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Formulation of Health Beneficial Synbiotic Sports Drink based on Hydrolyzed Whey Permeate

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ABSTRACT

An isotonic beverage based on whey permeate with higher levels of electrolytes and carbohydrates is presented as an alternative hydration source to conventional sports drinks. In this study, a functional beverage was prepared from hydrolyzed whey permeate inoculated with Lactobacillus plantarum and Lactobacillus casei at a concentration of 1 × 108 CFU/ml. The lactose present in whey permeate was hydrolyzed using the enzyme β-galactosidase. In this context, the effects of added prebiotics (inulin and oligofructose) and lactose hydrolysis on physicochemical and microbiological factors during storage in refrigeration were examined. The resulting product exhibited good viability for the starter culture, maintaining a concentration of 1 × 108 CFU/ml after 4 weeks of refrigeration. The addition of prebiotics significantly increased the phenolic content and antioxidant properties of the beverage ($p < 0.05$). Oligofructose and inulin improved the sensory attributes. The treatment containing both probiotics and prebiotics showed the highest sensory scores throughout the storage period ($p < 0.05$). Lactose hydrolysis, along with improved acceptability of the sports beverage, may provide a suitable option for individuals with lactose intolerance. Based on the results obtained, this beverage can be a good alternative to sports drinks and allergenic products containing lactose.

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1. Introduction

Sports drinks are liquids designed to provide hydration, electrolytes, energy, and essential nutrients needed by athletes. These drinks typically contain water, electrolytes (such as sodium and potassium), carbohydrates, and vitamins. Isotonic drinks based on whey have been suggested as an alternative source of hydration to sports drinks due to their higher electrolyte concentration and similar carbohydrate content. Whey, a byproduct obtained from the ultrafiltration process of sweet whey, contains lactose (as the main component) along with several water-soluble vitamins, which makes it nutritionally important [1].

Fermentation is an effective method for producing functional hydro-electrolytes based on whey permeate, which can also serve as an important carrier for probiotics. Moreover, lactic acid bacteria produce various inhibitory substances that can extend the shelf life of fermented products [2].

Probiotic bacteria have limited survival due to their sensitivity to environmental conditions during food processing as well as within the gastrointestinal tract, which is considered one of the main challenges in the production and processing of probiotic products. In this regard, the use of prebiotic substances—which stimulate the growth of probiotics in the intestine and can help improve their survival during the product's shelf life—seems essential in the production of probiotic foods and is highly suitable for large-scale and economical production of probiotic products. Among these compounds, inulin and oligofructose can be mentioned [2].

Prebiotics are non-digestible carbohydrates that selectively stimulate the growth and activity of certain gut bacteria, exerting beneficial effects on the host. Examples of prebiotics include inulin, fibrous gums, raftiline, pyrodextrin, xylan, stachyose, maltodextrin, lactilol, xylo-oligosaccharide, lactosucrose, raffinose, lactulose, and fructooligosaccharides. Among the non-digestible oligosaccharides, inulin and oligofructose are well-known prebiotics with proven beneficial effects. They are selectively fermented by beneficial gut bacteria such as Bifidobacteria and Lactobacillus, stimulating the growth of these beneficial microbes in the intestine [3].

Although there are numerous studies on the preparation of drinks from whey enriched with probiotics and prebiotics [4, 5], no study was found regarding the formulation of a hydro-electrolytic drink based on hydrolyzed whey permeate that is simultaneously enriched with probiotic bacteria and prebiotics. The aim of this study was to produce a functional sports drink based on whey permeate, a byproduct obtained from cheese production, enriched with prebiotics (inulin and oligofructose) and probiotic bacteria including Lactobacillus plantarum (strain 1) and Lactobacillus casei (strain 2). This product could be introduced as a completely natural and low-cost alternative to commercial sports drinks.

2. Materials and Methods

Whey permeate (an ultrafiltered and concentrated product from skimmed cow's milk using reverse osmosis) was obtained from Ramak Dairy Company (Karaj, Iran). Inulin (6 g per 100 g, degree of polymerization ≥ 23) and oligofructose (96 g per 100 g, degree of polymerization 8) were sourced from BENEO–Orafti®, Brazil. The Lactobacillus plantarum starter culture (PTCC 1058) and Lactobacillus casei (PTCC 1608) were obtained from Takgene Zist Company (Tehran, Iran), with an initial live cell count of 1×10^9 CFU/mL. MRS agar culture medium was purchased from Ibersco (Tehran, Iran).

2-1. Lactose Hydrolysis

Hydrolysis of whey permeate with an initial pH of 6.45 was performed using the enzyme β-galactosidase (of microbial origin from Kluyveromyces lactis) at an enzyme concentration of 1.11 g/L. The process was carried out in a rotary shaker (150 rpm) at 37°C. The activity of this enzyme was equivalent to 2000 neutral lactase units per gram of enzyme. One unit of neutral lactase is defined as the amount of enzyme that releases 1.3 micromoles of o-nitrophenol per minute from o-nitrophenol-β-D-galactoside. A 1N KOH solution was then used to adjust the acidic pH of the enzyme solution and optimize enzyme activity, bringing the pH to 6.5. The resulting samples were incubated at 37°C for 4 hours.

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The enzyme was subsequently inactivated by heating the solution at 60°C for 5 minutes. This temperature is similar to that reported by Beucler et al. (2005), where they used lactase at 37°C for 3 hours for hydrolyzing whey permeate, followed by enzyme inactivation at 63°C for 5 minutes. The prepared samples were then rapidly cooled to room temperature and filtered to remove turbidity [6].

To assess the initial lactose content and sugars resulting from lactose hydrolysis, high-performance liquid chromatography (HPLC) was used. A Waters-brand HPLC system equipped with a UV-vis detector was used to determine the lactose hydrolysis level. The system utilized a Eurokat H column and a mobile phase of 0.01N sulfuric acid with a flow rate of 0.05 mL/min. The quantity of sugars was calculated based on the area under the curve [7].

2-2. Preparation of Probiotic Culture

The strains of probiotic bacteria Lactobacillus plantarum (PTCC 1058) and Lactobacillus casei (PTCC 1608) with specified batch numbers were obtained from the Takgene Center. MRS broth and MRS agar media were also procured from Ibersco. To prepare the bacterial inoculum from the stock culture, the probiotic bacterial strains were first activated by culturing them in MRS broth at 37°C for 24 hours. A second subculture was then prepared by transferring the first culture into fresh MRS broth and incubating again at 37°C for another 24 hours. Sterile tubes were prepared, and 5 mL of sterile liquid medium was added to each. Different volumes from the second culture were transferred into cuvettes, and the optical density was measured using a spectrophotometer at 600 nm—this wavelength is specific to bacterial measurement and corresponds to the growth curve. These readings were then used to estimate the bacterial count.Finally, the cuvette tube containing half McFarland standard (equivalent to 1.3×10^8 bacteria per milliliter) was selected for preparing the inoculum dose. Additionally, one drop of the MRS broth culture was streaked onto solid MRS agar medium to isolate a pure single colony.

2-3. Preparation of Sports Drink

100 mL of ultrafiltered and concentrated whey permeate was placed into 250 mL Erlenmeyer flasks. Two different treatment groups were prepared as follows:

(I) Hydrolyzed permeate (v/v) with 1% Lactobacillus plantarum and Lactobacillus casei bacteria, and

(II) Hydrolyzed permeate (v/v) with 1% Lactobacillus plantarum and Lactobacillus casei bacteria along with 1% prebiotics (inulin + oligofructose).

After the addition of the probiotic culture and prebiotics, the samples were incubated at 42°C for fermentation. The incubation time was set between 4 to 4.5 hours, until the pH reached 5. After fermentation, the products were stored in a refrigerator at 4°C and kept under these conditions for 28 days.

2-4. Probiotic Bacteria Count

Assessing the probiotic population in a functional product is essential to determine how the total probiotic population, comprised of two probiotic strains, changes over the course of the storage period.

2-4-1. Lactobacillus plantarum Count

To count viable cells, the tenfold serial dilution method and the pour plate technique were used. For dilution preparation, 10 mL of the homogenized sample was weighed into sterile zip-lock bags containing 90 mL of sterile trisodium citrate (2 g per 100 g). The mixture was homogenized for two minutes using a stomacher device (SCIENTZ-09, China). Then, using a 100–1000 µL micropipette, 1 mL was transferred to test tubes containing 9 mL of 0.1% sterile peptone water to prepare a series of tenfold serial dilutions. Each subsequent dilution was made by adding 1 mL of the previous dilution to 9 mL of sterile peptone water. From each dilution tube, 0.1 mL was taken using a sterile micropipette and plated on solid MRS agar. Plates were incubated for 72 hours at 37°C. Colony counts of Lactobacillus plantarum were recorded for samples stored for 1, 14, and 28 days in the refrigerator. Plates with a countable number of colonies—typically between 30 and 300—were selected. Colonies counted were spherical, concave, with a

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diameter of 1–1.5 cm, smooth and clearly defined edges, and without halos. Colony numbers were recorded after calculating the number per milliliter using the following formula:

Formula (1): Colonies/mL = (Number of colonies × 1) / Dilution factor

2-4-2. Lactobacillus casei Count

Counting of Lactobacillus casei was carried out using the pour plate and standard plate count methods on MRS agar. Appropriate dilutions were prepared, and after inoculating the MRS agar plates, they were incubated at 37°C for 72 hours, and colony counts were performed [8].

2-5. Physicochemical Properties

2-5-1. Measurement of Acidity and pH

To ensure flavor stability of the product during storage—so that the taste on the first day of purchase and on the twenty-eighth day remains similar—changes in pH and titratable acidity must be evaluated. The sample with the least variation was selected. After production of the beverage, pH changes were measured at 25°C using a pH meter (Metrohm model 827, Switzerland). The percentage of titratable acidity was calculated based on the volume of 0.1N NaOH used to titrate 18 grams of the sample until reaching a final pH of 8.3 [9].

2-5-2. Evaluation of Free Radical Scavenging Activity

The DPPH free radical scavenging ability of the treatments was determined using the method of Sanchez et al. with a spectrophotometer at 517 nm. DPPH is a lipophilic radical with maximum absorption at 517 nm. In the DPPH assay, hydroxyl groups of antioxidant compounds donate hydrogen atoms to DPPH radicals, reducing them and changing the solution color from deep purple to light yellow, thereby decreasing absorbance.

The absorbance at 517 nm reflects the amount of remaining DPPH. To prepare the solution, 39.43 mg of DPPH was dissolved in 100 mL of methanol, then diluted with methanol at a 1:10 ratio.

0.1 mL of beverage samples with varying concentrations were mixed with 3.9 mL of 0.1 mM DPPH methanolic solution to reach a total volume of 4 mL. The mixture was vortexed for 30 seconds (Stuart model SA7), then left in the dark at room temperature for 30 minutes for the reaction to occur. Absorbance was then measured at 517 nm using a spectrophotometer against a methanol blank. The percentage of free radical inhibition was calculated using the following formula:

Formula (2): %IP = [(A_control - A_sample) / A_control] × 100

Where:

%IP is the percentage of free radical inhibition,

A_control is the absorbance of the control (containing all reagents except the sample),

A_sample is the absorbance of the sample (containing various concentrations of plant extract, methanol, and DPPH solution) [10].

2-5-3. Determination of Total Phenolic Compounds

Quantification of total phenols was performed using a spectrophotometric method based on the Folin–Ciocalteu reagent. This method is among the most widely used for measuring phenolic compounds. The principle of the method is the reduction of the Folin reagent by phenolic compounds in an alkaline medium, forming a blue-colored complex that shows maximum absorbance at 760 nm. In this method, 0.5 mL of the beverage sample was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent in a test tube. After 5 minutes, 2 mL of sodium carbonate solution (75 g/L) was added to the mixture. The absorbance of the colored solution was measured after 2 hours using a spectrophotometer at 760 nm. Total phenolic content was calculated using a gallic acid standard curve ($R^2 = 0.9912$). For constructing the gallic acid calibration curve, concentrations of 25, 50, 100, 150, 200, 250, 300, 350, and 400 mg/L were used. Results were reported as mg of gallic acid equivalents (GAE) per 100 mg of sample [11].

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3. Sensory Evaluation of the Samples

The sensory effects resulting from the addition of Lactobacillus plantarum, Lactobacillus casei, and prebiotics to the beverage samples were evaluated through a hedonic sensory test.The synbiotic beverage samples were poured into 10 mL glass containers, which were randomly coded to prevent bias during the evaluation process. After overnight storage at 4°C, twelve trained taste panelists assessed the samples using a 5-point hedonic scale. Each evaluator rated the color, taste, aroma, mouthfeel, and overall acceptance of each 10 mL synbiotic beverage sample using the following categories: (1) Very Bad, (2) Bad, (3) Average, (4) Good, and (5) Very Good [12].

4. Statistical Analysis

The experimental design was carried out using a factorial arrangement in the form of a completely randomized design with three replications. To identify statistically significant differences among the mean values of the obtained data, analysis of variance (ANOVA) followed by Duncan's multiple range test was performed using SPSS software version 22. A significance level of $p < 0.05$ was considered for all statistical analyses.

5. Results and Discussion

5-1. Lactose Hydrolysis

Figure 1 shows the chromatogram of lactose hydrolysis after mixing with β-galactosidase enzyme. Peaks corresponding to glucose, galactose (the two main components of lactose), and pentose (most likely arabinose) were observed at 11.7, 14.7, and 20 minutes, respectively. Based on the resulting chromatogram, it was confirmed that all the lactose present in the whey permeate was completely hydrolyzed into its constituent sugars. Martínez et al. (2011) achieved similar results when using β-galactosidase produced by Kluyveromyces lactis for the hydrolysis of lactose in regular yogurt [13].

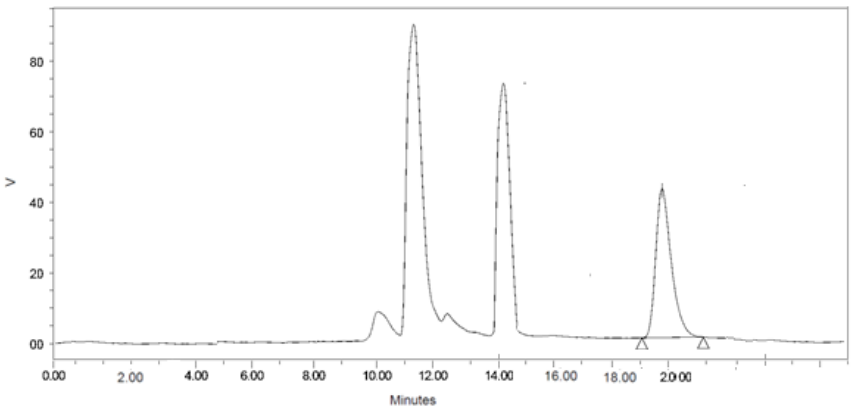

Figure 1. chromatogram of sugar analysis by the HPLC method

5-2. Acidity and pH

Table 1 shows the acidity and pH values of the different beverages over a 28-day storage period at 4°C, based on hydrolyzed whey permeate in the presence and absence of inulin and oligofructose.

Table 1. pH and titratable acidity of samples during storage

Treatment	Storage time	Hydrolyzed permeate/probiotic	Hydrolyzed permeate/probiotic/prebiotic
pH	1st day	4.82 ± 0.189^{dA}	4.84 ± 0.19^{dB}

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	7st day	4.72 ± 0.10^{cA}	4.72 ± 0.12^{cB}
	14st day	4.56 ± 0.03^{bA}	4.69 ± 0.23^{bB}
	28st day	4.25 ± 0.32^{aA}	4.50 ± 0.15^{aB}
acidity (%)	1st day	0.68 ± 1.19^{dA}	0.78 ± 1.09^{dB}
	7st day	0.85 ± 0.28^{cA}	0.88 ± 0.08^{cB}
	14st day	1.05 ± 0.15^{bA}	0.97 ± 0.15^{bB}
	28st day	2.68 ± 0.10^{aA}	1.88 ± 0.10^{aB}

Numbers are expressed as means ± standard deviation. Different lowercase letters indicate significance in the column ($p < 0.05$). Different capital letters indicate significance in the row ($p < 0.05$).

According to Table 1, a significant difference was observed between the various treatments when comparing the means ($p \geq 0.05$). With the increase in the amount of inulin and oligofructose in the beverage, the acidity significantly decreased, and pH significantly increased ($p \geq 0.05$). Moreover, over time, the acidity significantly increased and pH similarly decreased ($p \geq 0.05$). In conclusion, it can be stated that the addition of inulin and oligofructose to the beverages causes changes in both acidity and pH.

According to the results obtained during the fermentation stage, the acidity increased due to the microbial activity. The bacteria Lactobacillus plantarum and Lactobacillus casei produce lactic acid and a range of organic acids such as acetic acid. The decrease in pH and the increase in acidity are associated with the production of organic acids. Based on studies, a pH level of around 3.5–4.5 in food formulations leads to a reduction in the pH of the digestive tract, which in turn increases the stability of probiotics. This also prevents the growth of pathogenic microorganisms [14].

The main factor responsible for the decrease in pH and the increase in acidity during the storage period may be the breakdown of organic acids and the production of short-chain fatty acids [15]. In the study by Shah et al. (2001), the effects of prebiotics such as lactulose, inulin, and oligofructose in probiotic yogurt showed similar results regarding acidity and pH [16].

5-3. Phenolic Compounds and Antioxidant Properties

Changes in total phenolic content and antioxidant activity in fermented beverages stored at 4°C for 28 days are shown in Table 2.

Table 2. Total phenolic content and percentage of DPPH measured for 28 days at 4 ° C

Treatment	Storage time	Hydrolyzed permeate/probiotic	Hydrolyzed permeate/probiotic/prebiotic
TOTAL PHENOL (mg GAE/ 100 mL)	1st day (before fermentation)	17.5 ± 0.05^{aA}	19.5 ± 0.05^{aA}
	1st day (after fermentation)	16.5 ± 0.05^{bB}	25.04 ± 0.16^{aB}
	7st day	20.19 ± 0.75^{bC}	25.96 ± 0.38 ^{aC}
	14st day	23.63 ± 1.30^{bD}	26.56 ± 1.8 ^{aD}
	28st day	25.20 ± 0.14 ^{bE}	28.43 ± 1.25^{aE}
DPPH (%)	1st day (before fermentation)	0.02 ± 0.24^{bA}	0.09 ± 0.24^{aA}
	1st day (after fermentation)	2.14 ± 1.24^{bB}	4.64 ± 1.22^{aB}
	7st day	6.15 ± 1.15^{bC}	9.65 ± 1.05^{aC}

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	14st day	8.02 ± 1.45^{bD}	15.32 ± 1.15^{aD}
	28st day	15.37 ± 0.25^{bE}	18.72 ± 1.25^{aE}

Numbers are expressed as means ± standard deviation. Different lowercase letters indicate significance in the column (p <0.05). Different capital letters indicate significance in the row (p <0.05).

Phenolic compounds in both beverage samples significantly increased after fermentation ($p < 0.05$); however, fermented beverages containing inulin and oligofructose had the highest levels of phenolic compounds. This may be due to the hydrolysis of glycosylated phenolic compounds and the release of free phenolic compounds during fermentation [17].

The results indicate that fermentation of the beverages by lactic acid bacteria (LAB) preserves the bioactive components of the synbiotic beverage [18]. Although a diet rich in dietary fiber and polyphenols has positive effects on human health, their bioactivity can be influenced by molecular interactions between them [19].

Antioxidant activity showed a similar trend. The total phenolic content of the functional beverage had a positive correlation with antioxidant activity, as shown in Table 2. The increase in antioxidant activity was observed in both fermented beverages, but the level was significantly higher in the beverage containing prebiotics. These findings are consistent with the study by Pereira et al. (2012), who evaluated the effects of lactic acid fermentation on cashew apple juice [20]. The increase in antioxidant activity in fermented beverages may be due to the elevated concentration of antioxidant compounds such as polyphenols, flavonoids, and beta-carotene produced during fermentation by lactic acid bacteria [21].

The increase in total phenolic content during the fermentation process and the resulting improvement in antioxidant properties have been confirmed by many researchers. This is attributed to the hydrolysis of glycosylated phenolic compounds and the formation of free phenols. The mentioned microorganisms are capable of increasing phenol levels during fermentation. However, in the sample without prebiotics, the level of phenolic compounds showed less change over time compared to the sample containing prebiotics. In a similar study, researchers reported that the total phenolic content of cranberry, grape, and strawberry juice significantly increased after fermentation with Serratia vaccinii—for example, after 5 days of fermenting strawberry juice, total phenol content rose from 385 to 771 mg gallic acid per liter [22].

5-4. Counting Lactobacillus casei and Lactobacillus plantarum

Figure 2 shows the survival of probiotics during cold storage over a 28-day period.

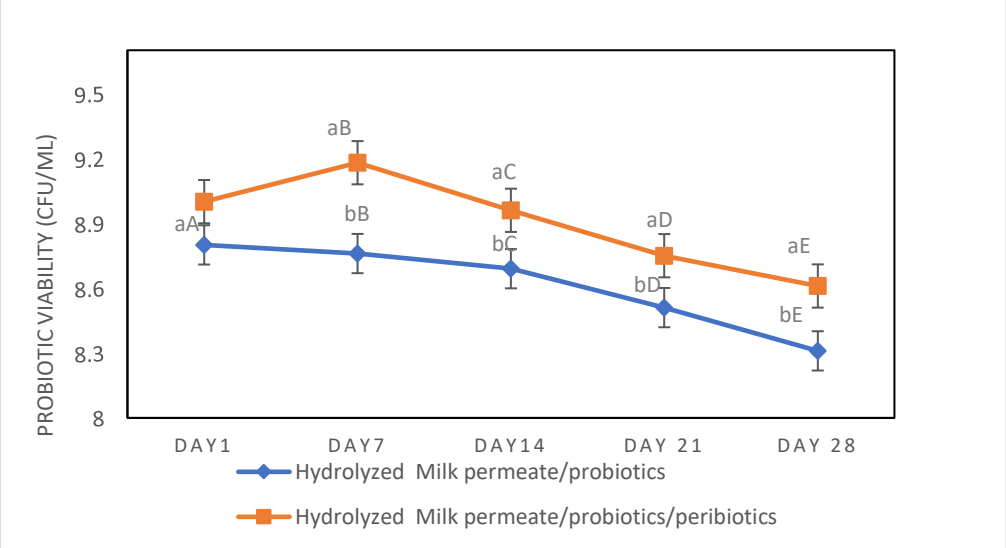

Figure 2. Survival of probiotic bacteria in beverages during storage

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Results show that probiotic bacteria maintained good viability during storage, with counts reaching 10^8 CFU/mL. The decline in probiotic levels over the storage period may be associated with a decrease in pH and the accumulation of organic acids resulting from bacterial growth and fermentation. In this regard, Jayamanne and Adams (2009) reported a significant reduction in the viability of Bifidobacterium lactis in products over cold storage time [23]. Based on the findings, probiotic levels in all treatments reached approximately 10^8 CFU/mL by the end of the fourth week. This level meets international standards (10^8 CFU/mL) for the number of viable probiotic bacteria per milliliter of product at the time of consumption [24]. These results are consistent with previous studies [25].

The addition of prebiotics (inulin and oligofructose) significantly enhanced ($P < 0.05$) the viability of L. casei and L. plantarum in synbiotic beverages. The stimulatory effect of prebiotics on the growth and survival of probiotic bacteria in the product aligns with findings from similar research [26]. In this context, Nouri et al. (2017) reported that rye sprout extract, due to its prebiotic activity, increased the growth and viability of probiotic bacteria [27].

5-5. Sensory Properties

Table 3 presents the sensory evaluation scores of the beverage samples over the storage period. The panelists reported significant sensory changes among different samples over the 28-day period ($P < 0.05$).

Table 3. Predominant sensory scores (1–5) * for taste, odour, and overall acceptability of sport drink samples: HWP/Pro (Hydrolyzed Whey Permeate and Probiotic) and HWP/Pro/Pre (Hydrolyzed Whey Permeate, Probiotic, and Prebiotic) for 28 days storage at 4 °C.

parameter	Group	Day(s)				
		1	7	14	21	28
Taste	HWP/Pro	5 ± 0.00^{D}	4.10 ± 0.23^{aC}	4 ± 0.00^{aC}	3.5 ± 0.00^{aB}	**3 ± 0.00^{aA}**
	HWP/Pro/Pre	5 ± 0.00^{D}	5 ± 0.00^{bD}	4.70 ± 0.55^{bC}	4.52 ± 0.85^{bB}	**4.01 ± 0.08^{bA}**
Odour	HWP/Pro	5 ± 0.00^{D}	4.82 ± 0.23^{D}	4 ± 0.00^{aC}	3.6 ± 0.83^{aB}	**3 ± 0.00^{aA}**
	HWP/Pro/Pre	5 ± 0.00^{C}	4.80 ± 0.00^{C}	4.56 ± 0.21^{bB}	4.22 ± 0.24^{bA}	**3.99 ± 0.82^{bA}**
Overall Acceptability	HWP/Pro	5 ± 0.00^{D}	4.5 ± 0.00^{aD}	4 ± 0.00^{aC}	3.54 ± 0.16^{aB}	**3.25 ± 0.23^{aA}**
	HWP/Pro/Pre	5 ± 0.00^{C}	5 ± 0.00^{bC}	4.77 ± 0.16^{bB}	4.50 ± 0.33^{bAB}	**4.20 ± 0.16^{bA}**

Data are means ± SD. Means in a column shown with different lowercase letters are significantly different ($p < 0.05$). Means in a row shown with different uppercase letters are significantly different a row ($p < 0.05$).

Flavor scores in both groups were evaluated as acceptable after fermentation. This can be attributed to the hydrolysis of lactose in the whey permeate into its monosaccharide components prior to fermentation. The beverage samples containing inulin and oligofructose received the highest sensory scores throughout the storage period ($p \leq 0.05$). Moreover, as time progressed, the flavor score significantly decreased ($p \leq 0.05$). This reduction is likely related to the increased acidity and decreased pH of the samples during storage. Sensory scores for aroma were lower compared to flavor, which may be due to the acidic odor of the samples.

6. Conclusion

A sports drink based on hydrolyzed whey permeate containing the probiotics L. casei and L. plantarum, along with prebiotic compounds including inulin and oligofructose, was formulated to not only improve the sensory attributes and taste of the product through lactose hydrolysis, but also to enhance the

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functional properties of the beverage. A satisfactory level of probiotic viability was observed during storage up to day 28. The hydrolysis of lactose into its monosaccharide components significantly improved the sensory characteristics of the beverage, while the incorporation of prebiotics (inulin and oligofructose) had a positive impact on the survival of probiotic bacteria during storage. Therefore, the formulated functional isotonic beverage could serve as a suitable alternative to existing sports drinks. Additionally, it may be considered an appropriate beverage option for individuals with lactose intolerance.

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مقاله علمی_پژوهشی

فرمولاسیون نوشیدنی ورزشی سین بیوتیک فراسودمند بر پایهٔ پرمیت آب پنیر هیدرولیزشده

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چکیده

نوشیدنی ایزوتونیک مبتنی بر پرمیت آب پنیر با محتوای بالاتر الکترولیت‌ها و کربوهیدرات‌ها، به عنوان یک منبع هیدراتاسیون جایگزین برای نوشیدنی‌های ورزشی معمولی مطرح است. در این مطالعه، نوشیدنی فراسودمند بر پایه پرمیت آب پنیر هیدرولیزشده و تلقیح‌شده با لاکتوباسیلوس پلانتاروم و لاکتوباسیلوس کازئی به غلظت ۱/۳×۱۰۸ CFU.ml-1 تهیه شد. لاکتوز موجود در پرمیت آب پنیر با استفاده از آنزیم β-گالاکتوزیداز هیدرولیز گردید. در این راستا، تأثیر پری‌بیوتیک‌های افزوده شده (اینولین و الیگوفروکتوز) و هیدرولیز لاکتوز بر روی فاکتورهای فیزیکوشیمیایی و میکروبی در طول نگهداری در دمای یخچال بررسی شد. محصول به‌دست‌آمده زنده‌ماندنی خوبی را برای کشت استارتر با مقدار ۱۰۸ CFU.ml-1 پس از ٤ هفته نگهداری در شرایط یخچال نشان داد. افزوده شدن پری‌بیوتیک‌ها به نوشیدنی موجب افزایش قابل توجهی در محتوای فنلی و خاصیت آنتی اکسیدانی محصول شد ($p < 0/05$) . الیگوفروکتوز و اینولین امتیازات حسی را بهبود بخشیدند. تیمار حاوی هر دو پروبیوتیک و پری‌بیوتیک‌ها بالاترین امتیازات حسی را در طول مدت نگهداری نشان دادند ($p < 0/05$). هیدرولیز لاکتوز به همراه بهبود پذیرش نوشیدنی ورزشی، می‌تواند برای افرادی که دچار عدم تحمل لاکتوز هستند، گزینه مناسبی باشد. بر اساس نتایج به‌دست‌آمده، این نوشیدنی می‌تواند جایگزین خوبی برای نوشیدنی‌های ورزشی و مواد آلرژی‌زای حاوی لاکتوز باشد.

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