.88 ± 0.03 ^{bC}	12.36 ± 1.11 ^{aA}	11.36 ± 0.02 ^{aB}	10.59 ± 0.07 ^{aC}
BMB-FMB *	76.63 ± 0.48 ^{aB}	76.81 ± 0.13 ^{aAB}	77.17 ± 0.32 ^{aA}	−3.62 ± 0.13 ^{aC}	−3.05 ± 0.01 ^{aB}	−2.60 ± 0.03 ^{aA}	8.63 ± 0.26 ^{bA}	8.05 ± 0.13 ^{bB}	7.13 ± 0.09 ^{bC}
BMB-FMB **	70.82 ± 0.40 ^{bB}	71.13 ± 0.16 ^{bB}	71.84 ± 0.04 ^{bA}	−1.58 ± 0.25 ^{bB}	−1.44 ± 0.04 ^{bA}	−1.73 ± 0.03 ^{bC}	14.35 ± 0.30 ^{aA}	14.49 ± 0.17 ^{aA}	14.15 ± 0.04 ^{aA}
SMB-FMB *	81.68 ± 0.87 ^{aB}	82.67 ± 0.03 ^{aA}	81.89 ± 0.04 ^{aB}	−4.25 ± 0.20 ^{aC}	−3.86 ± 0.02 ^{aB}	−3.18 ± 0.01 ^{aA}	5.36 ± 0.73 ^{bB}	5.89 ± 0.05 ^{bA}	5.27 ± 0.01 ^{bB}
SMB-FMB **	70.28 ± 0.09 ^{bB}	70.97 ± 0.13 ^{bA}	71.03 ± 0.14 ^{bA}	−3.07 ± 0.08 ^{bC}	−2.97 ± 0.03 ^{bB}	−2.95 ± 0.08 ^{bA}	9.33 ± 0.07 ^{aA}	8.87 ± 0.16 ^{aB}	8.58 ± 0.01 ^{aB}

*: without Sukkari date sugar, **: with Sukkari date sugar, ^{a,b}: there is no significant difference ($p > 0.05$) between any two means within the same column that have the same superscripted letters, ^{A,B,C}: there is no significant difference ($p > 0.05$) between any two means within the same row that have the same superscripted letters.

4. Discussion

The longest fermentation time was for the skimmed milk-MM sample, in which the pH was recorded as 5.00, and the TA% was 0.49% after 3.0 h of fermentation. These results were matched with the previous study made by [17]. In their study, they followed the acidity production during fermentation of a composite finger millet–dairy beverage, and they reported that the production of acid was significantly ($p < 0.05$) affected by the temperature of incubation, the addition of reconstituted skim milk, and type of the bacterial starter culture. In parallel with our results regarding the skimmed-milk-based sample, they found that the pH of skimmed milk during fermentation was not lowered sufficiently when the millet was added. The long fermentation time in the skimmed milk-MM sample is mainly associated with a higher protein content than other samples, which resulted in a higher buffering capacity [33]. In contrast, the shorter fermentation time for buttermilk-MM may be because the initial acidity of this sample before fermentation was the highest. In addition, the faster acidification rates of the buttermilk-MM may be due to the higher content of peptides or amino acids of lower molecular weight in buttermilk compared to in skimmed milk [34].

Changes in total solids values between samples are mainly due to the base type used for each beverage preparation. Thus, the amounts of total solids appeared higher in the BMB-FMB and SMB-FMB, followed by in the WHB-FMB, and, finally, in the WB-FMB. The same observation was previously found in [35]. The differences in protein values were observed due to the fortification of millet grains and fermentation. These factors enhanced protein bioavailability, improved digestibility, and improved starch hydrolysis, thereby improving glycemic response [2].

It is well known that lactic acid bacteria play a significant role in most fermented foods [36,37]. The lowering of the pH of the food brought about by lactic acid fermentation slows down or prevents its spoilage by other micro-organisms and will render the food safe from the growth of pathogenic micro-organisms [38,39]. In response, lactic acid bacteria (LAB) have been used as starter cultures in controlled fermentations, successfully contributing to product quality and safety, increased shelf-life, and improved texture and sensory properties, adding value to the product [40].

The microbiological observation is strongly related to the available nutrients for different starter culture strains used in the fermentation involved in the sample. The opposite observation was made for the remaining samples, as there was bacterial growth on TC, M17, and MRS selective media during 15 days of cold storage (4 ± 1 °C). The lack of growth in the WB-FMB sample may be because of insufficient nutrients available for the growth of starter strains in the water which was the basis of this sample. On the other hand, the chemical composition of BMB-FMB is rich in nutrients that accelerate the growth of the starter culture, as shown by the pH and TA% values. Adding pearl millet may increase the growth of initiator cells in general [41].

From the nutritional point of view, it was previously reported that millet grains are rich sources of phytochemicals and micronutrients with high antioxidant effects [42–44] which

can protect against the oxidative stress related to various chronic conditions, including cardiovascular disease, cancer, neurodegenerative disorders, arthritis, and diabetes [45]. These findings have increased interest in using millet grains as a portion of food sources due to their potential to produce healthy value-added products. Moreover, millet possesses various substances with antioxidant properties that are increased by processing techniques such as fermentation. The report of [46] could support the data collected during storage for the TFC and TPC. They summarized that, although different kinds of millet are rich in polyphenols with hypoglycemic effects, their contents change after different processing methods, including thermal treatments, roasting, milling, and fermentation, which may affect the final products' antioxidant and hypoglycemic properties. Previously, Singh [47] found that fermentation increased the content of biologically active ingredients and changed the ratio of nutritional to anti-nutritive constituents in millets. Moreover, [48] found that *Rhizopus azygoporus* fermentation significantly increased the polyphenols in pearl millet, including phenolic acids and flavonoids. They found that the polyphenol extracts of millet affected antioxidant and anti-inflammatory factors, the insulin signal pathway, and enzyme activities related to postprandial blood glucose in an in vivo study. During storage, *L. paracasei* subsp. *paracasei* may increase milk protein breakdown, causing the release of phenolic amino acids and non-phenolic compounds, which can interfere with TPC determination [49].

Hydrolysis curves indicated that millet–milk prepared in skimmed milk and buttermilk suppressed the increasing glucose levels during in vitro carbohydrate hydrolysis compared to in millet–milk samples prepared in whey and water. The bitter taste in the buttermilk-based fermented millet–milk beverage was related to the increase in fermentation time. The more the fermentation time increases, the more the proteolytic degree increases, which could have a fetal effect when a bitter taste is observed. *L. paracasei* subsp. accompanied the fermentation of buttermilk-based millet–milk. *Paracasei* is a proteolytic strain [31]. So, to benefit from the action of those bacterial strains in increasing the release of antioxidants and to limit the bitterness, more study is needed to determine the fermentation time suitable for fermentation.

Regarding the color property difference between fermented millet–milk beverages sweetened with Sukkari date powder, a previous study on the effect of Sukkari date powder on the color of fermented dairy beverages was evaluated [50], and their results were parallel to our findings. They found that, with increasing contents of Sukkari date powder, the value of L* (as an indicator for light vs. dark) decreased significantly, and the b* value (as an indicator for yellow vs. blue) increased.

5. Conclusions

Using dairy by-products is an economically and nutritionally sound method for creating beverages based on skimmed milk, sweet whey, or sweet buttermilk with plant materials. This study used three major dairy by-products (sweet whey, sweet buttermilk, and skimmed milk) and pearl millet grains to make dairy-fermented millet–milk beverages. Commercial yogurt starter culture (YC-381) containing *L. delbrueckii* subsp. bulgaricus, *S. thermophilus*, and a pure strain of *L. paracasei* subsp. *paracasei* was used in the preparation of the samples. Samples prepared with dairy by-products were compared to samples prepared with the ordinary method using water to produce the millet–milk beverage. Evaluation of the different samples during 15 days of cold storage revealed that using dairy by-products improved the quality of the beverages. The sensory acceptance and antioxidant properties were higher in samples containing dairy by-products, except for in the BMB-FMB sample, which had a bitter taste in fresh samples. The degree of proteolysis flow in the BMB-FMB sample indicated that the high proteolytic ability of starter cultures used in the current study was behind the presence of better taste. All samples had low glycemic index and glycemic load. Therefore, it can be concluded that fermented millet–milk beverages prepared using dairy by-products are more beneficial for antioxidant properties and sensory acceptance. It

is important to consider the type of starter culture strains used in the fermentation and the fermentation conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9110927/s1. Figure S1. Summary of the fermented millet beverages preparation. Figure S2. Hydrolysis curves plotted on 20 min time intervals during 160 min. of in vitro digestion.

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