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## THE EFFECT OF RUNNER BEAN FLOUR ON THE VIABILITY OF *LACTOBACILLUS PLANTARUM* AND ON THE QUALITY CHARACTERISTICS OF PLANT-BASED PROBIOTIC BEVERAGES

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**Abstract.** In this study, the effect of runner bean flour on the viability of probiotic bacteria and the quality characteristics of plant-based probiotic beverages has been investigated. The germinated and non-germinated seeds were roasted, ground, sifted, and added to beverages based on soya milk, almond milk, and coconut milk. Then the plant-based beverages were inoculated with *Lactobacillus plantarum* and examined for chemical, rheological, sensory properties and probiotic bacteria count. The results have shown that bean germination increased the acidity, TEAC percentage, pH, total phenolic content, and improved the sensory properties. The acidity and pH of the plant-based probiotic beverage samples containing germinated beans were higher than those of the plant-based probiotic beverage samples containing non-germinated beans. The highest viscosity was observed in the almond beverage sample containing germinated and non-germinated beans, and the second highest was that of soya milk containing germinated and non-germinated beans. The highest sugar content was observed in the almond milk sample containing germinated beans and in the coconut milk sample containing germinated beans. The lowest amount of sugar was in the soya milk and coconut milk samples containing non-germinated beans. The highest amount of TEAC and total tocopherol content were in the coconut milk sample containing germinated beans, and the lowest amount of TEAC was in the soya milk sample containing germinated and non-germinated beans. The largest quantity of probiotic bacteria was observed in the beverage based on almond milk and coconut milk, and the smallest quantity of probiotic bacteria was in the beverage based on soya milk during the storage period of 28 days. The highest grade for the sensory attributes (taste, smell, appearance, and overall acceptability) was received by the coconut milk beverage containing germinated beans, the second highest being that of the almond milk samples containing germinated beans, and the lowest grade for these attributes was given to the soya milk beverage sample containing germinated and non-germinated beans. These results have shown that plant-based probiotic beverages based on coconut milk and almond milk containing germinated beans can be popular with consumers.

**Keywords:** runner beans, vegetable milk, *Lactobacillus plantarum*, probiotic beverage.

### Introduction. Formulation of the problem

Probiotic products in today's markets are usually found as dairy resources. However, due to special diets, including vegetarianism (consuming no meat), problems caused by high cholesterol levels of milk, and lactose intolerance in some people, the demand for non-dairy probiotic products has increased and led to focusing on developing plant-based probiotic products. An example is Rigobelo's research, with plant-based products as priority objects [1]. Probiotic beverages can be made from a variety of raw materials such as cereals, legumes, fruit, and

vegetables [2]. Vegetable milks are a good substitute for dairy beverages: ordinary milk contains whey protein and casein, which can cause allergies. The benefits of vegetable milks such as soya milk, almond milk, and coconut milk are due to the lack of cholesterol and lactose [3]. Legumes are high in protein (18–32%). Functional properties of legume proteins, such as water retention, fat binding, and gel formation, can expand their potential use in promoting a variety of foods [4]. Bean is an annual herbaceous plant with a segmented stalk. Beans rank first in the world in terms of areas of their cultivation, and are also the seventh major food product in the world.

Runner bean, with the scientific name *Phaseolus coccineus*, is an annual herbaceous plant, one of the different species of the legume family, and one of the most important legume products in the world. Its important features include its high protein content (20 to 35%) and faster cooking compared to other bean cultivars [5].

#### **Analysis of recent research and publications**

Different species of microorganisms are used as probiotics. The most common ones are bifidobacteria and lactic acid bacteria such as lactobacilli, lactococci, and streptococci [6]. Lactic acid bacteria are the main microorganisms used to produce traditional and new fermented food products. They are responsible for both the protective and the sensory properties of the product, such as colour, taste and texture [7]. *Lb. plantarum* is one of the most widespread and important species of lactic acid bacteria. The proven health effects of foods containing *Lb. plantarum* include reduction of gastrointestinal infection, lower risk of inflammatory bowel disease, and reduced irritating effects on the immune system. Studies have shown that *Lb. plantarum*, as a natural inhibitor in biologically processed foods, inhibits the growth of pathogenic bacteria and spoilage microorganisms during storage and increases the shelf life of a product [8]. *Lb. plantarum* strains cannot cause inflammation and are resistant to stomach acid and bile salts, so they have the potential of being introduced as probiotics [9]. Their sensitivity to processing conditions (such as high temperatures), storage conditions of the product (humidity, access of oxygen, temperature), and acidic conditions and bile salts in the gastrointestinal tract, makes it necessary to search for methods to increase the bioavailability of probiotics, so that a sufficient quantity of them can enter the intestinal environment and manifest their beneficial effects there [10].

Soya milk is a natural product of soya beans. It started being used about 2000 BCE. This product is one of the most popular milk substitutes for people with lactose intolerance or those allergic to cow's milk proteins [11]. Soya milk contains a variety of substances that can take part in lipid metabolism, including high quality proteins, unsaturated fatty acids, saponins, as well as phytosterols, lecithin, and isoflavones of soya [12]. Coconut milk is a liquid form of grated brown coconut fruit [13] and has the following nutritional properties: 3% of protein, 17–14% of fat, and 2% of carbohydrates. Coconut milk is cholesterol-free, but contains many vitamins, minerals, and electrolytes, including potassium, calcium, and chloride. One of the benefits of coconut milk is its ability to strengthen the immune system [14]. Besides, it has a large amount of lauric acid, which is found in significant amounts in breast milk and sebaceous gland secretions [13]. Almond milk is a good source of minerals (such as calcium), vitamins, and potassium. The basic functions of almond milk include strengthening the immune system, preventing cancer, maintaining overall kidney health, and reducing the

risk of cardiovascular disease. Almond milk is currently on the market in Europe as a nutritious alternative for customers with lactose intolerance and sensitive to cow's milk protein, and for people who are looking for a plant-based drinking milk substitute. The unique property of almond milk is high amounts of monounsaturated fatty acids. Besides, almonds have a balanced combination of protein, fat, and other beneficial plant compounds such as fibre, vitamins, antioxidants, and minerals [15].

Chavan *et al.* examined the development of fermented probiotic beverages (soya, almond, coconut milks) based on germinated and non-germinated cereals, and showed that fermentation with *Lb. acidophilus* improved the overall acceptability and functional properties of the drink during fermentation. The pH and acidity, the number of bacteria, and the polyphenol content increased in all the three samples. Sensory evaluation showed that the coconut milk-based probiotic beverage had a higher score than the soya milk and almond milk-based probiotic beverages [16].

Nakkarach and Withayagiat developed a non-dairy synbiotic beverage from riceberry malt extract (RME). The objectives of the study were to select suitable lactic acid bacteria (LAB) and to compare the stabilities of products made using free and encapsulated cells. Five LAB (*Enterococcus faecalis* N1-33, *Lactobacillus acidophilus* TISTR450, *Lactobacillus johnsonii* KUN119-2, *Lactobacillus plantarum* TC24, and *Lactobacillus reuteri* KUB-AC5) were tested for their ability to grow in RME, for their survival under simulated gastrointestinal tract conditions, and for their antimicrobial activity. *L. plantarum* TC24 had the greatest probiotic potential [17].

Mridula and Sharma examined development of non-dairy probiotic drinks made with the use of sprouted wheat, barley, pearl millet, green gram, separately with oat meal, a stabiliser and sugar, also using *L. acidophilus*-NCDC14 with soya milk. The acidity, pH, and probiotic count in probiotic drink samples based on wheat, barley, pearl millet, and green gram were found dependent on the level of flour from sprouted cereal and soya milk. The overall sensory acceptability grades for all the probiotic drink samples with soya milk were higher up to 6 g of wheat, barley, and green gram, and 4 g of pearl millet flour per a 100 ml portion of liquid (i.e. distilled water and soya milk in the ratio 1:1). Due to their good sensory acceptability grade (more than seven) and good probiotic count, wheat, barley, and green gram (6 g), and pearl millet (4 g), in combination with of soya milk and distilled water (in the ratio 50:50 ml), can be considered as an alternative for development of non-dairy probiotic drink using sprouted cereals and green gram [18].

**The purpose** of this study was to investigate the viability of probiotic bacteria in plant-based probiotic beverages containing runner bean flour and the quality characteristics of plant-based probiotic beverages containing runner bean flour.

**The objectives** of this study were:

- evaluating the viability of probiotic bacteria in non-dairy probiotic drinks containing runner bean flour;
- evaluating the quality characteristics of non-dairy probiotic drink containing runner bean flour;
- determining which of the plant-based beverages (soya milk, coconut milk, or almond milk) better helps maintain the survival of probiotics.

### Research materials and methods

**Materials.** Runner beans and soya beans were taken at the central market of cereals and grains of Mashhad, coconuts at the Mashhad fruit market, and almonds from trees native of the city of Quchan. Probiotic bacteria *Lb. plantarum* PTCC 1058 were purchased freeze-dried from the Persian Type Culture Collection and kept in a refrigerator under sterilised conditions. The culture media (MRS agar, MRS broth and buffered peptone water), NaOH 0.1 N, lead acetate, activated carbon, potassium oxalate, hydrochloric acid, phenolphthalein, methylene blue, and a DPPH reagent were used in this study. All chemicals and culture media used were purchased from Sigma Aldrich (USA) and Merck (Germany).

**Germination of runner beans and preparation of plant-based milk.** To prepare the bean seeds for germination, they were cleaned and washed and soaked in water at the ratio 1:2 at 30°C for 8h. After this, the water was drained, and the soaked bean seeds were kept under germination conditions for 24h, at the temperature 30°C and relative humidity 95%. The germinated beans were dried in hot air and then dried in an oven at 55±5°C till the moisture content was 8%. The germinated and non-germinated seeds were roasted in an oven at 130°C for 5 minutes and ground in an electric mill. Finally, they were sifted with a sieve shaker (mesh 60) and kept in a closed container at room temperature until consumption.

To prepare the vegetable milks, 20g of soya beans, 25g of almonds, and 25g of coconut were soaked in water at the ratios 1:6, 1:2 and 1:4, respectively, at room temperature for 12 h, and then mixed with a mixer. While mixing, to improve the taste of the drink, 6g of sugar was added, and then the drink was filtered through a double-layer filter cloth. The milk obtained was boiled for 5 min, and then cooled [16].

**Preparation of the probiotic bacteria culture.** To activate the *Lb. plantarum* PTCC 1058, which was purchased freeze-dried from the Persian Type Culture Collection, a needleful of lyophilised bacteria was added to 5ml of an MRS liquid medium and grown for 24–48h in a shaker incubator at 37°C. The sample was then inoculated in 95ml of an MRS liquid medium and cultured under the above conditions. At the end, the biomass was separated by refrigerated centrifugation at 8,000 rpm for 5 minutes at 4°C and washed in two steps with sterile 0.1% peptone water solution. It was

stored in sterile 0.1% peptone water solution and kept refrigerated until used [19].

**Preparation of the probiotic beverage.** 6g of whole germinated and non-germinated bean powder was mixed with 100ml of plant-derived milk to prepare the germinated soya milk probiotic drink (GSPD), non-germinated soya milk probiotic drink (NGSPD), germinated almond milk probiotic drink (GAPD), non-germinated almond milk probiotic drink (NGAPD), germinated coconut milk probiotic drink (GCPD), non-germinated coconut milk probiotic drink (NGCPD). A probiotic culture was added to the liquid part containing the formulation mixture at the ratio 1ml to 100ml, with the cell count 104cells/ml [16].

**Acidity and pH measurements.** Regarding lactic acid, the acidity was measured by titration against sodium hydroxide 0.1N using phenolphthalein solution as the reagent [20]. The pH meter was calibrated with pH: 7 and pH: 4 buffer solutions, respectively, and after that, the samples' pH values were read [21].

**Sugar content.** It was measured using the Lane-Eynon method according to standard number 2685 [21].

**Antioxidant activity. Trolox equivalent antioxidant capacity (TEAC).** For antioxidant activity, probiotic drinks were diluted in the ratio 1:100 (water:probiotic beverage). Free radical scavenging activity based on TEAC was calculated by the method described by Gat and Ananthanarayan [22].

**Total phenolic content (TPC).** Polyphenols were determined using the Folin-Ciocalteu reagent [22]. The absorbance of clear supernatant solution was read at 765nm using gallic acid as the standard. The results were expressed as equivalent to 1mg of gallic acid per 100g of dry weight.

**Viscosity.** To measure the viscosity of the samples, a rotary viscometer (Visco Elite-L model, made in the USA) was used. 100ml of the sample was poured into a beaker, and its temperature was maintained at 10°C using water and ice during the experiment. The viscosity of the samples was measured by spindle number 2 at 200 rpm.

**Probiotic bacteria count.** Under sterile conditions and under a biological safety hood, 10g of each sample was mixed with 90cm<sup>3</sup> of 0.1% sterile peptone water, and then a series of dilutions were prepared by adding 1cm<sup>3</sup> of each dilution to 9cm<sup>3</sup> of sterile peptone water. 0.1cm<sup>3</sup> of each dilution was cultured on MRS agar (containing 0.15% bile salt) and incubated at 37°C for 72h. Then plates containing 30–300 colonies were counted [23].

**Sensory evaluations.** The 9-point hedonic scale was adopted to rate each sensory attribute of the plant-based probiotic beverage samples (appearance, taste, consistency, and overall acceptability) from 1 to 9, grading 1 for “undesirable”, 5 for “neutral,” and 9 for “highly desirable.”

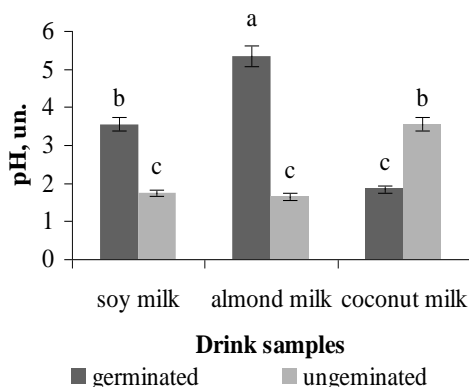
**Statistical analysis.** This study was performed on a completely randomised design. All the experiments

were replicated three times, and the average values were reported. The experimental results were determined by the analysis of variance (ANOVA) method ( $p < 0.05$ ) using SPSS software (version 20, SPSS Inc., Woking, Surrey, UK).

### Results of the research and their discussion

**Acidity and pH of the plant-based probiotic beverages.** The results of using runner bean flour to produce plant-based probiotic beverages from soya beans, almonds, and coconuts in both germinated and non-germinated forms have shown that there is a significant difference in acidity and pH in the samples of the plant-based beverages prepared, with the significance level  $p < 0.05$ .

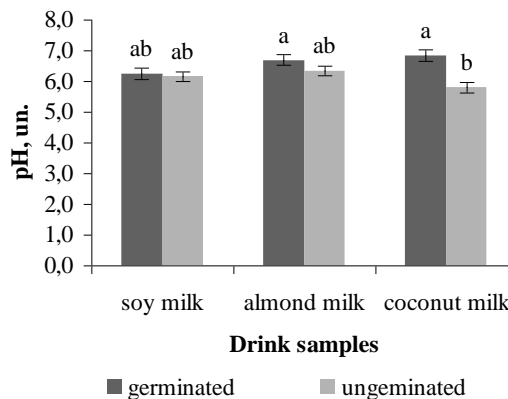
Examination of the acidity in the samples of soya milk, coconut milk, and almond milk containing germinated and non-germinated beans has allowed establishing that the acidity in the plant-based probiotic samples containing germinated beans was higher than that of the plant-based probiotic samples containing non-germinated beans (fig. 1). The highest acidity level was observed in the almond milk sample containing germinated beans, and the next highest was that of the soya milk containing germinated beans, with no significant difference from the coconut milk containing non-germinated beans. The lowest acidity level was observed in the sample of almond milk with germinated beans, without a significant difference (at the significance level 95%) from the soya milk containing germinated and non-germinated beans. These results have shown that bean germination increased the acidity of the soya milk and almond milk samples, but caused a decrease in the acidity of the coconut milk.



**Fig. 1. Acidity level in the plant-based probiotic drink samples of soya milk, almond milk, and coconut milk containing germinated and non-germinated beans**

pH is one of the most important factors that affect the quality of any probiotic product during storage. The pH determination results are shown in Fig. 2. Examination of the pH of the soya milk, coconut milk, and almond milk containing runner beans with and without germination has shown that the samples

differed, although not greatly, from each other, with the significant level 95%. The highest pH was observed in the coconut milk and the almond milk containing germinated beans, and the lowest pH was observed in the coconut milk with non-germinated beans.



**Fig. 2. pH in the plant-based probiotic samples of soya milk, almond milk, and coconut milk containing germinated and non-germinated beans**

Mousavi *et al.* in their results on a probiotic beverage showed that with a longer shelf life and increased flaxseed concentration, the pH decreased and the acidity increased. They stated that *Lb. acidophilus* was able to grow on flaxseed and produce lactic acid [24].

Korbekandi *et al.* examined changes in the pH and acidity of symbiotic beverages stored at 8°C and 30°C, and showed that higher temperatures stimulated bacterial metabolism and released more acid [25].

The results of this study were consistent with the results of Chavan *et al.* Their results showed that the acidity of plant-based beverages containing germinated and non-germinated cereals increased significantly with an increase in the amount of germinated and non-germinated cereals and legumes. Also, the acidity level in the probiotic almond milk was the highest, and the lowest level of acidity was observed in the probiotic drink containing distilled water of the germinated drink mixture. This resulted from the increase in nitrogen due to higher production of lactic acid in probiotic beverages, which increased the acidity of plant milk-based beverages [26]. Neither did they observe a significant difference between the germinated and non-germinated probiotic drink samples. The reason for no significant differences in the pH of the probiotic drinks is the hydrolysis of starch to sugars during germination: it is easily consumed by microorganisms and converted into lactic acid.

Nakkarach and Withayagiat in their research on plant-based probiotic drinks stated that after production, the acidity of the samples increased and the pH decreased. The acidity increases with a decrease in the pH during the beverage production process [17]. This decrease in the acidity prevents the growth of pathogenic microorganisms

during storage, thus making plant-based probiotic drinks safer to consume [27].

Mridula and Sharma in their research on preparation of probiotic plant-based drinks showed that when making plant-based probiotic drinks using germinated wheat, barley, millet and green peas separately with oats, sugar, and a stabiliser using soya, the level of acidity in the samples depended on the amount of soya bean added and the amount of millet flour. In the samples containing soya, with an increase in the level of millet flour, the acidity increased linearly, and the pH decreased [18].

*Sugar and viscosity of the plant-based probiotic beverages.* The results of using runner bean flour to produce probiotic beverages from soya beans, almonds, and coconuts containing germinated and non-germinated beans have shown that there was a significant difference in the sugar content and viscosity among the samples of plant-based beverages produced ( $p < 0.05$ ).

Viscosity is resistance to flow, and the higher its index is, the lower the fluidity of the beverage produced. Examination of the viscosity in the probiotic beverage samples from soya beans, almonds, and coconuts containing germinated and non-germinated beans has shown that there was no significant difference in the viscosity of probiotic beverages with non-germinated and germinated beans ( $p < 0.05$ ). The highest viscosity was in the sample of almond drink containing germinated and non-germinated beans, followed by that of the soya milk containing germinated and non-germinated beans ( $p < 0.05$ ). These results have shown that bean germination caused no difference in the viscosity of the beverage samples (fig. 3).

The increase in the viscosity can be attributed to the formation of strong gel in the product, with an increase in compounds in the beverage composition, such as fibre, protein, and sugar. During fermentation, probiotic bacteria produce various metabolites such as exopolysaccharides. Using soya beans, almonds, and coconuts also increases the content of these compounds in a product. Their interaction can change the viscosity of plant-based drinks.

Bernat *et al.* developed a probiotic fermentation product based on almond milk and inulin and showed that the use of these compounds in plant-based beverages increased the viscosity [28].

Milk compounds, food additives, and hydrocolloids have a very important effect on the viscosity of beverages. The property of water to make bonds with proteins and polysaccharide compounds makes it possible for the final product to have high consistency, better sensory properties, and lower hydration [29]. Almonds contain fibre and protein, which causes more water to be absorbed by these compounds into the product [30]. As a result, the viscosity of the drink prepared from almond milk was higher. It seems that the high water holding capacity and the ability of the gel to form soya flour proteins increased its viscosity [31].

*Sugar content of the non-dairy probiotic drinks.* Examination of the sugar content in the plant-based probiotic drinks from soya beans, almonds, and coconuts

containing both germinated and non-germinated beans has shown that the sugar content in the non-germinated plant-based probiotic drinks was higher compared to the drinks from soya beans, almonds, and coconuts with germinated beans (fig. 4). The highest amount of sugar was in the almond milk drink containing germinated beans and in the coconut milk containing germinated beans, without a significant difference ( $p < 0.05$ ), and the next highest content was in the soya milk containing germinated beans. The lowest sugar content was observed in the soya milk and coconut milk containing non-germinated beans with no significant difference at the significance level 95%. These results have shown that bean germination increased the sugar content of the plant-based probiotic drinks from soya beans, coconuts, and almonds.

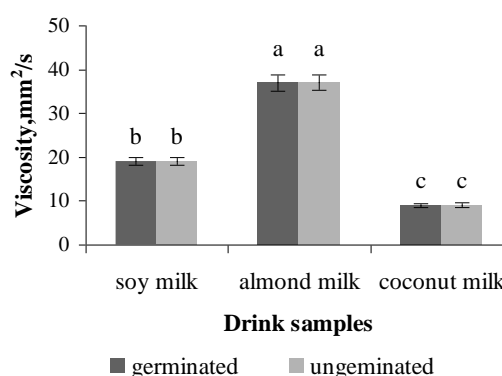


Fig. 3. Viscosity in the probiotic soya milk, almond milk, and coconut milk containing germinated and non-germinated beans

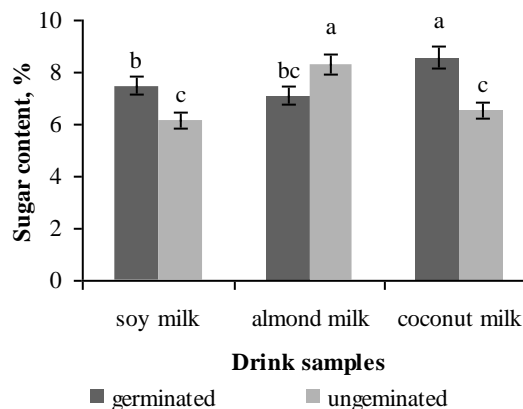


Fig. 4. Sugar content in the probiotic drinks based on soya milk, almond milk, and coconut milk containing germinated and non-germinated beans

*Antioxidant activity of the plant-based probiotic drinks.* The results of using runner bean flour to make probiotic drinks from soya beans, almonds, and coconuts, in both the germinated and the non-germinated form, have shown that among the beverage samples produced, the values of Trolox equivalent antioxidant capacity and total phenolic content were significantly different ( $p < 0.05$ ) (table 1).

**Table 1 – Trolox equivalent antioxidant capacity (TEAC) and total phenolic content (TPC) in the probiotic beverages based on soya milk, almond milk, and coconut milk**

Sample	Germinated			Non-germinated		
	soya milk	coconut milk	almond milk	soya milk	coconut milk	almond milk
TEAC (% P.S.C)	1.95 <sup>c</sup>	6.15 <sup>a</sup>	4.95 <sup>b</sup>	1.40 <sup>c</sup>	4.20 <sup>b</sup>	3.25 <sup>bc</sup>
TPC(mM G.A.E)	3.30 <sup>c</sup>	5.10 <sup>a</sup>	4.25 <sup>b</sup>	2.45 <sup>d</sup>	4.6 <sup>ab</sup>	3.4 <sup>c</sup>

Different letters denote a significant difference ( $p < 0.05$ )

Measuring the DPPH free radical scavenging activity is widely used as a valid yet simple test to evaluate the antioxidant activity of fermented beverages. This test allows quickly evaluating the antioxidant activity of a large number of samples.

Besides, the high sensitivity of DPPH radicals makes it possible to determine the radical scavenging activity of low concentrations of antioxidant compounds.

Examination of the Trolox equivalent antioxidant capacity (TEAC) in the probiotic plant-based beverages from soya beans, almonds, and coconuts containing both germinated and non-germinated beans has shown that the TEAC in the plant-based probiotic beverage containing germinated beans was higher than in the plant-based probiotic drinks from soya beans, almonds, and coconuts containing non-germinated beans. The highest value of TEAC was in the sample of the coconut milk drink containing germinated beans. The second highest was in the almond milk and coconut milk containing germinated beans, with no significant difference ( $p < 0.05$ ), and the next highest was that of the soya milk containing germinated beans. The lowest TEAC value was observed in the samples of soya milk drinks containing germinated and non-germinated beans, without a significant difference, at the significance level 95%. These results have shown that bean germination increased the TEAC value in the soya bean, coconut, and almond-based probiotic drinks.

Studying the total tocopherol content in the plant-based probiotic beverages made from both germinated and non-germinated soya beans, almonds, and coconuts has shown that the total tocopherol content in the samples containing germinated beans was higher compared to the drinks made from non-germinated soya beans, almonds, and coconuts. The highest total tocopherol content was in the sample of the coconut milk drink containing germinated beans, followed by the samples of almond milk containing germinated beans and non-germinated coconut milk, with no significant difference ( $p < 0.05$ ). The lowest value of TEAC was observed in the sample of the soya milk beverage containing non-germinated beans. These results have shown that bean germination increased the content of total tocopherol in the soya, coconut, and almond-based probiotic beverages.

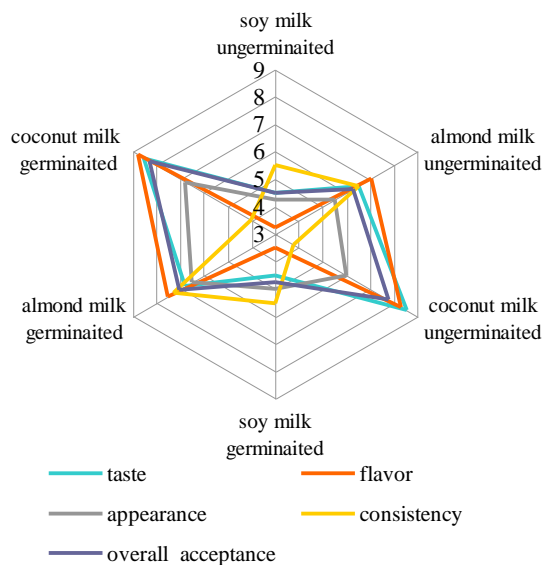
Many researchers have confirmed that fermentation improves antioxidant properties. This effect is mostly attributed to bioactive peptides

resulting from the activity of microorganisms, extracellular secretion of microorganisms, and activation of phenolic compounds in the material [32].

The results of this study are consistent with the results obtained by Chavan *et al.* [16]. The value of TEAC in the mixture of plant-based drinks without germinated cereals and legumes in all samples increased significantly with an increase in the amount of the mixture of germinated beverages in probiotic drinks. The TEAC value was relatively low in the soya-based probiotic drink and higher in the coconut-based probiotic drink. The researchers established that any substance present in lower concentrations significantly delayed or inhibited the oxidation of that substance. They also found that the TPC increased significantly with an increase in the amount of the germinated beverage mixture in all samples. They explained the higher phenolic compounds in the coconut milk (compared to other probiotic drinks) by higher levels of gallic acid, which is considered the equivalent of phenolic compounds [16].

*Sensory characteristics of the plant-based probiotic drinks.* Using germinated and non-germinated runner bean flour to make plant-based probiotic drinks from soya beans, almonds, and coconuts has resulted in a significant difference in the sensory ratings of the plant-based beverages produced ( $p < 0.05$ ). The results of sensory evaluation of the probiotic drinks made from soya beans, almonds, and coconuts containing germinated and non-germinated beans are shown in Fig. 5. Analysis of the taste, smell, appearance, and overall acceptability characteristics has shown that the samples of plant probiotic drinks containing germinated beans were not significantly different from the samples of plant probiotic drinks prepared from soya beans, almonds, and coconuts containing germinated beans ( $p < 0.05$ ). The highest grade for the sensory attributes of taste, smell, appearance, and overall acceptability was given to the drink prepared from germinated coconuts. It was followed by the samples of germinated almond milk and non-germinated coconut milk, with no significant difference ( $p < 0.05$ ). The lowest grade for the sensory properties was received by the beverage containing germinated and non-germinated soya beans. These results have shown that the plant-based probiotic drinks from germinated coconuts and almonds were more popular with consumers. For the sensory attribute of consistency, the highest grade was given to the sample of germinated and non-germinated almond

flour, and then to that containing germinated and non-germinated soya beans, with the significance level 95%.



**Fig. 5. Sensory properties of the plant-based probiotic drink samples (soya milk, almond milk, and coconut milk) containing germinated and non-germinated beans**

The results of this study were consistent with the results of Chavan *et al.* In this study, it has been observed that the sensory rating of all probiotic drinks based on plant milk was good and they were acceptable as beverages. The best sensory characteristics were observed in the probiotic drink based on coconut milk, compared to the samples of probiotic drinks containing distilled water, soya milk, and almond milk [16].

Kemsawasd and Chaikhram, who studied preparation of soya-based probiotic drinks, showed that all probiotic drinks had similar grades for all sensory attributes. They established that oral sensation caused by the soya-containing samples deteriorated during storage due to the acerb taste and increased acidity or sourness [33].

Mridula and Sharma experimented with plant-based probiotic drinks using germinated wheat, barley, millet, and green peas separately with oats and sugar with soya. Their results showed that in plant-based probiotic drinks, the mean grades for different sensory properties of sugar-free and soya-containing samples were less than 7, which was not desirable for

marketing the products. This showed that sugar and soya were important not only for the optimal growth of probiotics in the wheat-based probiotic beverage samples, but also for consumer acceptance [18].

*Survival of probiotic bacteria in the plant-based drinks.* Analysis of the results obtained using runner bean flour to make probiotic drinks from soya beans, almonds, and coconuts containing germinated and non-germinated beans has shown that there was a significant difference among the drink samples produced in terms of the survival rate of probiotic bacteria at different times (1, 7, 14, 21, and 28 days) ( $p < 0.05$ ) (table 2).

Survival of probiotic bacteria in probiotic beverages containing soya milk, almond milk, and coconut milk with germinated and non-germinated beans has shown that the survival rates of probiotic bacteria in the beverage samples containing germinated and non-germinated beans did not differ significantly ( $p < 0.05$ ). The highest survival rate of probiotic bacteria was in the coconut milk drink containing germinated and non-germinated beans, and in the almond milk containing germinated and non-germinated beans. The lowest survival rate of probiotic bacteria was observed in the samples with soya milk containing germinated and non-germinated beans. The same tendency was observed during storage of the plant-based drinks. These results have shown that the survival rate of probiotic bacteria in the soya milk-based drinks was lower than that of the almond milk and coconut milk. The storage of the plant-based drink for 28 days reduced the quantity of probiotic bacteria, and by day 21, the probiotic bacteria count in these products had become acceptable. Chavan *et al.* in a study of a plant-based drink with germinated and non-germinated cereals showed that an increase in the concentration of the mixture of germinated cereals in the probiotic drink resulted in an increase in the probiotics count observed in all samples. These results suggested that a mixture of water, soya, almond milk, and coconut milk was suitable for bacterial growth [16].

Kemsawasd and Chaikhram showed that the survival of probiotic bacteria was influenced by the nature of probiotic strains, storage time, and especially the presence of constituents of plant-based drinks.

**Table2 – Total count of probiotic bacteria in the plant-based probiotic drink samples (soya milk, almond milk, and coconut milk) containing germinated and non-germinated beans (CFU/ml)**

Day	Non-germinated			Germinated		
	Almond milk	Coconut milk	Soya milk	Almond milk	Coconut milk	Soya milk
1	$2 \times 10^9$ a	$5 \times 10^9$ a	$2 \times 10^8$ ab	$4 \times 10^9$ a	$6 \times 10^{10}$ ab	$4 \times 10^8$ ab
7	$2 \times 10^8$ ab	$3 \times 10^8$ ab	$4 \times 10^7$ b	$4 \times 10^8$ ab	$4 \times 10^8$ ab	$5 \times 10^7$ b
14	$3 \times 10^7$ b	$2 \times 10^7$ b	$4 \times 10^6$ bc	$5 \times 10^7$ b	$4 \times 10^7$ b	$8 \times 10^6$ bc
21	$3 \times 10^6$ bc	$4 \times 10^6$ bc	$3 \times 10^6$ bc	$5 \times 10^6$ bc	$9 \times 10^6$ bc	$2 \times 10^6$ bc
28	$1 \times 10^6$ bc	$2 \times 10^6$ bc	$7 \times 10^5$ bc	$3 \times 10^6$ bc	$4 \times 10^6$ bc	$9 \times 10^5$ bc

Different letters denote a significant difference ( $p < 0.05$ )

These researchers established that mulberry leaves contained many bioactive compounds with antioxidant properties. Flavonoids, alkaloids, polysaccharides, phenolics, and sterols in plant constituents are able to protect probiotic cells in food matrices from oxidative damage and prolong the survival of probiotics during storage [33]. Mridula and Sharma established that soya and sugar added both had a positive effect on the probiotic count in probiotic drink samples containing germinated beans based on plant compounds and increased the survival of probiotic bacteria in plant-based drinks [18]. Mousavi *et al.* in their research on a plant-based probiotic drink from flaxseed showed that the number of probiotic bacteria of *L. acidophilus* was directly related to the flaxseed concentration. It was found that flaxseed significantly increased the number of probiotic bacteria. The number of *L. acidophilus* decreased during storage. However, the reduction rate in the samples containing flaxseed was less than in the control sample [24]. Withayagiat and Nakkarach showed that the number of viable cells in the plant-based symbiotic drink made from rice malt increased during the first 4 days of incubation and decreased after 2 weeks, but was within the range set for probiotic products. The researchers also considered how the temperature of the storage of a product effected on the survival of probiotic bacteria, and established that the survival rate of the bacteria was higher in the beverage stored at 8°C [17].

Various factors effect on the survival of probiotic bacteria: the formulation of a product (probiotic bacterial strains, microbial interactions, pH and acidity, presence of hydrogen peroxide, molecular oxygen, presence of growth enhancers and food additives, salt, microencapsulation), incubation time, conditions of the production process (inoculation temperature, heat treatment, inoculation type, storage temperature), and packaging [10].

### Conclusion

The purpose of this study was to investigate whether the high functional properties of flour from runner beans, both germinated and non-germinated, make it practical to use it in production of probiotic beverages (soya milk, almond milk, and coconut milk). The results have shown that bean germination increased the values of such parameters as acidity, pH, TEAC, and total phenolic content, and improved the sensory characteristics. The level of acidity and pH in the plant-based probiotic beverage samples containing germinated beans was higher than that of the plant-

based probiotic beverages (soya milk, almond milk, and coconut milk) containing non-germinated beans. The highest acidity was in the sample of the almond milk drink containing germinated beans, and the lowest acidity was in the sample of the almond milk drink containing germinated beans and soya milk containing germinated and non-germinated beans.

Viscosity is an important feature largely responsible for the acceptability and marketability of various beverages. In this study, it has been observed that using almond milk and soya milk helps the formation of weak gel and strengthens the internal network of the drink, thus increasing this physical parameter. The amount of sugar in the plant-based probiotic drink samples containing germinated beans was higher than in the soya milk, almond milk, and coconut milk drinks containing germinated beans. The highest sugar content was observed in the samples of almond milk and coconut milk drinks containing germinated beans. The lowest amount of sugar was observed in the samples of soya milk and coconut milk drinks containing non-germinated beans.

The DPPH free radical scavenging activity has been studied as an indicator of antioxidant activity. The use of almond milk, soya milk, and coconut milk made antioxidant compounds more active. The highest TEAC and total tocopherol content were observed in the sample of coconut milk drink containing germinated beans, and the lowest value of TEAC was in the sample of soya milk drink containing germinated and non-germinated beans. In this study, it has been observed that all the parameters under study had a significant effect on the number of probiotic bacteria. Studying the survival of probiotic bacteria in the soya milk, almond milk, and coconut milk-based drinks containing germinated and non-germinated beans has shown that the highest count of probiotic bacteria was in the almond and coconut milk-based drinks, and the lowest count of probiotic bacteria was in the drink based on soya milk during 28 days of storage.

As for the taste, smell, appearance and overall acceptability characteristics, they were rated the highest in the coconut milk-based drink containing germinated beans, then in the almond milk-based drinks containing germinated beans. The lowest grades for the sensory attributes were given to the soya milk-based drinks containing germinated and non-germinated beans. These results have shown that the probiotic drinks based on coconut milk and almond milk containing germinated beans found better consumer acceptance.

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