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Comparison of the effect of inulin and wheat fiber on the viability of probiotics in the sports drink enriched with calcium and vitamin D

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ABSTRACT

The purpose of this research is to produce a synbiotic sports drink based on whey. Whey provides the necessary osmolality in a sports drink due to its mineral content. In this research, the effect of two types of long-chain and short-chain inulin fiber and wheat fiber on the survival of probiotics in sports drinks was investigated and compared during 28 days of storage at refrigerator temperature. The presence of these compounds in the drink has a positive effect on the viability of probiotics in the drink, and the effect of wheat fiber on the survival of probiotic bacteria was greater than that of inulin fibers. The bacterial colonies such as Escherichia coli, coliform, mold, yeast and acid-resistant bacteria were not observed in the 28-day storage of the drink in the refrigerator. Also, microorganism was not observed in the general count of microorganisms until the 21st day. This result showed the efficiency of the thermal process (at a certain temperature and storage time). Vitamin D3 and calcium levels were within the standard recommended range. The results showed that it is possible to use calcium in the amount of 124 mg/100 grams and vitamin D in the amount of 0.02 ppm without any adverse effect in the formulation of the drink. Therefore, it is possible to benefit from its valuable properties by enriching the drink and using it in the diet of children and the elderly.

1-Introduction

Sports drinks with positive ions such as sodium and potassium were produced as a complete drink for consumption after exercise [1]. So, the need to provide the body with essential electrolytes such as sodium to delay thirst and replace electrolytes lost through sweating and provide some of the energy needed through drink consumption becomes clear [2]. Today, dairy drinks made from milk and cheese have become increasingly popular not only in terms of scientific research but also in the global trade market [3]. Previous studies have shown that whey protein is more effective at retaining fluids than casein protein or traditional sports drinks. This suggests that whey protein concentration is an important factor in post-exercise hydration [4]. Whey and whey contain protein and lactose and are often used in sports drinks as a source of protein and energy [5].

Probiotics are live, beneficial microorganisms that, when consumed by humans or animals, exert beneficial effects on the host's health by affecting the body's microbial flora. Most probiotics belong to a large group of bacteria that are part of the human intestinal microflora, where they live a harmless life. The most common probiotic microorganisms are divided into two groups: bacteria and fungi. Some of these microorganisms are selected strains of the bacteria Lactobacillus and Bifidobacterium [6]. Prebiotics are defined as non-digestible food substances that benefit the host by selectively stimulating the growth or activity of a number of probiotic yeasts or bacteria in the large intestine. Inulin is a natural compound extracted from various fruits and vegetables. Inulin is mainly obtained by hot water extraction from bitter chicory, then the extracted liquid is purified and crystallized. The degree of polymerization of inulin ranges from 2 to 60 units, with an average of 12 units for natural inulin [7]. Wheat fiber is a white powder with fine grain size and a purity of about 97%, the majority of which (94%) is insoluble fiber. The main components of this fiber include cellulose, hemicellulose and a large amount of lignin. One of the characteristics of wheat dietary fiber is the formation of a three-dimensional fibrous network in the final product, which helps improve the texture and stability of the product [8].

Consuming synbiotic products (simultaneous presence of prebiotics and probiotics) has greater beneficial effects on the consumer's health. In addition, in synbiotic products, the survival of probiotic bacteria during the storage period of the product and their passage through the digestive tract are increased [9].

Although food production in the world today is sufficient and there should be no hungry people in the world, a large part of the world's population suffers from hidden hunger despite having access to energy. This term is used for deficiencies of micronutrients such as iodine, zinc, iron, folic acid, calcium, vitamins A and B, the symptoms of which are not felt for a long time. Fortification involves adding nutrients to a food that does not naturally contain that nutrient [10].

Vitamin D is a fat-soluble vitamin and comes in two general forms: D2 and D3. Vitamin D2 (ergocalciferol) comes from food, and form D3 (cholecalciferol) is produced in the skin by exposure to sunlight. The effect of vitamin D deficiency has been described as the cause of metabolic bone diseases including rickets, osteomalacia, osteoporosis, and finally weakness and decreased muscle mass [11].

Calcium is considered a major component of the human diet due to its numerous roles in the body. Calcium is a mineral that strengthens bones and has a protective role against blood pressure abnormalities and colon cancer. Other vital functions of calcium include maintaining normal heart function, regulating nerve conduction, and helping with blood clotting [12].

The purpose of this research is to produce a whey-based sports drink, evaluate the effect

of long-chain and short-chain inulin fiber and wheat fiber on the viability of the probiotic Bifidobacterium, and enrich the drink with calcium and vitamin D3.

2- Materials and methods

2-1 Materials

Commercial pectin gum, sugar and citric acid were purchased from Amol market. Whey solution (with acidity 9, dry matter 6.58, pH 5.7 and protein 0.66) was obtained from Dousheh Amol Dairy Company (Haraz Dairy). Orange concentrate (Babol Ab Co.) was purchased. Probiotics were purchased from Christian Hansen Co. Tricalcium citrate was purchased from Pas-Adak Co.

2-2 Beverage formulation

To produce the drink, first 4% sugar, 25.7% whey solution, 0.14% citric acid, and 5% orange concentrate were mixed together and mixed in an appropriate amount of water (until reaching 100% by weight) using a Thermomix (Vorwerk, Germany). Then, after the mixture reached a temperature of 50°C, 0.25% stabilizer was added to the desired mixture and homogenized at high speed for 5 minutes. According to the supplier's suggested dosage, which is based on Iran's fortification standards, calcium was added to the beverage at a rate of 130 mg per 100 g of beverage and vitamin D at a rate of 0.025 ppm. Then, long-chain inulin, short-chain inulin, and wheat fiber were added to the desired beverage at the same concentration of three percent in accordance with the standard Iranian enrichment criteria. Finally, the desired samples were pasteurized using a Thermomix device until reaching a temperature of 85°C. And finally, after curing and cooling to ambient temperature (25°C), Probiotic bacteria Lactobacillus acidophilus and Bifidobacterium bifidum were separately inoculated into the drink according to the manufacturer's recommended dosage (10^7-10^8 cfu/ml). The samples were stored at 4°C until the experiments.

2-3 Physicochemical properties of the beverage

pH was determined according to the Iranian National Standard No. 2852 by a digital pH meter (METROM model – made in Switzerland), soluble solids or Brix was determined using a handheld refractometer (ATAGO – Japan) [13]. The amount of beverage dry matter and total sugar was calculated according to the Iranian National Standard No. 1249 [14]. Protein content was measured using the formalin method according to Iranian National Standard No. 14987 [15].

2-4 Vitamin D measurement

Vitamin D was measured by high-performance liquid chromatography (HPLC) under the number 13579 [16].

2-5 Calcium Measurement

Calcium measurement was performed by spectrophotometry according to the Iranian National Standard No. 10780 [17].

2-6 Viability of probiotics

The viability of probiotic bacteria was evaluated in accordance with the Iranian National Standard No. 5272 and measured by the total microbial population count method [18].

2-7 Microbial tests

In order to confirm the microbial quality of beverages, the total number of microorganisms, Escherichia coli, coliform, acid-fast bacteria, mold, and yeast were examined immediately after production and after 7, 14, 21, and 28 days [19].

2-8 Statistical analysis

In this study, the data results were analyzed using SPSS version 20 software. In order to analyze the data and examine the significance of the difference between the means, the ANOVA analysis of variance

method (completely randomized design) and Duncan test at a confidence level of 95% were used. The results were reported based on the mean ± standard deviation of three replicates.

3- Results and discussion

3-1 Physicochemical test

3-1-1 pH

The pH was determined by the inverse logarithm of the hydrogen ion concentration using a pH meter. The effect of long-chain and short-chain inulin fibers on pH was not significant compared to the fiber-free beverage (control) ($P > 0.05$), However, as a result of adding wheat fiber to the beverage, the pH increased significantly ($P < 0.05$). In accordance with the results of the present study, researchers observed that by adding inulin to various products such as UF low-fat cheese [20], Reduced-fat cottage cheese [21], kefir [22, 23, 24], yogurt [25], low-calorie dark chocolate [26], and cherry-red grape juice [27] do not significantly change the pH and acidity of the product. In other results that contradict the results of the present study, Hosseini (2016) reported that adding inulin reduces the pH of the samples in a study of the effect of some quality parameters in synbiotic apple juice-cherry juice [28].

In accordance with the present study, Javidi and Goli (2021) reported that adding wheat fiber to low-fat yogurt increased the pH of yogurt [29]. Also, in another study by Nateghi et al. (2021), in investigating the effect of wheat extract and fiber on the physicochemical properties of orange juice, they reported that on all days the control sample (without wheat extract and fiber) had the highest pH, although on some days there was no statistically significant difference compared to the sample containing wheat fiber [30].

3-1-2 Brix

The Brix degree indicates the percentage of solids in a solution by weight to the total weight of the solution, or in other words, the percentage by weight of solids in the solution. The higher the Brix degree of a solution, the higher the concentration of solids in that solution and the lower the amount of water in the solution. Given that the specific gravity (volumetric weight - weight per unit volume) of materials varies at different temperatures, measurements should always be made at a constant and specific temperature for correct comparison and correct use of the tables available for different materials. This constant and specific temperature, which is called the laboratory temperature, is 20 degrees Celsius. Usually, for practical applications, measurements at 18 to 22 °C are also acceptable. According to the above definition, the most accurate method for determining the Brix degree of a solution is to weigh a certain volume of the specific solution (substance) [30].

The lowest Brix value was found in the control sample and the sample containing wheat fiber, but as a result of adding long-chain and short-chain inulin fiber, the Brix value increased significantly ($P < 0.05$). According to the present study, Hosseini (2016), reported that adding inulin to a synbiotic beverage containing apple and cherry juice in the first days increases the Brix of the beverage, but over time, microorganisms use the sugars and reduce the Brix [28]. Nateghi et al. (2021), in investigating the effect of wheat fiber on the physicochemical properties of orange juice, reported that the lowest Brix was for the control sample and the highest Brix was for the sample containing 1.5% wheat fiber. However, there was no significant difference between this treatment and the treatments containing 2 and 0.5% wheat fiber ($P > 0.05$) [30].

3-1-3 Dry matter

The lowest amount of dry matter was in the control sample, but as a result of adding all three types of fiber (wheat and long-chain and short-chain inulin), the amount of dry matter in the beverage increased significantly ($P < 0.05$), although there was no statistically significant difference

between these three types of fiber ($P > 0.05$). In accordance with the present study, Ababaf et al. (2020) reported that with increasing inulin content, the dry matter content of inulin-containing samples increased compared to the control samples. Inulin had a significant effect on this factor. The highest dry matter content was obtained for inulin at 2% and the lowest for the control sample [31]. The results of Rinaldoni et al. (2012) showed that increasing the concentration of inulin increases the dry matter of yogurt [32].

In accordance with the present study, Zomorodi et al. (2013) reported in their study of wheat fiber enrichment in doogh that with increasing wheat fiber percentage, the dry matter of doogh increased significantly. The reason for the increase in dry matter can be attributed to the hydration or water absorption property of the fibers. The hydration properties of dietary fibers depend on the chemical structure of the polysaccharides present and some other parameters, including porosity, particle size, ionic state, pH, and temperature [33].

Sahan et al. (2008) also showed that increasing beta-glucan increases the dry matter of yogurt, which confirms the results of this study. Since the cell wall of wheat grain is a combination of arabinoxylans and beta-glucans, increasing wheat fiber also increases the dry matter of doogh [34]. Zomorodi et al. (2013) showed in a study that increasing wheat fiber reduced the moisture content in fruit yogurt. The hydration properties of fibers can be measured by measuring water absorption and water holding capacity. The ability of the water holding capacity of fibers depends largely on the sucrose content of the dietary fiber [33].

3-1-4 Protein

The lowest protein content was found in the control sample and the sample containing wheat fiber, but as a result of adding long-chain and short-chain inulin fiber, the protein content increased significantly ($P < 0.05$). Contrary to the present study, Ghobadi et al (2016) reported that the addition of wheat fiber and red beet fiber increased the protein content [35]. Yarahmadi et al. (2023) reported in a study on the use of barley malt pulp fiber as a fat substitute in mayonnaise that adding fiber increased the protein content ($P < 0.05$) [36]. According to the results of the study by Romero-Lopez (2011), increasing the amount of orange pulp fiber from 10 to 15% reduced the protein content in muffin samples [37].

3-2 Osmolality

The results of measuring the levels of calcium and vitamin D are shown in Table 2. To prove that a sports drink is isotonic, the osmolality of the sports drink must be between 250 and 330 milliosmoles per liter, according to the Iranian national standard. Also, its physicochemical properties, including total sugar and Brix, must be in accordance with the Iranian National Standard (No. 6693) [38]. The osmolality of the drink in question was 365 milliosmoles/liter, which is higher than the standard value for isotonic drinks and is in the range of hypertonic drinks (minimum 340 milliosmoles/liter). According to the Iranian national standard, the sugar content in isotonic drinks is less than 8 grams per hundred milliliters, but in hypertonic drinks, the minimum is 8 grams per hundred milliliters. The total sugar content of the drink in this study was 8 grams per hundred milliliters, which was within the hypertonic range.

The first calcium fortification was carried out in England in 1942. Since then, other countries have also fortified foods with calcium. The World Health Organization recommended a daily intake of 1500 mg for 11- to 18-year-olds in 1994 [39].

According to the Functional Foods Executive Guideline, it has been reported that in the fortification of dairy products, in addition to calcium, fortification with vitamin D3 is also necessary. According to the recommendations of FAO and WHO, the average daily calcium requirement of

each person is 1000 mg. The requirement of Iran is also the same amount, while most people only receive 57% of the actual requirement [40].

According to the Iranian National Standard, the amount of calcium for a source claim is 150 mg per serving of a beverage and for a rich or enriched claim is 300 mg per serving. Each serving of the beverage is equal to 240 ml. The measured calcium content was 124 mg/100 g of the beverage, which is equivalent to approximately 298 mg of calcium in one serving of the beverage. These results indicate that the calcium content is within the standard recommended range [11].

According to the Iranian national standard, the amount of vitamin D for a source claim is 2.3 micrograms per serving of a beverage, and for a rich or enriched claim, it is 4.5 micrograms per serving. Each serving of the drink is 240 ml. The measured vitamin D content is 0.02 ppm (4.8 micrograms per serving). With 124 mg/100 g of beverage, which is equivalent to approximately 298 mg of calcium in one serving of beverage. These results indicate that the calcium content is within the standard recommended range [11].

Tamer et al. (2013) conducted research on enrichment applications in the jelly industry using three different concentrations of four minerals (calcium, iron, zinc, and phosphorus) and seven vitamins (Vitamins B1, B2, B6, niacin, pantothenic acid, vitamin C, and vitamin E) and stated that using jelly fortification, the vitamins and minerals needed by children aged seven to 14 years can be compensated [41]. Cortes (2013) reported that calcium-enriched candies provided about 20% of the daily calcium requirement of consumers and that their calcium content did not decrease significantly during storage [42].

3-3 Microbial load

Changes in the microbial status of whey-based orange drink during 28 days of storage at refrigerator temperature (4°C) are shown in Table 3. Given that the pH of the drink is less than 4, it is an acidic drink. According to the Iranian National Standard No. 3845, acid-resistant acidophilic microorganisms in beverages include Lactobacillus, Leuconostoc, Acetobacter, and Gluconobacter. The high acid content and resulting decrease in the pH of the beverage means that only lauric acid bacteria, yeasts, and molds are able to grow and multiply. The high acidity and low pH of the beverage are not suitable for the growth of pathogens. Acetic acid bacteria such as Gluconobacter also cause juice spoilage in cases of packaging defects and increased dissolved oxygen. Acid-resistant spore-forming bacteria also cause juice spoilage by producing alcohol. These bacteria are resistant to the heat of pasteurization [43].

The initial microbial load in the pasteurized beverage was 0 Log CFU/ml. During 28 days of refrigerated storage of the beverage, no bacterial colonies including Escherichia coli, coliform, mold and yeast, and acid-fast bacteria were observed. Also, in the total count of microorganisms, no microbes were observed until the 21st day. This result showed the efficiency of the thermal process (at a given temperature and holding time), which is consistent with the results obtained by Vegara et al. (2013) in pomegranate juice [44]. The total count of microorganisms on the last day showed 9 Log CFU/ml, resulting in a visible increase in microbial growth during storage, indicating an increasing trend during storage, but this amount of microorganisms was not significant.

In line with the present results, de Oliveira Ribeiro et al. (2020), in the production of a non-fermented probiotic beverage with juçara fruit, reported that the population of molds and yeasts was less than 1 log CFU/ml, and the samples were free of Salmonella and the concentration of coliforms was less than 3 log CFU/ml, indicating that there were good hygienic conditions in the processing and during the storage of the beverage according to Brazilian regulatory standards [18].

3-4 Viability of probiotics during storage

According to the results, the viability and growth of Bifidobacterium bifidum was higher than that of Lactobacillus acidophilus. So that with three replicates and even at different inoculation concentrations, Lactobacillus acidophilus did not grow in the beverage samples. One of the most important issues in the production of probiotic products is the lack of change in the population of probiotic bacteria during the storage period of the product, as it has been proven that if the amount of these bacteria decreases during the storage period of the product, the probiotic effects of these bacteria will not be exerted on the host. For probiotics to be effective in humans, the number of viable microorganisms must exceed 10^6 CFU/ml to provide an adequate daily dose of 10^9–10^6 CFU/ml viable bacteria [45].

Figure 1 shows the trend of changes in Bifidobacterium counts in whey-based orange drink during refrigerated storage. On the seventh day of storage, the lowest and highest probiotic counts belonged to the control or fiber-free sample (6.69 log CFU/ml) and the sample containing wheat fiber (6.95 log CFU/ml), respectively. However, during 4 weeks of storage at 4°C, the number of probiotic bacteria in all beverage samples decreased by approximately two logarithmic units. Therefore, to provide this number of microbes in the probiotic beverage, it is recommended to add a larger number of probiotics to the beverage at the time of inoculation.

The main cause of death of microorganisms during storage of samples is high acidity and low pH and production of metabolites such as organic acids and lack of sugars. The highest population of probiotics after four weeks was still related to the drink sample containing wheat fiber. The difference in the growth rate of bacteria is related to their growth environment, which shows different reactions in different environments [46].

The wheat fiber-containing beverage was evaluated as suitable despite the decrease in the number of probiotics because after 21 days of refrigeration, the survival of probiotic bacteria in the refrigerator was more than 10^6 CFU/ml, which was in accordance with the limit recommended by the Iranian standard (10^6 CFU/ml).

In examining fibers, the results showed that wheat fiber performed better than inulin fiber and maintained greater probiotic viability. Also, in comparing long-chain and short-chain inulin fibers, the results showed that long-chain inulin performed better than short-chain inulin. However, overall, compared to the control sample (without fiber), the addition of fibers increased the viability and growth of probiotics.

In accordance with the present results, Rezaei et al. (2012) reported that adding 2% inulin to yogurt maintained the viability of Lactobacillus acidophilus and Bifidobacterium [47].

Tayebi & Ehsani (2020) reported that the presence of inulin in milk has a positive effect on the viability of probiotic bacteria in yogurt, and the effect of commercial inulin on the survival of probiotic bacteria was greater than that of inulin powder extracted from chicory root [7].

4- Conclusion

The aim of the present study is to produce a synbiotic sports drink based on whey enriched with calcium and vitamin D. In this study, the effect of long-chain and short-chain inulin fiber and wheat fiber as prebiotic compounds on the physicochemical properties and viability of probiotics in a sports drink during 28 days of storage at refrigerated temperature was investigated and compared. The results showed that the use of wheat fiber, unlike inulin fiber, significantly increased the pH of the beverage. As a result of adding inulin fibers, unlike wheat fiber, a significant increase in Brix and protein levels was observed. A significant increasing trend in the amount of dry matter in the beverage

was observed as a result of adding all three types of fibers. Probiotic viability in beverage samples decreased during 28 days of storage at refrigerated temperature, but overall, the addition of fibers increased probiotic viability compared to the control sample, with wheat fiber performing better than long-chain and short-chain inulin. So that after 21 days of storing the drink in the refrigerator, the probiotic viability in the drink containing wheat fiber was maintained at the defined level (for probiotic products, at least 10^6 cells per milliliter). According to the measurements taken, the levels of vitamin D3 and calcium were within the standard recommended range, which means that the sports drink can be enriched with calcium and vitamin D3 at 124 mg/100 g and 0.02 ppm, respectively, and benefit from its health-promoting properties without having an adverse effect on the quality properties of the product.

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Table 1. Physicochemical characteristics of beverages

Sample	Protein	dry matter	brix	pH
Control	0.77 ± 0.01^{b}	8.96 ± 0.04^{b}	8.5 ± 0.01^{b}	3.59 ± 0.005^{b}
Wheat fiber	0.78 ± 0.01^{b}	10.89 ± 0.03^{a}	8.5 ± 0.1^{b}	3.63 ± 0.015^{a}
Short chain inulin	0.87 ± 0.01^{a}	10.71 ± 0.57^{a}	10.46 ± 0.05^{a}	3.61 ± 0.011^{b}
Long chain inulin	0.87 ± 0.01^{a}	10.93 ± 0.03^{a}	10.4 ± 0.1^{a}	3.61 ± 0.018^{b}

alues with different letters within a column denote statistically significant differences ($P<0.05$).

Table 2. Analysis results of fortified drink

Calcium (mg per 100 grams)	Vitamin D (ppm)	Osmolality (milliosmol/kg)	Total sugar (%)
124	0.02	365	8

Table 3. The results of microbial test of drinks stored for 28 days					
Test	first day	7th day	14th day	21st day	28th day
Escherichia coli	ND	ND	ND	ND	ND
Coliform	ND	ND	ND	ND	ND
mold and yeast	ND	ND	ND	ND	ND
Acid resistant bacteria	ND	ND	ND	ND	ND
Total count	ND	ND	ND	ND	9Log

(ND = not detected)

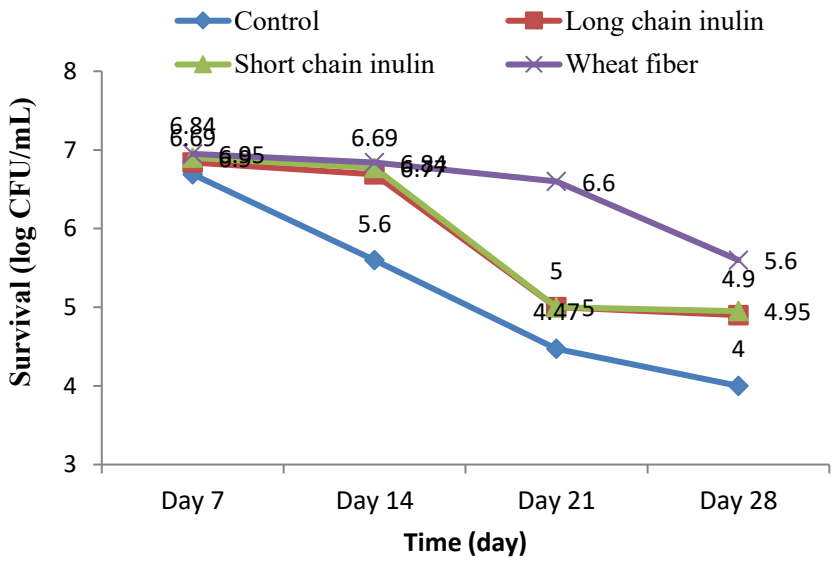

Fig. 1 The number of *Bifidobacterium* (log_{10} CFU/mL) in whey-based orange beverages during 28 days of storage at 4 ºC.

مجله علوم و صنایع غذایی ایران

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مقاله علمی_پژوهشی

مقایسه اثر فیبر اینولین و گندم بر زنده‌مانی پروبیوتیک در نوشیدنی ورزشی غنی‌شده با کلسیم و ویتامین D

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چکیده

هدف از این پژوهش، تولید یک نوشیدنی ورزشی سین‌بیوتیک بر پایه آب‌پنیر می‌باشد. آب‌پنیر به علت داشتن مواد معدنی، اسمولالیتی لازم را در نوشیدنی ورزشی فراهم می‌کند. در این پژوهش مقایسه اثر دو نوع فیبر اینولین بلندزنجیر و کوتاه زنجیر و فیبر گندم بر زنده‌مانی پروبیوتیک‌ها در نوشیدنی ورزشی طی ۲۸ روز نگهداری در دمای یخچال بررسی و مقایسه گردید. حضور این ترکیبات در نوشیدنی تاثیر مثبتی روی قابلیت زنده‌مانی پروبویتیک‌ها در نوشیدنی دارد که تاثیر فیبر گندم روی بقاء باکتری‌های پروبیوتیک بیش‌تر از فیبرهای اینولین بود. در نگهداری ۲۸ روزه نوشیدنی در یخچال، هیچ کلونی باکتریایی از جمله اشرشیاکلی، کلی‌فرم، کپک و مخمر و باکتری‌های مقاوم به اسید مشاهده نشد. همچنین در بررسی شمارش کلی میکروارگانیسم‌ها تا روز ۲۱ ام، هیچ‌گونه میکروبی مشاهده نگردید. این نتیجه کارایی فرآیند حرارتی (در دما و زمان نگهداری مشخص) را نشان داد. میزان ویتامین D_3 و کلسیم در بازه توصیه شده استاندارد قرار گرفت. نتایج نشان داد که می‌توان از کلسیم به میزان ۱۲۴ میلی‌گرم در ۱۰۰ گرم و ویتامین D به میزان ۰/۰۲ پی‌پی‌ام بدون تاثیر نامطلوب در فرمولاسیون نوشیدنی استفاده نمود. بنابراین می‌توان با غنی‌سازی نوشیدنی و استفاده از آن در رژیم غذایی کودکان و سالمندان از خواص ارزشمند آن بهره برد.