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ORIGINAL PAPER

Peach pomace: a potential probiotic carrier for fiber enrichment in milk

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Abstract

Industrial fruit processing generates abundant by-products that can be recycled or reused as a source of dietary fiber (DF). In this study, we aimed to extract DF from peach pomace (PP), an industrial waste, using an eco-friendly method called microwave-assisted extraction (MAE). Physicochemical, antioxidant, and microbiological analyses were conducted on the DF samples. The DF exhibited a total phenolic content of 55.15 mg gallic acid/g and an antioxidant capacity of 62.5 mg Trolox/g. Microbiological analyses showed that all microorganism counts remained below the limit of detection. The potential prebiotic activity of the produced DF and commercial inulin was investigated under in vitro conditions, using *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* as a test probiotic bacterium. Milk samples with varying levels of DF were inoculated with the probiotic bacteria, and microbiological enumerations, physicochemical analyses were conducted for 48 days. No significant difference in the enumeration results of milk samples with different DF levels was observed during the storage period for both probiotic bacteria. On day 48, samples inoculated with *L. acidophilus* had a count of 8.33 ± 0.22 log CFU/mL with 0.5% DF, while samples inoculated with *B. animalis* ssp. *lactis* had a count of 8.63 ± 0.20 log CFU/mL with 0.5% DF. The pH values decreased, and the titratable acidity values increased with the concentration of DF during storage. This research demonstrates that PP is an important natural source for DF production, and the obtained DF enhances the metabolic activity of probiotic microorganisms, making it a potential functional product with prebiotic properties.

Keywords Peach pomace · Microwave · Dietary fiber · Milk · Prebiotic

Introduction

The consumption and processing of fruits generate many by-products including high amounts of valuable bioactive compounds such as antioxidants, phenolic compounds, carbohydrates and dietary fiber. The amount of fruit produced worldwide in 2020 has exceeded 900 million metric tons, resulting in large amounts of fibrous by-products called pomace (about one-third of total fruit production). The management and disposal of this by-product, which occurs in the fruit processing industry, is one of the biggest problems of the agro-fruit industries [1]. Pomace, rich in biodegradable organic ingredients and nutrients, is highly prone to enzymatic and microbial degradation and causes environmental pollution [2]. For this reason, many industries can utilize these by-products as renewable resources, which are low-cost and in abundance, to develop new functional ingredients, natural additives, and generate value-added products for reducing negative environmental impacts [3]. In addition to preventing environmental pollution by evaluating industrial wastes and transforming them into high-value-added products, other advantages such as reducing external dependency, generating commercial income, obtaining products having functional properties such as coloring agents and dietary fiber can be achieved [4, 5]. Recently, products having functional properties that are effective in the prevention of chronic diseases such as obesity, hypertension and

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diabetes have gained importance. One of these products is dietary fiber.

Dietary fiber (DF) states edible plant parts that cannot be completely digested by digestive enzymes. According to the definition by the Codex Alimentarius, DF is a carbohydrate polymer containing ten or more monomeric units that cannot be hydrolyzed by endogenous enzymes. DF consists of cellulose, hemicellulose, inulin, fructo-oligosaccharides, pectin, gums, resistant starch, polydextrose, dextrin, lignin, and other related components of plant origin [6]. It is an important food additive that is widely used because of its various positive effects on health such as reducing the risk of coronary heart disease, diabetes, and some forms of cancer, lowering blood sugar and blood pressure, giving a feeling of fullness for a long time, increasing intestinal motility and preventing the absorption of high cholesterol [7]. The physiological effects of DF vary depending on their structural and physical properties. Due to these differences, properties such as viscosity, water holding capacity, solubility and fermentability of DF also differ from each other [8]. In the classification of DF, especially its solubility comes to the forefront and the solubility of DF is related to its health benefits. In this sense, it is classified as soluble (SDF), which dissolves in water, or insoluble (IDF), which does not dissolve in it. Studies have reported that consumption of SDF reduces the risk of cardiovascular disease by lowering blood cholesterol [9]. Consumption of IDF reduces the risk of colon cancer by increasing the peristalsis of the colon and stool volume. Hence, it is very important to consume a balanced of both fractions of DF, soluble and insoluble, to obtain optimum benefits of these compounds [10]. The ratio between SDF and IDF fractions in fruits and vegetables is well-balanced. Especially, the antioxidant capacity of DF from fruits is high since it is in bound form with bioactive compounds such as polyphenols. At this point, it is thought that the consumption of obtained DF in the antioxidant-rich diet management, which has been the preference of consumers in recent years, will play an important role. In addition, DF increases metabolic interactions between bacterial species in the gastrointestinal microflora and allows them to be fermented. If this fermentation encourages the growth of beneficial bacteria in the colon, their prebiotic activity can be mentioned [11]. However, not all DFs have prebiotic properties. Pectin, resistant starch, some hemicelluloses, inulin, and mucilage have prebiotic activity due to fermentability [12]. DF is also used as a supplementary material in many products, such as meat products, beverages, and drugs to address issues like allergic reactions. Researchers have reported that adding these DFs to bread, breakfast cereals, cakes, cookies, beverages, meat, and dairy products has yielded favorable results. Supplementation with it can result in fitness-promoting foods, low in calories, fat, and cholesterol. Also, DF as a functional ingredient is used to improve physical and structural properties, such as texture, oil holding capacity, viscosity, sensory characteristics, hydration and shelf-life in the development of new products. Therefore, the extraction of DF from pomace becomes an important research topic. Many different methods are used in the extraction of DF from pomace including chemical, biological, ultrasound-assisted, enzymatic hydrolysis, combination methods and so on. Chemical extraction is the most widely used in the industry among these methods [13]. However, alternative methods are needed because they cause nutrient losses, structural changes and environmental pollution. One of these methods is microwave-assisted extraction as green technology.

Microwave-assisted extraction (MAE) is a process of utilizing electromagnetic waves with frequencies from 0.3 to 300 GHz as a heating source [14]. MAE has gained prominence in recent years due to its numerous advantages over other extraction techniques. This method stands out for its speed and efficiency, significantly reducing extraction times compared to traditional approaches. The application of microwave energy in MAE raises the solvent's temperature, thereby increasing the solubility of the target compounds and expediting the extraction process. This efficiency often leads to higher extraction yields, making MAE a preferred choice for researchers. Furthermore, the technique is environmentally friendly as it typically requires less solvent, thus reducing the environmental impact. MAE allows for fine-tuning of extraction conditions, enhancing selectivity for specific compounds, and is particularly effective in preserving the integrity of thermally sensitive substances. Safety considerations are also addressed as MAE employs a closed-vessel system, minimizing the risk of exposure to hazardous materials. The basic principle of this method is the conversion of electromagnetic energy absorbed by the sample into thermal energy to degrade the molecular structure of the cell wall. It was widely applied as a complementary method to extract some functional natural products and bioactive compounds from plant sources [15, 16]. In this sense, MAE was used to extract DF from different sources [17]. When the studies in the literature are examined, MAE provides an increase in extraction efficiency by supporting water permeability, which enables faster extraction of DF from plant materials [18].

In literature, peach pomace (PP) is reported as a possible source of DF. The peach is a well-known agricultural product, and it is used frequently in the food industry. A considerable part of peach is processed to produce juice, generating great volumes of by-products [19]. The by-products of peach are especially the outer parts of it, which are removed during processing at the cutting and pressing processes, and are essentially considered as stalks, peel, seed, and pomace. PP is a solid waste generated in high quantities every year (21.6 million tons in 2018) which may reach from 15 to 28% of raw material [20, 21]. The PP contains approximately 54%

total DF, 7.5% protein, and less than 3% fat as basic components in its composition [22]. PP becomes an important raw material to produce of DF due to its high content.

Recent studies have primarily focused on the content of DF in fruit and vegetable processing by-products, the production of bioactive compounds and DF from these product [23, 24] s. However, no study has been found to date that explores the production of DF from PP using MAE. Furthermore, there has been limited investigation into the prebiotic potential of this DF under various conditions. The aim of this study is to produce DF from PP, which is an industrial waste within the extent of waste utilization, and to evaluate the prebiotic activity of this DF in broth medium and in food medium as a supplement. The goal is to obtain commercially valuable products from the waste generated by the fruit juice industry. In the context of waste evaluation, it is believed that a high-value product can be derived from PP using MAE, a green technology, making this study compatible with economic practices focused on sustainable resource optimization. Moreover, it is expected that this research will contribute to scientific knowledge in the fields of food science, nutrition, and microbiology.

Materials and methods

Materials

The PP used in the study was provided by Dimes Food Ind. and Trade. Inc. (Turkey) and it was stored at − 18 °C in the laboratories of Tokat Gaziosmanpasa University Food Engineering Department until the production stage. Before production, the PP samples were thawed at 4 °C. The PP was corresponding to 74.4%, the peel was 19.8%, the stone and other parts were 5.8% of the fruit weight. The pH value of PP was 4.06 ± 0.02 and its titratable acidity was 1.07 ± 0.07% in terms of malic acid. The brix of PP was determined 11.0 ± 0.1% and it had 85.2% moisture content. The color values of the PP were *L** (brightness) 49.28 ± 0.12, *a** (redness) 8.92 ± 0.16, and *b** (yellowness) 37.37 ± 0.69. Inulin, whose prebiotic effectiveness has been previously proven [25], was provided by Smart Chemistry Ind. and Trade. Inc. (Turkey) to compare the prebiotic effectiveness of dietary fiber. The milk utilized in the research for investigating the potential use of DF obtained from PP as a prebiotic in the food matrix, was provided as Ultra-High Temperature (UHT) milk from Pınar Milk and Dairy Products Ltd. Inc. (Turkey).

Chemicals, media and reagents

Folin–Ciocalteu reagent (Merck, 1.09001, Germany); sodium carbonate (Na_2CO_3) (Merck, 106395, Germany); sodium hydroxide (NaOH) (Merck, 106498, Germany); potassium persulfate ($K_2S_2O_8$) (Merck, 105091, Germany); sulfuric acid (H_2SO_4) (Merck, 109981, Germany); gallic acid (Sigma, 842649, Germany); 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma, 194437, Germany); Trolox (Sigma, 648471, Germany) were used for chemical analyses. All chemicals used in this study were of analytical grade.

Peptone (Merck, 1.07224, Germany); Ethanol (Tekkim, TK200650, Turkey); Dichloran Rose Bengal Chloramphenicol (DRBC) Agar (Merck, 100466, Germany), Man, Rogosa and Sharpe (MRS) Agar (Lab M, LAB093, England); Plate Count Agar (PCA) (Lab M, LAB149, England); Man, Rogosa and Sharpe (MRS) broth (Lab M, LAB093, England) were provided for microbiological analyses. In addition, glucose-free MRS broth medium was prepared in laboratory with reference to [26]. The components used in the prepared medium are listed in Table 1.

Microwave-assisted extraction of dietary fiber

In the production of DF, the dehydration of samples was performed with a laboratory-scale microwave oven (Arcelik, MD554, 600 W, Istanbul, Turkey). Firstly, 50 g of PP was mixed with 80 mL of distilled water and stirred using a

Table 1 Glucose-free ingredients of MRS broth medium

Ingredients	Quantity	Brand
Peptone	10 g	Merck, 1.07224, Germany
Beef extract	10 g	Lab M, MC 019, UK
Yeast extract	5 g	Lab M, MC 001, UK
Tween 80	1 g	Merck, 8.22187.0500, Germany
Dipotassium phosphate	2 g	Merck, 1.05101.1000, Germany
Sodium acetate trihydrate	5 g	Tekkim, 6131-96-4, Turkey
Triammonium citrate	2 g	Alfa Aesar, 6132-04-3, Germany
Magnesium sulfate heptahydrate	0.2 g	Tekkim, 10034-99-8, Germany
Manganese sulfate tetrahydrate	0.05 g	Merck, 1.05999.1000, Germany
Distilled water	1 L	–

magnetic stirrer for 10 min. Following the mixing process, the samples were filtered through coarse filter paper. Then, 80 mL of distilled water was added to the filtrate, stirred for another 10 min, and the filtration process was repeated. This filtration step was performed twice. The resulting filtrate was spread on the glass apparatus in the form of a thin layer and dried in a microwave oven. The temperature and power values applied in the drying process were determined by preliminary experiments. The temperature and power values of the microwave oven applied in the drying process were increased gradually. In the first step of drying, it was started with 360 W for 3 min and then the power value was increased at 600 W for 3 min. After that, the power of the device was again decreased to 360 W and applied for 2 min. Finally, the power of the device was brought to 90 W and it was performed for 1 min and the drying process (70 °C, 5–7 h) was completed. The extraction process was carried out at the optimum point determined for extraction from preliminary trials [27]. The dried samples were powdered by passing through a laboratory grinder (Sinbo, SCM 2934, 110 Watt, Istanbul, Turkey) and stored until the analysis stage.

Prebiotic effect

Preparation of bacterial cultures

In this study, *Bifidobacterium animalis* ssp. *lactis* (*B. animalis* ssp. *lactis*) (5×10^9 CFU/g, Maflor B94, Istanbul, Turkey) and *Lactobacillus acidophilus* (*L. acidophilus*) were provided by Mamsel Pharmaceutical Ind. and Trade. Inc. and the microbiology laboratory of Tokat Gaziosmanpasa University Food Engineering Department (Tokat, Turkey), respectively. These microorganisms were cultured in MRS broth at 37 °C for 72 h under aseptic and anaerobic conditions. One loop of cultured media was transferred into 10 mL of fresh MRS broth and incubated at 37 °C for 24 h. The active cells were washed three times with 0.1% peptone water (PW, Merck, 1.07224, Germany). The cultures used in all analyses were used after washing in this way.

Prebiotic activity of extracted DF in vitro conditions

The prebiotic activity of the DF samples in the broth medium was investigated by *B. animalis* ssp. *lactis* (Maflor B94, Istanbul, Turkey) and *L. acidophilus*. In this section, glucose-free MRS broth medium was prepared [26], but without glucose and DF produced used as a carbon source instead of glucose. After sterilization under UV light for 15 min, DF samples were added to 10 mL glucose-free MRS broth medium at the ratio of 0.2%, 0.5%, 1%, 5% and 10% under aseptic conditions. A commercial MRS broth medium containing glucose was used for the control group. Finally, microorganism groups determined within the study were inoculated into the MRS broth medium and then incubated in anaerobic conditions at 37 °C. The cells in the cultures were enumerated for 5 days. Also, inulin, whose prebiotic efficiency has been proven by many researchers, was used as a carbon source to compare the prebiotic effectiveness of DF obtained from PP. Before the prebiotic activities were determined, total mesophilic aerobic bacteria (TMAB) [28], lactic acid bacteria (LAB) [26] and yeast & mold (Y&M) [29] counts were performed in order to determine the microbial suitability of DF and commercial inulin to be used in the study.

Prebiotic activity of extracted DF in milk

The prebiotic activity of the DF in the milk as food medium was determined by *B. animalis* ssp. *lactis* (Maflor B94, Turkey) and *L. acidophilus* (Food isolate-Tokat Gaziosmanpasa University Food Microbiology Lab., Turkey). After treatment with UV light for 15 min to decrease the initial microflora, 0.5%, 1%, and 5% DF were mixed separately with 180 mL UHT milk in the sterile glass jars under aseptic conditions. And then inoculated with *B. animalis* ssp. *lactis* and *L. acidophilus*, separately. Inoculated milk samples were stored at 4 °C for 48 days and samples were taken on the 0th, 6th, 12th, 24th and 48th days of storage. Firstly, 10 mL inoculated milk was mixed with 90 mL of 0.1% PW and homogenized by using a stomacher device (IUL, 707/470 Instruments, Spain) for 20 s. Appropriate dilutions were prepared from this homogenate by using 0.1% PW, and then inoculated into the MRS agar medium by spread plate technique. These microorganisms were incubated at 37 °C for 24–48 h in anaerobic jars (Oxoid, AN35US, United Kingdom). At the end of the incubation, cell counts were carried out and the results were given as log CFU/mL. In this experiment, the inoculated milk with both probiotic microorganism strains without DF was used as a positive control. As a negative control, an uninoculated milk medium containing 1% DF was used. This study was carried out in three replicates and two parallels.

Analytical determinations

Analyses of dietary fiber from peach pomace

Physicochemical analyses The pH, titratable acidity (TA), total dry matter (TDM), water activity (a_w) and color analyses of DF were determined according to the AOAC [30, 31]. pH values of the samples were analyzed by using a pH meter (WTW Inolab, Germany). TA was determined by titrimetric method and expressed as the percentage of malic acid (%). Total dry matter (TDM) was carried out by drying the weighed sample in a drying oven (Memmert 100-800, Germany). The a_w values of DF samples were performed with a water activity measurement device (AquaLab, 3TE, USA). Color measurements were analyzed in a Minolta colorimeter (Minolta CR-300, Japan). The color parameters L^* (darkness, lightness), a^* (redness, greenness), b^* (yellowness, blueness) were determined according to CIELAB color co-ordinates. All physicochemical analyses were performed in three parallels.

Antioxidant properties *Total phenolic compounds* The total phenolic compounds (TPC) of the samples were measured by the Folin–Ciocalteu method [32]. Approximately 500 µL of the sample was mixed with 2 mL of Folin–Ciocalteu reagent (10% v/v). This mixture was blended with 1 mL of Na_2CO_3 solution (7% v/v) and kept in a place without light at 25 °C for 30 min. At the end of this period, the mixture was measured at 760 nm wavelength (T80+, PG Instruments, United Kingdom). Standard curves were created from the concentrations of gallic acid using standard response to the absorbance values read at 760 nm. The concentration value of samples corresponding to the absorbance from the standard chart was calculated taking dilutions into account and defined as mg gallic acid/g.

Antioxidant activity The antioxidant activity of the DF samples was conducted in accordance with 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS). For the ABTS method, Trolox (±-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard. To determine antioxidant activity, 7 mM ABTS stock solution and 2.45 mM $K_2S_2O_8$ solution were blended with in equal proportions and then incubated in a dark place at 25 °C for 16 h. After that, 1 mL of this solution was mixed with 48 mL sodium acetate (20 mM sodium acetate, pH 4.5) as a buffer solution, and a 0.700 absorbance value was adjusted at 734 nm wavelength. Then 10 µL of extract and 2990 µL of adjusted solution were weighed and waited for 30 min at room temperature in the dark. After that the absorbance of extracts read at 734 nm. The obtained data were calculated on a standard curve as mg Trolox/L [33].

Water holding & oil adsorption capacities The extracted DF was mixed with distilled water and stirred for 1 h. After this process, the samples were centrifuged for 10 min at 4000×*g*. The precipitates remaining after separation of the supernatant were weighed and the water holding capacity (WHC) of the samples was calculated as gram water per gram dry sample.

For oil adsorption capacity (OAC), the extracted DF was mixed with virgin olive oil and stirred for 1 h. After this process, the samples were centrifuged for 10 min at 4000×*g*. The precipitates remaining after separation of the supernatant were weighed and the OAC of the samples was calculated as per gram olive oil for per gram dry sample.

Microbiological quality of DF The initial microflora of the DF was determined by enumeration of TMAB [28], LAB [26] and Y&M [29]. The samples (10 g DF) were homogenized using a stomacher device (IUL, 707/470 Instruments, Spain) for 20 s in 90 mL 0.1% PW. Appropriate dilutions were prepared from this homogenate by using 0.1% PW, and then inoculated into the medium determined in the reference methods according to analysis. Decimal dilutions were prepared using PW, followed by surface plating on PCA for TMAB counts, DRBC agar for Y&M counts, and MRS agar for LAB counts. The PCA plates were incubated at 30 °C for 48 h, while DRBC agar plates were incubated at 25 °C for 5 days, and MRS agar plates were incubated at 30 °C for 3 days within anaerobic jars [34]. Each experiment was replicated three times with two parallels.

Analyses of milk containing dietary fiber

The pH, TA, a_w, and color analyses [30, 31] were performed in the milk samples containing 0.5%, 1% and 5% DF at the 0th, 24th and 48th days of during the storage period.

Statistical analysis

The significant difference between the means of obtained analysis results was examined by ANOVA variance analysis and Tukey’s Test. All results were expressed as the mean ± standard deviation of three separate experiments. The results were evaluated with the SPSS statistical package program (SPSS 21.0 for Windows Version, SPSS Inc., Chicago, USA). The fit of the polynomial model equation was stated by the coefficient of determination R^2 and its statistical significance was checked by an F-test.

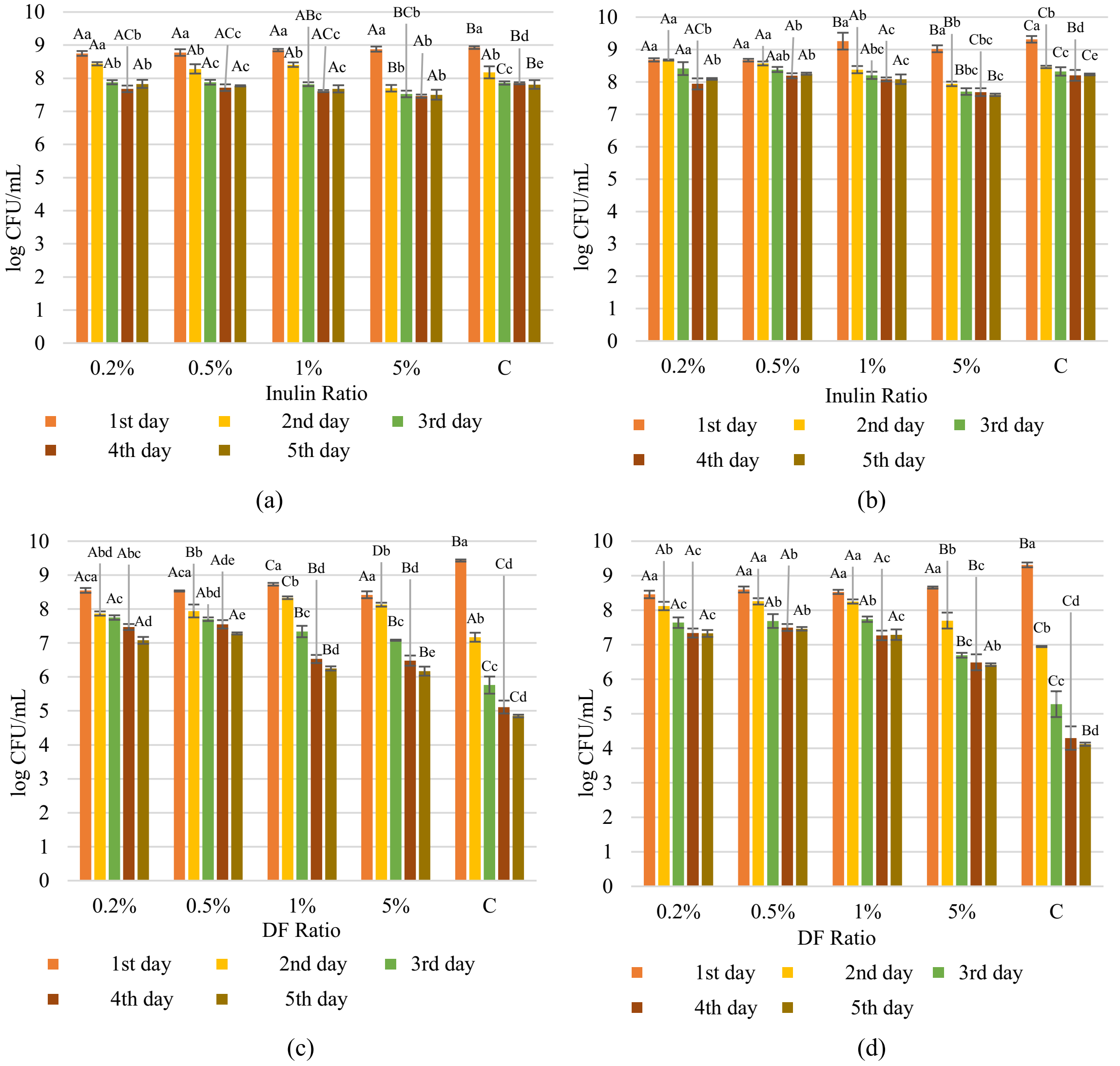
Results and discussion

Prebiotic effect

Prebiotic activity of extracted DF in vitro conditions

Prebiotics are aimed at increasing the survival rate and numbers of beneficial (probiotic) bacteria in the gastrointestinal system or in a symbiotic product [35]. In this study, the prebiotic effect of DF obtained from PP and commercially inulin was determined by using them as a carbon source substitute for glucose. TMAB, LAB and Y&M count results, which were carried out to determine the suitability of DF and commercial inulin, whose prebiotic activities will be determined, were found below the minimum detectable value (< 1 log CFU/g). *B. animalis* ssp. *lactis* and *L. acidophilus* were inoculated separately on glucose-free MRS broth with 0.2%, 0.5%, 1%, 5% and 10% DF added as carbon source to determine the prebiotic efficacy of DF in vitro conditions. In addition, the same analysis was performed under the same conditions with inulin, with proven prebiotic activity, to determine the prebiotic activity of DF. Enumeration results obtained on 5 days of incubation are given in Fig. 1.

It was determined that both *L. acidophilus* and *B. animalis* ssp. *lactis* were able to grow in different ratios of carbon sources as DF and inulin. Moreover, although probiotics were able to rapidly metabolize glucose in MRS media, bacterial growth was higher in media containing DF and inulin as carbon sources than containing glucose (control group). At the end of 5 days, the highest count value for *L. acidophilus* and *B. animalis* ssp. *lactis* as a living microorganism was obtained from media containing 0.5% DF. There was no statistical difference in enumeration results on the 1st day ($p > 0.5$), but there were statistical differences between the count results on the 2nd, 3rd, 4th and 5th days ($p < 0.5$) in the matter of *L. acidophilus* in media with DF. When the enumeration results of *B. animalis* ssp. *lactis* in media with DF were examined, no statistically significant difference was observed between different DF concentrations on the 1st day ($p < 0.05$), and the highest count value was obtained



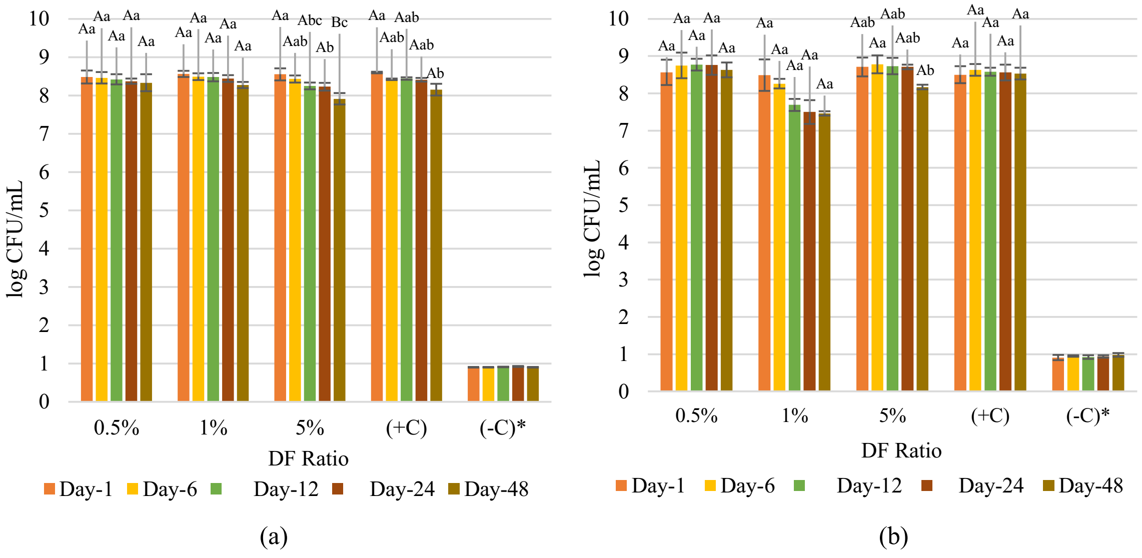
(a) (b) (c) (d)

Fig. 1 The changes of viable probiotic bacteria count (log CFU/mL) with different ratios of DF and inulin in-vitro conditions. **a** Count results of inulin inoculated with *L. acidophilus*; **b** count results of inulin inoculated with *B. animalis* ssp. *lactis*; **c** count results of DF inoculated with *L. acidophilus*; **d** count results of DF inoculated with *B. animalis* ssp. *lactis*. Y-axis represents colonies forming units per mL and the x-axis represents the inulin or DF ratio used in broth medium. C, control group (MRS broth with glucose as carbon source). Different letters mark significant differences ($p < 0.05$) among the variants of a given examples after 5 days of storage. For each storage day, different uppercase letters denote a significant difference ($p < 0.05$) between inulin or DF treatments. For each treatment of DF or inulin in broth medium, different lowercase letters denote a significant difference ($p < 0.05$) among storage days ($n = 4$) (Color figure online) from the media with 5% DF. Although bacterial growth was slower, the survival rate was higher in the presence of both DF and inulin compared to the control group with glucose. Our results showed that DF from PP can be used as prebiotics with lower concentrations compared to previous similar studies in the matter of inulin [36–38]. The counts of *B. animalis* ssp. *lactis* on the 5th day of inoculum were higher than *L. acidophilus* in commercial inulin and DF.

Prebiotic activity of extracted DF in milk

It is a known fact that diet has a significant impact on health and there is a strong relationship between them. Especially the positive effects of functional foods in the prevention and management of diseases have been proven [39]. Probiotics and prebiotics, one of the most important functional food ingredient groups, are widely used in milk and milk products [40]. Because the organoleptic properties of fermented milk products are significantly influenced by the proteolytic system in which LAB play a crucial role. LAB constitute a diverse group of bacteria known for their capacity to ferment sugars into lactic acid. This process, known as lactic acid fermentation, is an intracellular, anaerobic enzymatic mechanism for converting sugars into lactic acid. It represents one of the strategies used to generate the energy required for cellular life processes in anaerobic condition. The proteolytic system of LAB, composed of proteinases, peptidases, and specific transport proteins, is linked to the hydrolysis of casein, thereby supplying the cells with essential amino acids for their growth in milk. These essential amino acids and nitrogen components in milk, which bacteria need for lactic acid fermentation, are insufficient to meet all their requirements. As a result, these bacteria must break down milk proteins, primarily casein, to obtain these essential ingredients. Consequently, milk provides an ideal environment for the growth of probiotic microorganisms, leading to numerous studies exploring the use of LAB and other probiotics in various types of milk [41–43]. However, there have been no studies on the production of fermented milk using laboratory-produced DF as a carbon source. Therefore, in this study, milk samples with different proportions (0.5%, 1%, 5%) of DF were inoculated with *B. animalis* ssp. *lactis* and *L. acidophilus* probiotic bacteria to produce fermented milk and then the viability of probiotic bacteria was determined during the 48-day storage period. The enumeration results obtained during the storage process are given in Fig. 2.

According to Fig. 2, the selected probiotics grew on milk containing 0.5–5% DF which is used as carbon sources. However, as expected, the number of viable microorganisms in the negative control milk samples without probiotic microorganisms (− C) was below the minimum detectable limit (< 1 log CFU/mL). For *L. acidophilus*, there was no statistically significant difference between the milk groups with different concentrations of DF added and the control group on the 0th, 6th, 12th, and 24th days ($p > 0.05$).



(a)

(b)

Fig. 2 The changes of viable probiotic bacteria count (log CFU/mL) with different ratios of DF in UHT milk. **a** Count results of DF inoculated with *L. acidophilus*; **b** count results of DF inoculated with *B. animalis* ssp. *lactis*. The Y-axis represents colonies forming units per mL and the x-axis represents the DF ratio used in milk. (+C), probiotic-fortified UHT milk (positive control); (− C), UHT milk fortified with 1% DF (negative control); *: less than 1 log CFU/mL. Different letters mark significant differences ($p < 0.05$) among the variants of given examples after 48 days of storage. For each storage day, different uppercase letters denote a significant difference ($p < 0.05$) between inulin or DF treatments. For each treatment of DF or inulin in broth medium, different lowercase letters denote a significant difference ($p < 0.05$) among storage days ($n = 4$) (Color figure online)

However, statistical differences were determined between the milk samples containing 5% DF and the control group during storage ($p < 0.05$). It was concluded that the addition of DF at different ratios did not statistically affect the enumeration results of *L. acidophilus* between applied DF concentrations and storage days ($p > 0.05$). Probiotic foods must have a carrier matrix that supports maintaining probiotic viability in a sufficient population (> 10^6 CFU/mL or g) throughout the shelf life of the product. From this point of view, it was concluded that milk samples containing DF had a suitable carrier matrix for both probiotic microorganisms. When the counting results were examined, a decrease of viable probiotic microorganisms were observed depending on time in all sample groups. In a similar study [44], the effect of passion fruit and buriti fruit pulp on the growth of starter lactic acid and probiotic bacteria was examined and the pulp addition on bacterial growth was found to be statistically insignificant. Another study [45] focuses on the bio accessibility of bioactive compounds in fruit and vegetable by-products, including their prebiotic potential. There are also similar studies in the literature examining the effect of fruit pomace on the growth of probiotic bacteria and their prebiotic potentials [46, 47].

Table 2 Analysis results of dietary fiber obtained from peach pomace

Analyses	Dietary fiber
Physicochemical analyses	
pH	3.98 ± 0.01
Titratable acidity (TA%)	0.12 ± 0.00
Total dry matter (TDM)	90.79 ± 0.09
Water activity (a_w)	0.46 ± 0.02
Color analyses	
*L**	65.52 ± 0.26
*a**	3.92 ± 0.21
*b**	35.26 ± 0.09
Antioxidant properties	
Total phenolic content (TPC)	55.15 ± 3.70
Antioxidant capacity (ABTS)	62.50 ± 13.18
Water holding & oil adsorption capacities	
Water holding capacity (WHC)	19.30 ± 0.17
Oil holding capacity (OHC)	2.43 ± 0.25
Microbiological analyses	
Total mesophilic aerobic bacteria (TMAB)	–[a]
Lactic acid bacteria (LAB)	–
Yeast and mold (Y&M)	–

TA malic acid %; *TDM* %; *ABTS* mg Trolox/g; *TPC* mg gallic acid/g dry sample; *WHC* g/g; *OHC* g/g; *TMAB* log CFU/g; *LAB* log CFU/g; *Y&M* log CFU/g

[a] Less than 1 log CFU/g

Analytical determinations

Analyses of dietary fiber from peach pomace

Physicochemical analyses Analysis results of physicochemical, color, antioxidant, WHC, OAC, and microbiological analyses are shown in Table 2. The pH value was 3.98 ± 0.01 for the DF samples. It is [48] reported a similar pattern where the pH values of DF samples obtained from peaches harvested in different months were 3.96–4.10. In another similar study carried out with DFs extracted from different fruits and vegetables, the pH values of them were varied between 3.90 and 6.48 depending on the type of raw material [49]. The titratable acidity of DF was 0.12 g/L in terms of malic acid. Considering the similar studies on this subject, pH and TA values obtained within the scope of present study were within the expected value ranges [50]. Dry matter content is one of the major quality parameters of materials and differs according to the kind of raw material, cultivation conditions and processing parameters [51]. TDM of DF was confirmed as 90.79%. It was found that the TDF of PP was 13.96%. It was determined that there was a significant increase in the TDM value with the drying process applied to the pomace for the production of DF [52]. Water activity (a_w) is an important factor affecting the texture, rehydration properties, and structure of products. Also, it is very important to control the stability of powder products in terms of microbial, chemical and physical properties (lipid oxidation, browning) during storage [53]. The substances with higher a_w value tend to support the growth of microorganisms and every microorganism has a limit value which it will not grow. The a_w value of DF was specified as 0.46. Since there is no microbial proliferation below 0.6 a_w value, this product is microbiologically safe, and it has adequate storage stability from a safety viewpoint [54]. The color of DF was determined numerically in color analysis. The values obtained for the color parameters *L** (lightness), *a** (positive values, red color), and *b** (positive values, yellow color) of DF were determined 65.52 ± 0.26, 3.92 ± 0.21 and 35.26 ± 0.09, respectively. The lightness of DF was significantly higher. The *a** value above zero confirm that the red tone is dominating over the green in sample. The *b** value above zero affirms that the yellow tone is more dominant than the blue tone in DF. It is thought that the reason for the positive *a** and *b** values and especially the higher *b** value of DF is due to the fact that it is rich in carotenoid component, which gives yellow/orange color to the peach fruit. De Escalada Pla et al. [55] pointed out that *b** values of DF from PP by hot air drying and freeze-drying methods were 29.39 and 34.00, respectively [73]. In addition, the results in this section of the study have complied with the research results of about the DF content of apple pomace [56].

Antioxidant properties Epidemiological studies states that a diet rich in phytonutrients significantly reduces the risk of cardiovascular diseases and many sorts of cancer. This suggests that some dietary antioxidants may be effective agents that reduce cancer incidence and mortality [57]. Antioxidative compounds prevent free radical oxidation reactions and cell damage due to their potential antioxidant properties. They also contribute to the anti-inflammation capacity of human beings. For this reason, it is very important to determine the antioxidant composition of the food materials [58]. TPC and antioxidant capacity analyses are performed to determine antioxidant properties.

Studies in the literature show that fruits and vegetables are a rich source of polyphenols, including phenolic acids and anthocyanins [59]. The most common polyphenolic compounds in fruits are anthocyanins and polymeric tannins. Gallic and ellagic acids are the most common phenolic groups detected after a possible hydrolysis process, depending on the research [60]. These compounds are also present in the composition of DFs. While the IDF contains between 1.4 and 50.7% of phenolic substances on a dry weight basis, SDF contains between 2.9 and 62.8% [61]. TPC of DF was confirmed as 55.15 mg/g gallic acid equivalent in this study. It is also reported a similar pattern where the total phenolic content was 62.5–72.6 depending on the type of DF sample obtained from grape pomace [62]. ABTS radical cation scavenging method was used to specify the antioxidant capacity of the DF sample in this study. The antioxidant capacity of DF was defined as 62.5 mg Trolox/g. The ABTS value in this part of the study correlates with similar research results from different DF sources [63, 64]. In addition, when the studies are examined, especially at high temperatures and long-term drying processes cause the breakdown of antioxidants such as phenolic compounds. Studies have shown that antioxidant compounds become more sensitive to heat treatment, especially at drying temperatures above 60 °C [65]. In a study with chokeberry (*Aronia melanocarpa*) pomace, the exposure of the sample to high temperatures of up to 140 °C, along with mechanical treatment, did not result in significant alterations in the structure or content of DF. Nevertheless, it led to a notable reduction in the initial total polyphenol content, decreasing it by approximately 40%, ultimately reaching a final content of 3.3 ± 0.5 g/100 g [66]. Therefore, the microwave drying method applied in this study ensured that losses due to the breakdown of compounds were minimized.

Water holding & oil adsorption capacities DFs used in food product formulations have a number of functional properties such as gelling, water binding, and thickening, and are also used as potential fat replacers [67]. The functionality of carbohydrate-based DFs is stated because of providing the appropriate texture and viscosity for the product, increasing the WHC and OAC. WHC is identified as the quantity of water held within the DF matrix without any external force being applied. WHC is vital as it directly influences a food item's ability to retain moisture, which is often synonymous with palatability. A high WHC value indicates for the DF's ability to bind water, which can promote feelings of fullness and help manage weight. Additionally, it plays a significant role in digestive health by providing bulk and softening stools, aiding in regular bowel movements [17, 68]. Fibers that have high WHC can be used as a functional ingredient, especially in the production of diet foods, to prevent syneresis and to increase the consistency of the final product and give the product the desired texture [69]. OAC is a parameter that reflects the ability of fiber to retain oil and is considered in stabilizing high-fat products in food processing [6]. OAC is crucial, particularly in applications like frying and deep frying. OAC analysis is equally significant as it reflects the ability of DF to interact with lipids, particularly in food applications. This property can influence the texture, mouthfeel, and overall sensory attributes of food products. Moreover, it has implications for the control of fat digestion and absorption, making it relevant for managing dietary fat intake and its effects on health [68]. OAC values change depending on the source of DF, polysaccharide structure, particle sizes, and applied extraction conditions [70]. The value of WHC was 19.30 g of water per gram of dry fiber and the value of OAC was 2.43 g of oil per gram of dry fiber for DF extracted in this study. In a study focused on the extraction and characterization of DF and polyphenolic compounds from red grape pomace, a by-product of grape fruit processing, the DF exhibited WHC values ranging from 1.91 to 4.23 g water/g dry fiber, while the OAC values varied between 0.59 and 0.65 g oil/g dry fiber [71]. In another similar study conducted by De Moraes Crizel et al. [65], including extractions of DF from orange by-products, the WHC and OAC values were determined in order of 8.71 and 3.50 g/g. However, the WHC and OAC of the extracted DF in this study were higher than the values confirmed for orange DF samples [72]. It is thought that the reason for this difference in the analysis results was the different raw materials used in the production of DF and the different extraction conditions applied.

Microbiological analyses The initial microflora of DF extracted from peach pomace was tested. For this purpose, enumeration of TMAB, LAB, and Y&M were performed. TMAB, LAB, and Y&M counts were below the limit of detection (≤ 1 log CFU/g). No microbial growth was observed due to the a_w value of the DF sample within the study. Considering the composition of PP, it offers very appropriate medium conditions for microorganism growth. With the production of DF from this pomace, unfavorable conditions are created for microorganisms, and food safety is ensured.

Analyses of milk containing dietary fiber

When the milk groups containing DF at different concentrations were compared with the positive control groups consisting of only probiotic bacteria, no statistically significant differences were observed in the count results. Therefore, in addition to the microbial analyses related to the prebiotic activity of the DF used, the physicochemical analysis results have become more important. In this study, pH, TA (expressed as lactic acid), a_w, and color (L^*, a^*, b^* values) analyses were performed on milk samples that contained different concentrations of DF and were inoculated with *L. acidophilus* and *B. animalis* ssp. *lactis* on days 0, 24, and 48. The change in the analysis results of milk samples containing different concentrations of DF and inoculated with *L. acidophilus* during the storage period is presented in Fig. 3, while the change in the analysis results of milk samples inoculated with *B. animalis* ssp. *lactis* is shown in Fig. 4.

It is mentioned in the literature that for a DF to be classified as prebiotic, it should positively influence the host's immune system, provide inhibition of pathogenic bacteria, and enhance the formation of short-chain fatty acids. An increase in the formation of fatty acids is expected to lead to an increase in acidity and a decrease in the pH of the medium. The moderately decreased pH, while not extremely low, allows for various trophic effects in the gut microenvironment, leading to positive outcomes for human health [11, 73]. Therefore, pH and TA analysis results are significant importance.

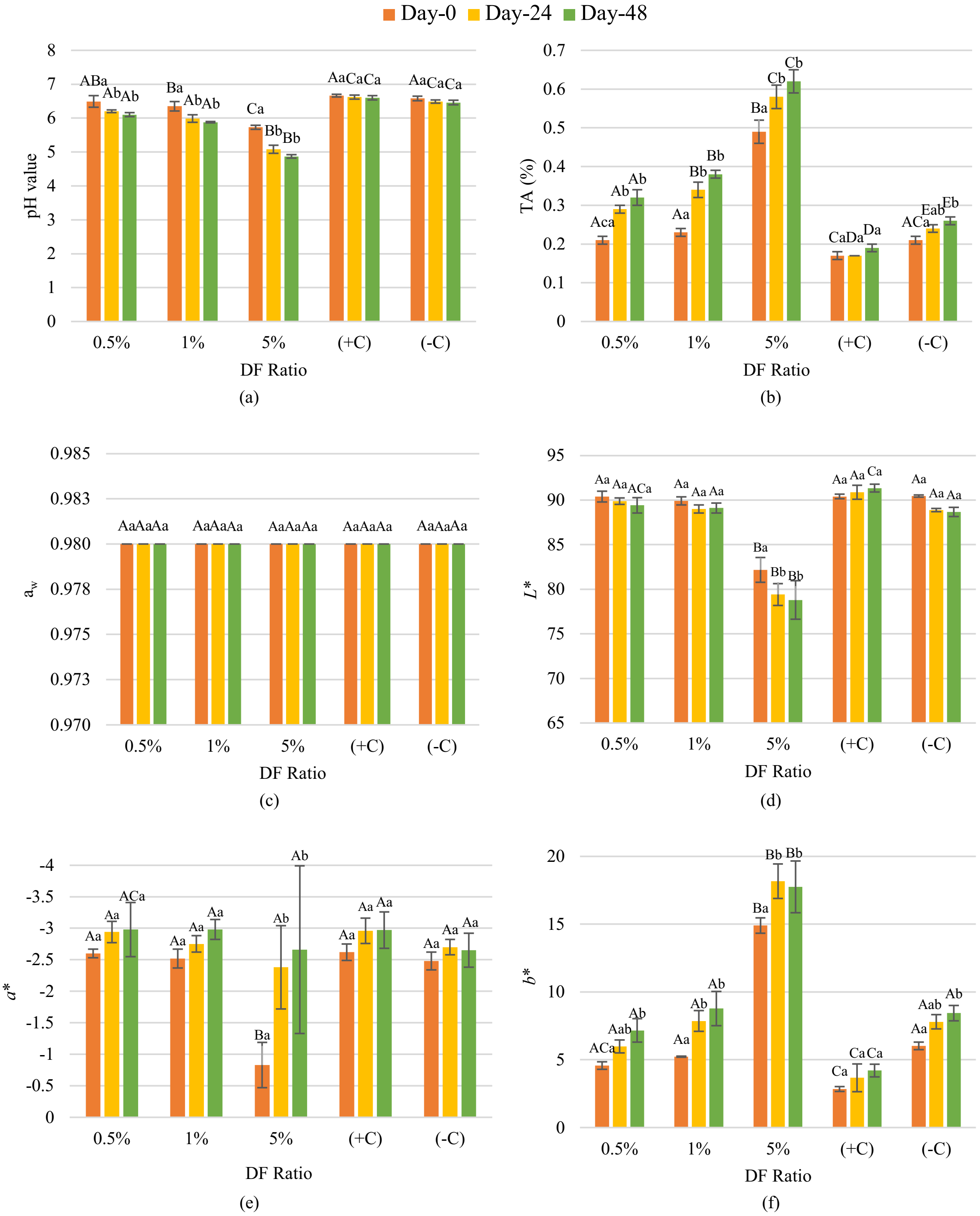
When the TA values in milk samples inoculated with *L. acidophilus* and containing different concentrations of DF were examined, it was observed that there was a rapid increase in titration acidity values (Fig. 3b). Furthermore, it was found that as the concentration of dietary fiber increased, the TA values also increased. Specifically, the TA values of milk samples containing 5% DF are higher compared to other concentrations and control groups, and the difference between them is statistically significant ($p < 0.05$). During the storage period, no statistically significant change is observed in the TA value of the positive control group ($p > 0.05$), while significant differences are observed in the groups containing DF ($p < 0.05$). The reduction in carbon sources of DF facilitated the growth of probiotics, consequently decreasing the pH of the medium. Therefore, an increase in the DF content has resulted in a decrease in the pH values of the samples.

When considering the a_w values of milk samples (Fig. 3c), it was determined that there was no statistically significant difference between DF concentrations and a_w values ($p > 0.05$). The color parameters L^* (darkness, lightness), a^* (redness, greenness), and b^* (blueness, yellowness) were determined according to CIELAB color co-ordinates. During the storage period, it was observed that there were statistically significant differences in the L^* color values among milk samples containing different DF concentrations ($p < 0.05$) (Fig. 3d). a^* parameter is relative to the green–red colors, with negative values toward green and positive values toward red and a^* values of milk samples in the present study were negative (Fig. 3e). It is believed that these negative a^* values are a result of the contribution of silage or green pasture consumption to the riboflavin content in milk. Riboflavin pigment is green, and an increase in riboflavin concentration would lead to the development of milk greenness [74]. Statistically significant differences were observed during the storage period in the b^* values between milk samples containing different concentrations of DF and the control groups ($p < 0.05$) (Fig. 3f).

Similar to literature, samples inoculated with *L. acidophilus*, there was a decrease in pH values and an increase in TA values in milk samples containing different ratios of DF and inoculated with *B. animalis* ssp. *lactis* (Fig. 4a, b) [44, 75]. However, it had been observed that in samples fermented with *B. animalis* ssp. *lactis*, when particularly compared to *L. acidophilus*, there is a faster decrease in pH values and an increase in TA values. Upon examining the color analysis results for L^*, a^*, b^* parameters, it was determined that there were statistically significant differences between the sample and control groups (Fig. 4d, f) ($p < 0.05$). The b^* values, which represents the yellowness of the samples with positive values, were increased with an increase in the DF content. In similar studies, it has been observed that the addition of fibers to the product also affects its color. In the study conducted by Hashim et al. [76], DF extracted from dates was added to yogurt samples at different ratios. It was observed through color analysis that over storage time, the L^* value decreased while the a^*, and b^* values increased. In other similar studies, it has been reported that yogurt samples supplemented with orange fiber and apple fiber resulted in a yellowish-brown color, while yogurt samples supplemented with asparagus resulted in a yellow-green color [77].

Conclusions

In this research, DF was obtained from PP using the MAE method as a green technology, and the prebiotic activity of the obtained DF was investigated. The ABTS, TPC, WHC, and OAC values obtained through the physicochemical analysis of the DF have proven the high quality and potential of PP as a source of DF. In the first stage of the study, the microbial suitability of laboratory-produced DF and commercial inulin, a prebiotic with proven activity, through TMAB, LAB, and Y&M microbiological analyses. All microbial analysis results remained below the detectable limit (< 1 CFU/g). In the second stage, the prebiotic activities of commercial inulin and DF at different concentrations



(a) (b)

(c) (d)

(e) (f)

Fig. 3 Physiochemical analysis results of fermented milk with *L. acidophilus*. **a** pH value; **b** TA, lactic acid %; **c** a_w; **d** *L** value (color); **e** *a** value (color); **f** *b** value (color). Y-axis represents analysis results for each sample and the x-axis represents DF ratio used in milk. (+C), probiotic-fortified UHT milk (positive control); (− C), UHT milk fortified with 1% DF (negative control). For each storage day, different uppercase letters denote a significant difference ($p < 0.05$) between DF treatments. For each treatment of DF in milk medium, different lowercase letters denote a significant difference ($p < 0.05$) among storage days (n = 3) (Color figure online)

were examined in vitro conditions. Both DF and inulin exhibited better prebiotic potential than the control group, with probiotic microorganisms, *L. acidophilus* and *B. animalis* ssp. *lactis*, remaining viable for an extended period. In the third stage, the prebiotic activities of DF and inulin were evaluated in milk as a food matrix in the same conditions. When considering the viable count of probiotic microorganisms in prebiotic products should be above 10^6 CFU/

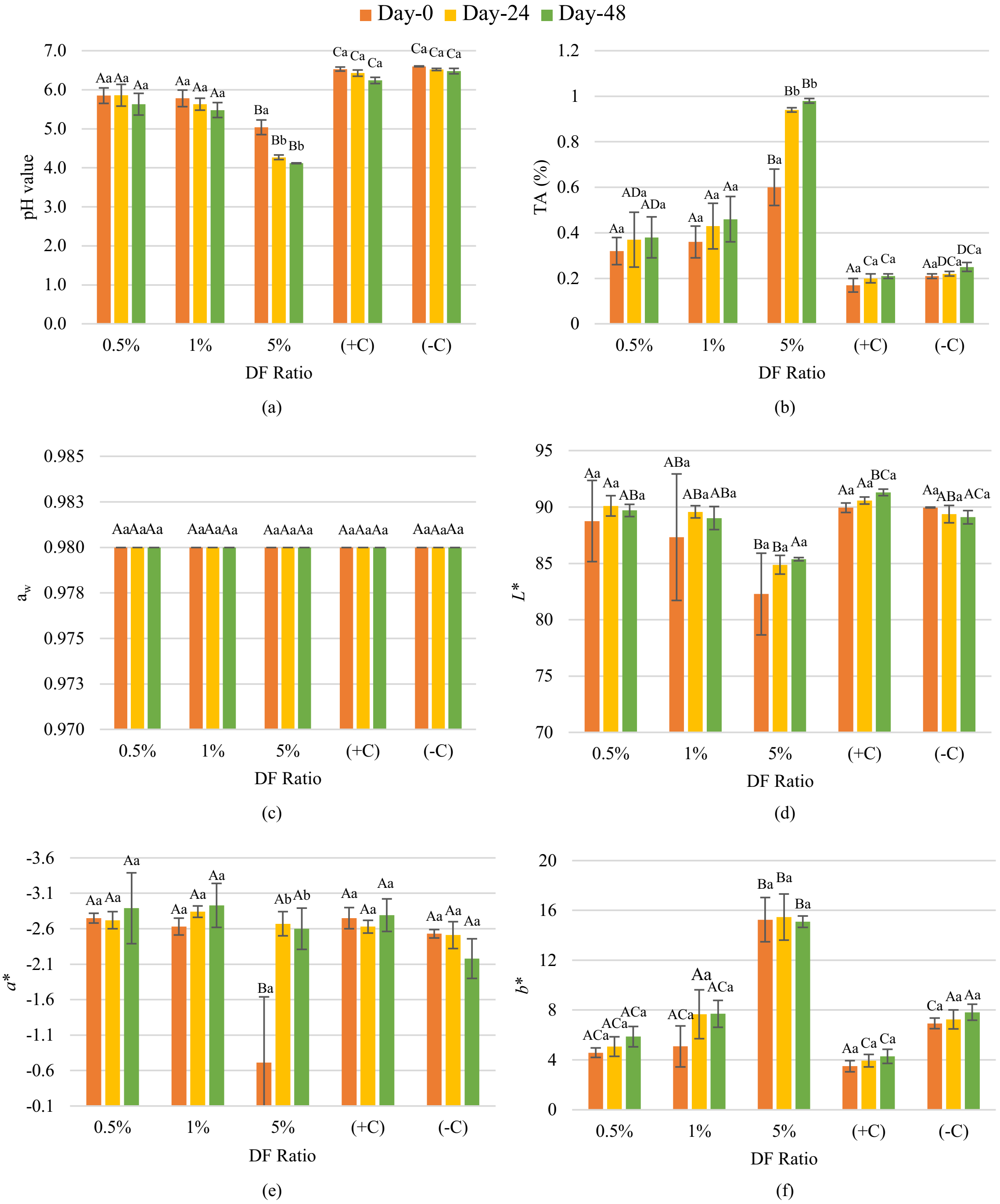


Fig. 4 Physiochemical analysis results of fermented milk with *B. animalis* ssp. *lactis*. **a** pH value; **b** TA, lactic acid %; **c** a_w ; **d** *L** value (color); **e** *a** value (color); **f** *b** value (color). Y-axis represents analysis results for each sample and the x-axis represents DF ratio used in milk. (+C), probiotic-fortified UHT milk (positive control); (− C), UHT milk fortified with 1% DF (negative control). For each storage day, different uppercase letters denote a significant difference ($p < 0.05$) between DF treatments. For each treatment of DF in Milk medium, different lowercase letters denote a significant difference ($p < 0.05$) among storage days ($n = 3$) (Color figure online)

mL or g, it has been determined that the obtained enumeration results for both microorganisms were above this value. The increase in DF concentration in milk samples did not significantly affect enumeration results, but both probiotic microorganisms displayed higher metabolic activity when supplemented with DF, leading to changes in titration acidity values and pH levels. DF, which resists digestive enzyme hydrolysis in the upper gastrointestinal tract, undergoes fermentation in the lower gut, promoting beneficial bacterial communities' growth. We hypothesize that DF from PP serves as a favorable substrate for probiotic growth. Additionally, our study demonstrates a sustainable solution for food waste management by converting PP, considered waste, into a functional product using a cost-effective microwave-assisted extraction method. The obtained DF can be used in various food formulations due to its potential health benefits. While the study's results are significant, it's essential to acknowledge some limitations. The findings are specific to PP and may not be directly generalizable to other fruit by-products due to variations in DF composition. Moreover, the in vitro conditions used to investigate the prebiotic activity of the produced DF necessitate further in vivo studies for real-world confirmation, and sensory analysis is necessary to ensure the product aligns with consumer preferences. Investigating its applicability in different food formulations and prebiotic activities in future studies can open new opportunities for sustainable waste management systems and introduce beneficial products to consumers.

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Data availability The author confirms that the data supporting this study are available within the article.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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