

Effects of germinated and ungerminated grains on the production of non-dairy probiotic-fermented beverages

Wanyu Liu¹, Shengjie Li¹, Ning Han², Hongwu Bian², Dafeng Song^{1*}

¹Key Laboratory for Food Microbial Technology of Zhejiang Province, School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou, Zhejiang, China; ²Institute of Genetics and Regenerative Biology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, China

***Corresponding Author:** Dafeng Song, Key Laboratory for Food Microbial Technology of Zhejiang Province, School of Food Science and Biotechnology, Zhejiang Gongshang University, No. 18, Xuezheng Str, Hangzhou 310018, Zhejiang, China. Email: dfsong@zjsu.edu.cn

Received: 4 April 2021; Accepted: 28 February 2022; Published: 19 April 2022

© 2022 Codon Publications

OPEN ACCESS



RESEARCH ARTICLE

Abstract

Non-dairy probiotic-fermented beverages were produced from germinated and ungerminated barley, highland barley and rice, which were germinated for 48–96 h at 30°C, then dried, baked, ground, mixed and blended in a ratio of 3:2:1. Varying amounts of the mixture (0, 2, 4, 6, and 8 g) were added to soybean milk, peanut milk or coconut milk. Distilled water was used as a control. Sucrose (4 g) was added to each solution, followed by inoculation with *Lactobacillus plantarum* strain ZJ5 for 6 h. After fermentation, the pH, acidity, bacterial counts and antioxidant activity were measured and sensory evaluations were performed. Increase in the concentration of grain mixture to 8 g increased the pH, acidity, probiotic count, antioxidant content and polyphenol content values as well. The antioxidant capacity of germinated and ungerminated grain probiotic beverages was 1.08–1.26 mM and 0.69–1.08 mM, while the total phenolic content was 1.10–3.91 mM and 1.03–2.79 mM, respectively. The sensory evaluation scores of coconut beverages were higher than those of other beverages. Probiotic beverages containing 6 g of cereal mixture had the highest acceptability scores. Beverages containing germinated, as opposed to ungerminated, grains had higher nutritional value and better sensory qualities. The results highlighted the importance of germinated grains to improve the quality of non-dairy probiotic-fermented beverages.

Keywords: grain; *Lactobacillus plantarum* ZJ5; germination; non-dairy; fermented drink

Introduction

Probiotics are beneficial microorganisms that inhibit intestinal pathogens (Quigley, 2019) and improve immune function (Maldonado Galdeano *et al.*, 2015) with utility for the treatment of diarrhea (Andrade *et al.*, 2017). Probiotic beverages are prepared by inoculation of raw material with lactic acid bacteria or other bacteria and addition of buttermilk powder or sweet whey powder after fermentation (Skryplonek and Jasinska, 2015). The use of probiotics not only inhibits the growth of harmful

bacteria (Wagner and Johnson, 2017) but also enhances nutrient absorption and improves flavor. *Lactobacillus plantarum* used in this study is a class of Gram-positive, anaerobic or facultative anaerobic lactobacillus that improves gastrointestinal function, lower cholesterol and possess antioxidant activity (Seddik *et al.*, 2017).

Grains contain starches, crude proteins, crude fats, reducing sugars, dietary fibers, mineral elements and other nutrients (Liu *et al.*, 2019). Addition of grains to the diet reduces blood lipid levels and blood pressure

(Holloender *et al.*, 2015). As Compared with ungerminated grains, germinated grains have higher contents of amino acids, antioxidants and nutrients. Germination is a practical and natural dietary intervention to increase the benefits, palatability and acceptability of grains (Nelson *et al.*, 2013).

Highland barley from Tibet, as a grain raw material, is rich in β -glucan, flavonoids and vitamins. β -glucan reduces blood cholesterol, regulates blood glucose levels and enhances immunity (Zhang *et al.*, 2019). The nutritional quality of highland barley differs according to the region of origin. For example, highland barley from Qinghai province has the highest protein content, while the Tibetan variety has the highest β -glucan content, the one from Sichuan province has the highest levels of starch and ash, and the variety grown in Yunnan province has the highest amylose, fat, and fiber contents (Xu *et al.*, 2016). Therefore, it is very important to select highland barley with high nutritional value for the development of non-dairy cereal products.

As an emerging functional food, non-dairy products are produced by probiotic fermentation of a variety of cereals. Non-dairy milk, which contains no cholesterol or lactose, is suitable for consumers with lactose intolerance (Gupta and Abu-Ghannam, 2012). Hence, probiotic fermentation of grains has a potential market value.

Following were the aims of this study: (1) To develop grain products with improved nutritional value; (2) compare the physicochemical properties of germinated versus ungerminated grains; (3) develop non-dairy beverages through probiotic fermentation of plant materials; and (4) increase the use of Tibetan highland barley in food.

Materials and Methods

Materials and bacterial strains

Rice, soybeans, peanuts and coconuts were purchased from a supermarket in Hangzhou, China. Barley (Meng 1) was provided by Liu Zhiping of the Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences (Hohhot, China). Highland barley (Gan keng 5) was provided by Gansu Yongxin Agricultural Science and Technology Co. Ltd. (Lanzhou, China). *Lactobacillus plantarum* strain ZJ5 was produced in-house (Song *et al.*, 2014). All chemicals were purchased from Sangon Biotech Co. Ltd. (Shanghai, China).

Grain germination and pulverization

Grains were washed with deionized water at room temperature and immersed in water at 30°C in a grain:water

ratio of 1:2. After 8 h, water was drained and grains were germinated at 30°C and 95% relative humidity for 96 h (highland barley) and 96 h (barley) or 48 h (rice) in our laboratory. Following germination, the grains were dried at $55 \pm 5^\circ\text{C}$ in an oven to reduce their moisture content to $\sim 8\%$ and then baked for 5 min at 130°C. The baked grains were mixed in a ratio of 3:2:1 (barley: highland barley:rice), milled in an electrical grinder and passed through a 100- μm mesh sieve (Donkor *et al.*, 2016).

Preparation of plant milk-like product

For milk preparation, soybeans, peanuts and deshelled coconut meat were washed with deionized water at room temperature and soaked for 12 h in water in ratios of 1:6, 1:4 and 1:4, respectively. After soaking, the materials were ground using a homogenizer, then boiled and filtered.

Preparation of strains

L. plantarum strain ZJ5 was inoculated in De Man, Rogosa and Sharp (MRS) broth, a universal culture medium suitable for the growth of *Lactobacillus* sp., for 24 h. The sample was then collected by centrifugation at 8,000 rpm, washed thrice in physiological saline solution and adjusted to a concentration of 10^4 cells/mL. The concentrations of the samples were confirmed by measuring absorbance at 600 nm with a spectrophotometer.

Probiotic beverage production

Following amounts of germinated and ungerminated grain flours were mixed in 100 mL of water, soy milk, peanut milk and coconut milk: 0 g (no grain flour), 2 g, 4 g, 6 g and 8 g. Then 4 g of sugar and 1 mL of *L. plantarum* ZJ5 suspension were added to each solution. The final products included germinated distilled water probiotic beverages (GDW), ungerminated distilled water beverages (UGDW), germinated soy beverages (GS), ungerminated soy beverages (UGS), germinated peanut beverages (GP), ungerminated peanut beverages (UP), germinated coconut beverages (GC) and ungerminated coconut beverages (UC). All beverages were fermented at 37°C for 6 h in triplicate.

Determination of acidity and pH

Acidity was determined by titration using 1% phenolphthalein as an indicator (Ough *et al.*, 1965). All experiments were repeated for three times.

Antioxidant capacity (AC)

Solutions were diluted according to the proportion of probiotic beverages: water = 1:100. Antioxidant capacity was measured using a total antioxidant capacity assay kit (Beyotime Institute of Biotechnology, Haimen, China) with the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method. The sample solutions were directly used for detection. The 10 mM Trolox standard solution was diluted with phosphate-buffer saline (PBS) to 0.15, 0.3, 0.6, 0.9, 1.2 and 1.5 mM. Then, 200 μ L of ABTS working solution was added to each well of a 96-well plate with 10- μ L distilled water or PBS as a blank control. In addition, 10 μ L of Trolox standard solution at various concentrations was included to generate a standard curve. Finally, 10 μ L of different samples were added to designated wells. After gentle mixing, the plate was incubated at room temperature for 2–6 min and absorbance was measured at 734 nm. Total antioxidant capacity of each sample was calculated with reference to the standard curve. All experiments were repeated for three times.

Determination of total phenolic content (TPC)

The Folin–Ciocalteu method was employed to assess the TPC of samples (Finley *et al.*, 2015). Briefly, 100 μ L of grain extract or standard, 100 μ L of MeOH, 100 μ L of Folin–Ciocalteu reagent and 700 μ L of Na₂CO₃ were added in a 1.5-mL micro-centrifuge tube, immediately vortexed, incubated for 20 min in the dark at room temperature, and then centrifuged at 13,000 rpm for 3 min. (Alvarez-Jubete *et al.*, 2010). Absorbance was measured at 765 nm and standard curves were generated from gallic acid standards to determine TPC, which was expressed as mg/L gallic acid equivalent (GAE) per 100 g of dry weight.

Sensory evaluations

Quality of beverages was randomly assessed by 20 evaluators. Beverages were put in coded glass bottles and scored for consistency, appearance and taste as follows: 9 = extremely good; 8 = excellent; 7 = medium; 6 = slightly good; 5 = neither like nor hate; 4 = slightly poor; 3 = moderately poor; 2 = destitute; 1 = extremely poor. The sensory evaluation test was approved by the Ethics Committee of the University of Shanghai (Shanghai, China).

Probiotics count

Fermented drinks were diluted with a gradient series of physiological saline solution and spread on MRS agar plates (pH 6.4 $\pm$ 2). The total number of colonies was evaluated following incubation for 24 h at 37°C.

Statistical analysis

Data were presented as mean $\pm$ standard error (SE). All experiments were repeated at least for three times. One-way analysis of variance was used to identify significant differences between groups. A probability (*P*) value of ≤ 0.05 was considered statistically significant.

Results and Discussion

Acidity

The acidity profiles of probiotic drinks are shown in Figure 1. The acidity of grain-based drinks significantly increased following the addition of mixed grain flour ($P < 0.05$). In addition, the acidity of distilled water, soy milk, peanut milk and coconut milk increased with increasing amount of germinated grain powder by 0.05–0.18%, 0.45–0.71%, 0.27–0.51% and 0.27–0.59%, respectively, as well as with increasing amount of ungerminated grain powder by 0.05–0.18%, 0.45–0.62%, 0.27–0.47% and 0.27–0.44%, respectively. As compared to probiotic drinks with ungerminated grain powder, drinks with germinated grain powder were more acidic. The most acidic beverage was germinated peanut drink, while the least acidic beverage was distilled water. Probiotic drinks containing ungerminated grain flour and coconut milk had the highest, while those with distilled water had the lowest acidity. Higher levels of nitrogen in germinated cereal flours promoted lactic acid production by *L. plantarum*, which increased the acidity of beverage.

pH

The pH values of beverages were assessed after 6 h of fermentation (Figure 2). The pH ranges of probiotic beverages with germinated grain powder in distilled water, soy milk, peanut milk and coconut milk were 6.18–7.65, 5.78–6.53, 6.29–6.92 and 5.72–6.87, while those of probiotic beverages with ungerminated grain powder were 6.12–7.65, 6.21–6.48, 5.82–6.92 and 6.32–6.87, respectively. No significant difference in the pH values of the beverages was observed ($P > 0.05$) because starch was hydrolyzed by amylase during germination into dextrin and monosaccharides, which were converted into lactic acid by *L. plantarum*.

Antioxidant capacity

The effects of mixed grain flour and milk on antioxidant capacity are described in Table 1. In the absence of germinated grain flour (0 g), the antioxidant capacity of distilled water, soy milk, peanut milk and coconut milk

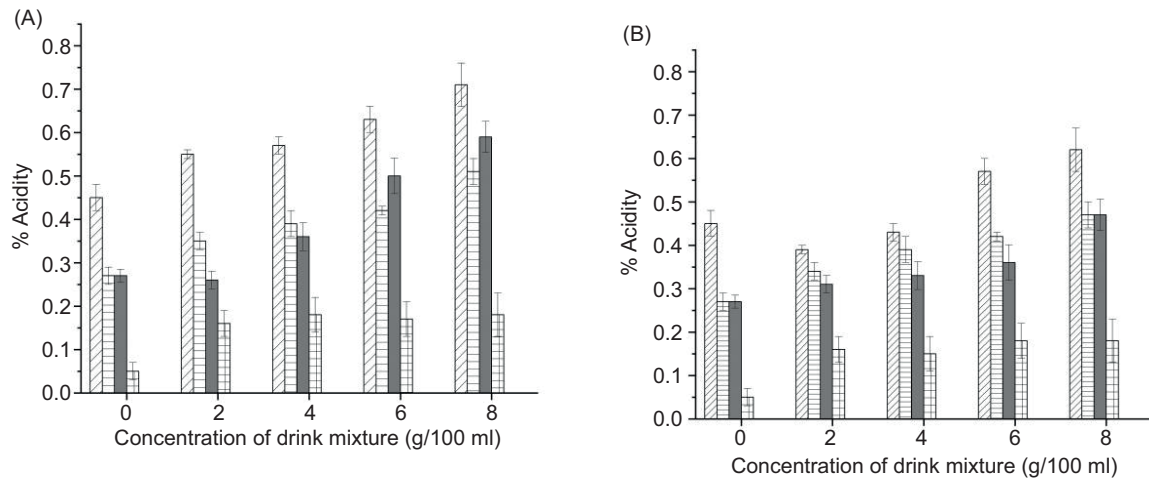


Figure 1. Acidity of the different milk probiotic drinks. (A) Germinated grain probiotic drinks; (B) Ungerminated grain probiotic drinks. Soybean milk probiotic fermented beverage, Peanut milk probiotic fermented beverage, Coconut milk probiotic fermented beverage, Distilled water probiotic fermented beverage (data are the mean $\pm$ standard deviation, $n = 3$).

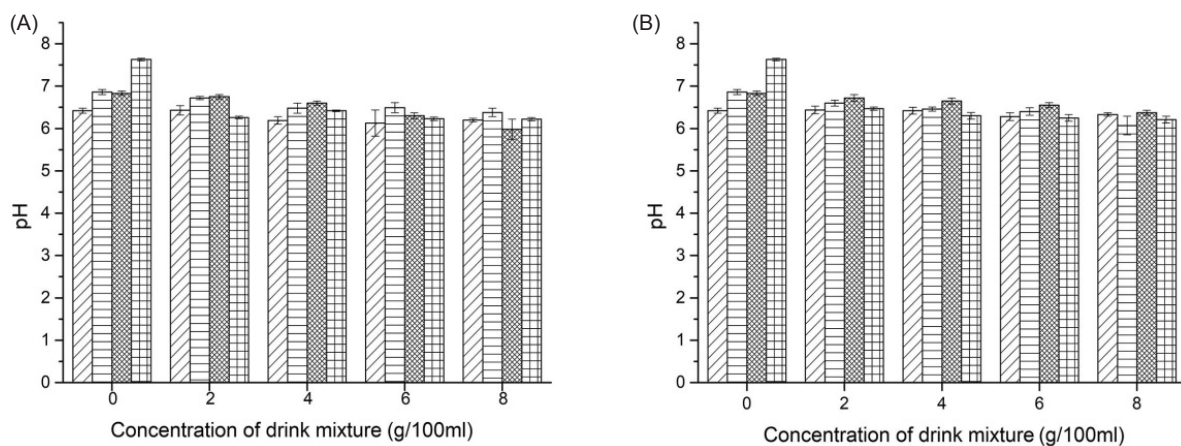


Figure 2. pH of the different milk probiotic drinks. (A) Germinated grain probiotic drinks; (B) ungerminated grain probiotic drinks. Soybean milk probiotic fermented beverage, Peanut milk probiotic fermented beverage, Coconut milk probiotic fermented beverage, Distilled water probiotic fermented beverage (data are the mean $\pm$ standard deviation, $n = 3$). After statistical analysis, no significant differences in the pH of the beverages were observed ($p > 0.05$).

was 0.13, 0.77, 0.86 and 0.78 mM Trolox, respectively. The antioxidant capacity significantly increased following the addition of germinated grain powder ($P < 0.05$). When 8 g of germinated grain powder was added, the antioxidant capacity reached the maximum values of 1.08, 1.26, 1.17 and 1.11 mM Trolox, respectively. The antioxidant capacity of drinks containing ungerminated grain flour was lower than that of germinated grain beverages ($P < 0.05$). Distilled water had the lowest antioxidant capacity (0.13–0.69 mM Trolox) because of enhanced matrix oxidation. A previous study (Donkor *et al.*, 2012) reported significant free radical scavenging activity of germinated cereals as compared with ungerminated cereals, especially germinated rye and sorghum.

The antioxidant capacity of germinated wheat and buck wheat also increased (Alvarez-Jubete *et al.*, 2010), consistent with the findings of the present study. The antioxidant capacity of germinated grains was greater than that of ungerminated grains.

Total phenolic content

The TPC of each sample is shown in Table 1. The TPC of probiotic beverages containing distilled water, soy milk, peanut milk and coconut milk with germinated grain flour was 0.89–1.10, 2.10–2.91, 2.17–2.68 and 1.64–2.32 mM GAE, while TPC with ungerminated grain

Table 1. Effect of grain concentration and milk on antioxidant assay and total phenolic content.

Grain concentration (g/100 mL)	GS	UGS	GP	UGP	GC	UGC	GDW	UGDW
Antioxidant capacity (mM Trolox)								
0	0.77±0.07 ^a	0.77±0.07 ^a	0.86±0.04 ^a	0.86±0.04 ^a	0.78±0.02 ^a	0.78±0.02 ^a	0.13±0.10 ^a	0.13±0.10 ^a
2	0.88±0.08 ^b	0.81±0.02 ^b	1.08±0.09 ^b	0.90±0.08 ^b	0.87±0.07 ^b	0.77±0.02 ^a	0.68±0.05 ^b	0.31±0.04 ^b
4	1.19±0.06 ^c	0.85±0.02 ^c	1.11±0.09 ^c	0.97±0.03 ^c	0.88±0.02 ^b	0.82±0.01 ^b	0.72±0.07 ^c	0.41±0.08 ^c
6	1.22±0.04 ^d	0.89±0.01 ^d	1.12±0.02 ^c	1.01±0.04 ^d	1.00±0.04 ^b	0.98±0.06 ^c	1.03±0.05 ^d	0.58±0.02 ^d
8	1.26±0.04 ^e	0.94±0.03 ^e	1.17±0.02 ^d	1.08±0.01 ^e	1.11±0.02 ^c	1.02±0.03 ^d	1.08±0.05 ^e	0.69±0.01 ^e
TPC (mM GAE)								
0	2.10±0.16 ^a	2.10±0.16 ^a	2.17±0.15 ^a	2.17±0.15 ^a	1.64±0.10 ^a	1.64±0.10 ^a	0.89±0.01 ^a	0.89±0.01 ^a
2	2.33±0.03 ^b	2.64±0.08 ^b	2.32±0.05 ^b	2.33±0.06 ^b	1.94±0.13 ^b	1.83±0.07 ^b	0.90±0.01 ^a	0.90±0.01 ^b
4	2.45±0.07 ^c	2.59±0.06 ^c	2.30±0.07 ^b	2.35±0.12 ^b	1.99±0.20 ^c	1.93±0.08 ^c	0.97±0.02 ^b	0.93±0.01 ^b
6	2.59±0.06 ^d	2.51±0.09 ^d	2.43±0.03 ^c	2.09±0.10 ^c	2.20±0.14 ^d	2.01±0.10 ^d	1.05±0.03 ^c	0.96±0.01 ^b
8	2.91±0.14 ^e	2.79±0.15 ^e	2.68±0.10 ^d	2.31±0.08 ^d	2.32±0.07 ^e	2.10±0.07 ^e	1.10±0.03 ^d	1.03±0.03 ^c
<i>Notes:</i> GS: germinated soy beverages; UGS: ungerminated soy beverages; GP: germinated peanut beverages; UGP: ungerminated peanut beverages; GC: germinated coconut beverages; UGC: ungerminated coconut beverages; GDW: germinated distilled water probiotic beverages; UGDW: ungerminated distilled water beverages; mM Trolox: millimolar Trolox equivalent; mM GAE: millimolar gallic acid equivalent. All values are mean ± standard deviation (n = 3). Different superscript letters following the values in the same column indicate differences for each level of grain concentrations ($P < 0.05$).								

flour was 0.89–1.03, 2.10–2.79, 2.17–2.31 and 1.64–2.10 mM GAE, respectively. The TPC of beverages increased significantly with increasing amounts of germinated or ungerminated cereal flour ($P < 0.05$). Probiotic beverages mixed with 8 g of mixed grain flour had the highest TPC. Germinated and ungerminated soy milk had the highest, while distilled water had the lowest TPC. A similar study conducted by Donkor *et al.* (2012) confirmed that relatively high TPC of germinated grains was positively correlated with antioxidant activity, which is consistent with the results of the present study and provides clear evidence that probiotic beverages with soy milk had the highest antioxidant capacity and TPC. Considering probiotic non-dairy beverages, Fiorda *et al.* (2016) found that kefir grains were well-adapted to bioreactor conditions, as confirmed by high levels of phenolic compounds (190 GAE/100 g) after 24 h of fermentation. Selection of grains with high contents of phenolic compounds is particularly significant to enhance the antioxidant properties of probiotic beverages.

Sensory evaluations

Sensory analysis evaluated the organoleptic properties of fermented beverages containing ungerminated and germinated grains. Appearance, taste, consistency and overall acceptability scores of probiotic beverages are listed in Table 2. Sensory evaluations differed in accordance with the grain powder content ($P < 0.05$). All

probiotic beverages produced with 4–8 g of grain had good consistency, while those containing 6 g of cereal mixture had improved appearance, flavor and taste. A comparison was therefore performed using 6 g of grain. The appearance scores of GC, UGC, GS and UGS were 8.25, 8.25, 7.25 and 7.00, while the taste scores were 7.25, 7.75, 7.5 and 7.5, respectively. Probiotic drinks with coconut milk achieved the highest overall acceptability scores as well as all other sensory attributes. Shilin *et al.* (2015) evaluated the organoleptic properties of several non-dairy probiotic beverages prepared from cereals and found that probiotic beverages with almond milk had the highest overall acceptance score of 5.2 ± 2.1 . In the present study, probiotic beverages containing coconut milk had an overall acceptance score of 7.75 ± 0.96 , indicating better market potential.

Probiotic counts

The probiotic counts of various probiotic beverages are shown in Figure 3. The probiotic bacteria counts of drinks containing germinated grain powder prepared with distilled water, soy milk, peanut milk and coconut milk were 7.56–8.75, 9.09–9.50, 9.16–9.49 and 9.15–9.48 log CFU/mL, while those of probiotic beverages containing ungerminated grain powder were 7.56–8.72, 9.09–9.48, 9.16–9.48, and 9.15–9.47 log CFU/mL respectively. In the absence of added grains, there were significant differences in probiotic counts between beverages

Table 2. Effects of grain concentration and milk on the sensory quality of probiotic products.

Grain concentration (g/100 mL)	GS	UGS	GP	UGP	GC	UGC	GDW	UGDW
Appearance								
0	7.00±0.82 ^a	7.00±0.82 ^a	5.50±0.58 ^a	5.50±0.58 ^a	8.25±0.96 ^a	8.25±0.96 ^a	5.00±0.00 ^a	5.00±0.00 ^a
2	7.00±0.00 ^a	6.75±1.26 ^b	5.50±0.58 ^a	5.25±0.50 ^b	7.00±1.63 ^b	7.00±0.82 ^b	4.50±0.58 ^b	4.25±0.50 ^b
4	7.25±0.50 ^b	6.50±1.00 ^c	5.00±0.00 ^b	4.75±0.50 ^c	7.25±1.26 ^c	8.00±0.82 ^c	4.50±0.58 ^b	4.50±0.58 ^c
6	6.75±0.50 ^c	6.75±1.26 ^d	5.25±0.50 ^c	5.00±0.00 ^d	7.25±0.96 ^c	7.75±0.96 ^d	4.50±0.58 ^b	4.50±0.58 ^c
8	7.00±0.82 ^d	6.75±1.26 ^d	5.50±0.58 ^d	4.75±0.50 ^e	7.25±0.96 ^c	7.50±0.58 ^e	4.25±0.50 ^c	4.25±0.50 ^d
Taste								
0	6.50±1.73 ^a	6.50±1.73 ^a	6.75±0.50 ^a	6.75±0.50 ^a	7.25±0.50 ^a	7.25±0.50 ^a	5.00±0.00 ^a	5.00±0.00 ^a
2	6.75±0.50 ^b	5.50±1.29 ^b	6.00±0.00 ^b	5.50±0.58 ^b	6.50±0.58 ^b	7.50±0.58 ^b	4.50±0.58 ^b	4.50±0.58 ^b
4	6.75±0.96 ^b	5.50±1.29 ^b	5.75±0.50 ^c	5.25±0.50 ^c	6.75±0.50 ^c	7.75±0.50 ^c	4.75±0.50 ^c	4.25±0.50 ^c
6	6.50±0.58 ^c	6.00±1.15 ^c	5.25±0.50 ^d	5.25±0.50 ^c	6.75±0.93 ^c	7.50±1.29 ^d	4.75±0.50 ^c	4.00±0.00 ^d
8	6.75±0.96 ^d	6.50±1.29 ^d	5.50±0.58 ^e	5.75±0.50 ^d	6.50±1.29 ^d	7.25±0.96 ^e	4.75±0.50 ^c	4.25±0.50 ^e
Consistency								
0	7.25±0.96 ^a	7.25±0.96 ^a	5.75±0.50 ^a	5.75±0.50 ^a	7.00±0.82 ^a	7.00±0.82 ^a	5.25±0.50 ^a	5.25±0.50 ^a
2	7.00±0.00 ^b	6.50±1.29 ^b	5.50±0.58 ^b	5.75±0.96 ^a	6.50±0.58 ^b	6.75±0.50 ^b	5.25±0.50 ^a	5.25±0.50 ^a
4	6.25±0.96 ^c	6.50±1.29 ^b	5.50±0.58 ^b	5.50±0.58 ^b	6.75±0.50 ^c	7.50±0.58 ^c	5.50±0.58 ^b	5.25±0.50 ^a
6	6.75±0.96 ^d	7.00±0.82 ^c	5.50±0.58 ^b	5.50±0.58 ^b	7.00±0.82 ^d	7.50±0.58 ^c	5.50±0.58 ^b	5.00±0.00 ^b
8	6.50±0.58 ^e	7.00±0.00 ^c	5.25±0.50 ^c	5.25±0.50 ^c	7.50±0.58 ^e	7.25±0.96 ^d	5.50±0.58 ^b	5.25±0.50 ^c
Overall acceptance								
0	6.75±1.26 ^a	6.75±1.26 ^a	6.25±0.96 ^a	6.25±0.96 ^a	6.75±0.50 ^a	6.75±0.50 ^a	5.00±0.00 ^a	5.00±0.00 ^a
2	7.00±0.00 ^b	6.00±0.82 ^b	5.00±0.00 ^b	5.50±0.58 ^b	7.25±0.50 ^b	7.50±0.58 ^b	4.50±0.58 ^b	4.50±0.58 ^b
4	7.00±0.82 ^b	6.00±0.82 ^b	5.25±0.50 ^c	5.00±0.00 ^c	7.50±0.58 ^c	7.75±0.50 ^c	4.50±0.58 ^b	4.25±0.50 ^c
6	6.75±0.96 ^c	6.25±1.50 ^c	5.50±0.58 ^d	5.25±0.50 ^d	7.75±0.96 ^d	7.50±0.58 ^d	4.50±0.58 ^b	4.00±0.00 ^d
8	6.75±0.70 ^c	6.75±1.26 ^d	5.25±0.50 ^e	5.50±0.58 ^e	7.25±0.50 ^e	7.75±0.50 ^e	4.50±0.58 ^b	4.00±0.00 ^d

Notes: GS: germinated soy beverages; UGS: ungerminated soy beverages; GP: germinated peanut beverages; UGP: ungerminated peanut beverages; GC: germinated coconut beverages; UGC: ungerminated coconut beverages; GDW: germinated distilled water probiotic beverages; UGDW: ungerminated distilled water beverages.

All values are mean ± standard deviation (n = 3). Different superscript letters following the values in the same column indicate differences for each level of grain concentrations ($P < 0.05$).

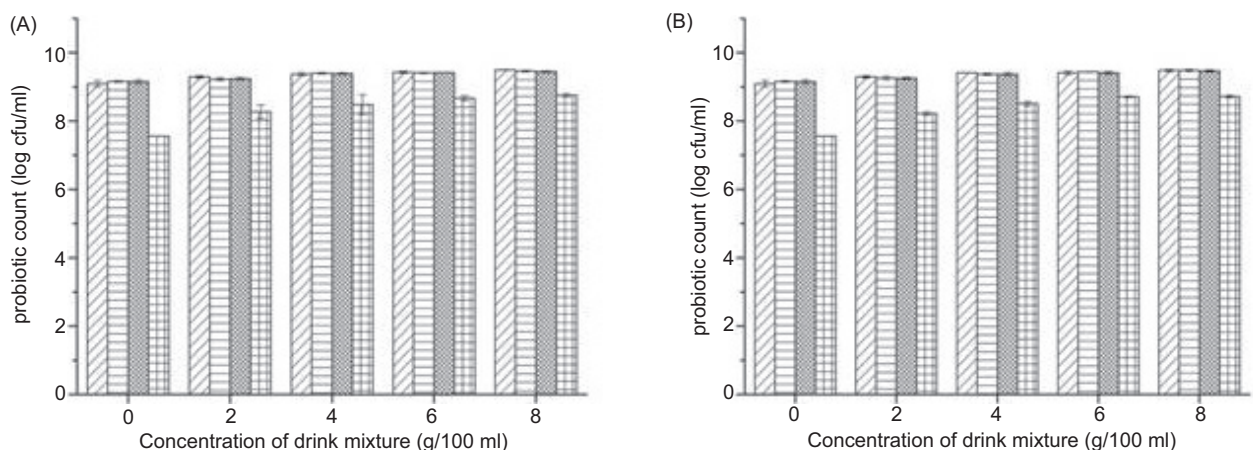


Figure 3. The Probiotic counts of different milk probiotic drinks. (a) Germinated grain probiotic drinks; (b) ungerminated grain probiotic drinks. Soybean milk probiotic fermented beverage, Peanut milk probiotic fermented beverage, Coconut milk probiotic fermented beverage, Distilled water probiotic fermented beverage (data are the mean ± standard deviation, n = 3).

prepared with soy milk, peanut milk and coconut milk ($P < 0.05$). The abundance of probiotic bacteria in all samples increased with the amount of mixed grain powder. Beverages prepared with germinated grain contained more probiotics than those prepared with ungerminated grain ($P < 0.05$). Overall, beverages containing coconut milk had the highest probiotic counts. The amount of *L. plantarum* was higher in drinks with increased amounts of grain mixture and following the addition of germinated grains. A previous study conducted by Shilin *et al.* (2014) to investigate the number of surviving *Lactobacillus rhamnosus* in non-dairy probiotic-fermented beverages of soybeans, almonds and peanuts after 24 h reported that almond beverages had the highest number of probiotic bacteria at 318.5 log CFU/mL versus 160.3 log CFU/mL and 65.8 log CFU/mL for soybean and peanut beverages, respectively, which were quite different from the results of this study. Types of probiotics and grain quality may affect the number of probiotics in probiotic beverages. Therefore, it is important to select suitable probiotics for grain fermentation.

Conclusions

In this study, changes made in various parameters showed benefits of adding grain powder to probiotic beverages. Cereals contain a variety of nutritional compounds that are beneficial to health. Germination of grains increases antioxidant activity and TPC, which prevent obesity and some diseases related to oxidation. Germinated grains had higher nutritional value and better sensory scores than ungerminated grains. Coconut drinks have good comprehensive sensory scores. Further studies should focus on coconut milk and germinated grains. Results of the present study highlighted the requirement of developing more favorable non-dairy probiotic-fermented beverages for consumers.

Acknowledgements

This work was supported by the National Science Foundation of China [31371613], China Agriculture Research System (CARS - 05-05A)

References

- Alvarez-Jubete, L., Wijngaard, H., Arendt, E.K. and Gallagher, E., 2010. Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chemistry* 119(2): 770–778. <https://doi.org/10.1016/j.foodchem.2009.07.032>
- Andrade, A., Matos, C., Lopes, J., Pires, T. and Joaquim, J., 2017. The use of probiotics and the effect in antibiotic associated diarrhea. *Clinical Therapeutics* 39: e47. <https://doi.org/10.1016/j.clinthera.2017.05.145>
- Donkor, O.N., Stojanovska, L., Ginn, P., Ashton, J. and Vasiljevic, T., 2012. Germinated grains—sources of bioactive compounds. *Food Chemistry* 135: 950–959. <https://doi.org/10.1016/j.foodchem.2012.05.058>
- Finley, J., Barrett, W., Joseph, M.B. and Burdette, C., 2015. Determination of total phenolic content using the folin-c assay. *Journal of AOAC International* 98: 1109–1110.
- Fiorda, F.A., Pereira, G.V.D., Thomaz-Soccol, V., Rakshit, S.K. and Soccol, C.R., 2016. Evaluation of a potentially probiotic non-dairy beverage developed with honey and kefir grains: fermentation kinetics and storage study. *Food Science and Technology International* 22: 732–742. <https://doi.org/10.1177/1082013216646491>
- Gupta, S. and Abu-Ghannam, N., 2012. Probiotic fermentation of plant based products: possibilities and opportunities. *Critical Reviews in Food Science and Nutrition* 52: 183–199. <https://doi.org/10.1080/10408398.2010.499779>
- Shilin, H. and Sharareh, H., 2015. Sensory evaluation of non-dairy probiotic beverages. *Journal of Food Research* 4(1): 186. <https://doi.org/10.5539/jfr.v4n1p186>
- Holloender, P.L.B., Ross, A.B. and Kristensen, M., 2015. Whole-grain and blood lipid changes in apparently healthy adults: a systematic review and meta-analysis of randomized controlled studies. *American Journal of Clinical Nutrition* 102: 556–572. <https://doi.org/10.3945/ajcn.115.109165>
- Liu, J., Huang, L., Wang, C., Liu, Y., Yan, Z., Wang, Z., Xiang, L., Zhong, X., Gong, F., Zheng, Y., Liu, D. and Wu B., 2019. Genome-wide association study reveals novel genomic regions associated with high grain protein content in wheat lines derived from wild emmer wheat. *Frontiers in Plant Science* 10: 464. <https://doi.org/10.3389/fpls.2019.00464>
- Maldonado Galdeano, C., Novotny Nunez, I., Carmuega, E., de Moreno De LeBlanc, A. and Perdigon, G., 2015. Role of probiotics and functional foods in health: gut immune stimulation by two probiotic strains and a potential probiotic yoghurt. *Endocrine Metabolic Immune Disorder Drug Targets* 15: 37–45. <https://doi.org/10.2174/1871530314666141216121349>
- Nelson, K., Stojanovska, L., Vasiljevic, T. and Mathai, M., 2013. Germinated grains: a superior whole grain functional food? *Canadian Journal of Physiology and Pharmacology* 91: 429–441. <https://doi.org/10.1139/cjpp-2012-0351>
- Ough, C.S., Amerine, M.A. and Sparks, T.C., 1965. Studies with controlled fermentations. xi. Fermentation temperature effects on acidity and ph. *American Journal of Enology and Viticulture (AJEV)* 20: 127–139.
- Ouwehand, A.C., Kurvinen, T. and Rissanen, P., 2004. Use of a probiotic bifidobacterium in a dry food matrix, an *in vivo* study. *International Journal of Food Microbiology* 95: 103–106. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.015>
- Quigley, E.M.M., 2019. Prebiotics and probiotics in digestive health. *Clinical Gastroenterology and Hepatology* 17: 333–344. <https://doi.org/10.1016/j.cgh.2018.09.028>
- Seddik, H.A., Bendali, F., Gancel, F., Fliss, I., Spano, G. and Drider, D., 2017. *Lactobacillus plantarum* and its probiotic and food potentialities. *Probiotics and Antimicrobial Proteins* 9: 111–122. <https://doi.org/10.1007/s12602-017-9264-z>
- Skryplonek, K. and Jasinska, M., 2015. Fermented probiotic beverages based on acid whey. *Acta Scientiarum Polonorum. Technologia Alimentaria* 14: 397–405. <https://doi.org/10.17306/J.AFS.2015.4.39>

- Song, D.F., Zhu, M.Y. and Gu, Q., 2014. Purification and characterization of plantaricin zj5, a new bacteriocin produced by *Lactobacillus plantarum* zj5. *PLoS One* 9: e105549. <https://doi.org/10.1371/journal.pone.0105549>
- Wagner, R.D. and Johnson, S.J., 2017. Probiotic bacteria prevent salmonella—induced suppression of lymphoproliferation in mice by an immunomodulatory mechanism. *BMC Microbiology* 17: 77. <https://doi.org/10.1186/s12866-017-0990-x>
- Xu, F., Dang, B., Yang, X.J., Wu, K.D. and Chi, D.Z., 2016. Evaluation of nutritional quality of different hulless barleys. *Journal of Triticeae Crops* 9: 1249–1257. <https://mlzwxb.alljournals.ac.cn/mlzwxb/ch/index.aspx>
- Zhang, K., Yang, J., Qiao, Z., Cao, X., Luo, Q., Zhao, J., Wang, F. and Zhang, W., 2019. Assessment of β -glucans, phenols, flavor and volatile profiles of hulless barley wine originating from highland areas of China. *Food Chemistry* 293: 32–40. <https://doi.org/10.1016/j.foodchem.2019.04.053>