Journal of Food Research; Vol. 4, No. 1; 2015
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Sensory Evaluation of Non-Dairy Probiotic Beverages

Shilin He[1] & Sharareh Hekmat[1]

[1] Division of Food and Nutritional Sciences, Brescia University College, Western University, London, ON., Canada

Correspondence: Sharareh Hekmat, Division of Food and Nutritional Sciences, Brescia University College, Western University, London, Ontario, Canada, 1285 Western Road, London, ON., N6G 1H2, Canada. Tel: 1-519-432-8353, ext. 28227. E-mail: hekmat@uwo.ca

Received: October 4, 2014 Accepted: November 4, 2014 Online Published: December 29, 2014

doi:10.5539/jfr.v4n1p186 URL: http://dx.doi.org/10.5539/jfr.v4n1p186

Abstract

This study measured the survival of *Lactobacillus rhamnosus* GR-1 in non-dairy probiotic beverages over a 28-day storage period and to determine which sample was most preferred on measures of appearance, consistency, flavour, texture, and overall acceptability. Three non-dairy samples and one control milk sample were prepared. The non-dairy samples were soy (1:3 product-to-water ratio), almond (1:3), and peanut (1:5). *L. rhamnosus* GR-1 remained viable (10^7 CFU/ml) in all samples over 28 days storage period and changes over time were dependent on the sample (P=0.03). The results of the sensory evaluation (n=90) showed that the soy and peanut samples were significantly different (P<0.03) from the control milk sample in appearance, consistency, flavour, texture, and overall acceptability. The almond sample was not rated significantly different (P>0.05) from the milk control in all categories. The results of this study suggest that probiotic almond milk may be a feasible substitute for conventional probiotic milk beverages, particularly for vegetarians who cannot consume dairy products and individuals with lactose intolerance.

Keywords: sensory evaluation, non-dairy, probiotic beverages, soy, almond, peanut

1. Introduction

1.1 Background and Rationale

Approximately 8% of Canadians are vegetarian (Iposos-Reid, 2004). Vegetarianism is a growing trend, which can be attributed to animal rights and ethics courses in post-secondary education, websites, magazines, newsletters, vegetarian cookbooks, and arrival of immigrants from countries where vegetarianism is commonly practiced (American Dietetic Association, 2003). Environmental concern, animal welfare, economic reasons, ethical considerations, world-hunger issues, and religious beliefs are additional reasons for consuming a vegetarian diet (American Dietetic Association, 2003; Fox & Ward, 2008). Plant-based diets are gathering growing appreciation and are recommended because of the many benefits that they confer, including lower levels of saturated fat, cholesterol, and animal protein, while offering higher carbohydrate, fibre, magnesium, boron, folate, antioxidant, carotenoid, and phytochemical intake (American Dietetic Association, 2003).

Fermented dairy products are the generic carrier matrices of probiotic microorganisms, with yogurt and fermented milk as the most commonly marketed products (Martins et al., 2013). Probiotic bacteria are beneficial bacteria that provide therapeutic effects on their host when ingested, and probiotic food products should contain at least 10^6 CFU/100g to transfer beneficial effects to the host (Hekmat & Reid, 2006; Rybka & Kailaspathy, 1995). *L. rhamnosus* GR-1 have been proven to help maintain a favourable microbial balance in the intestine and can survive in the intestinal tract without induction of systemic immune or inflammatory responses (Gardiner et al., 2002).The increasing consumption of probiotics parallels the growing trend in vegetarianism and diets that promote health and wellness (Martins et al., 2013). Therefore, the growing number of vegetarian individuals reinforces the importance of the need to develop non-dairy probiotic alternatives. Three non-dairy probiotic beverages were developed from soybeans, almond, and peanut.

Soybeans are composed of 40% high-quality protein and 20% oil (Liu, 2004). Soy contains high levels of a number of phytochemicals and is specially noted for the cholesterol-lowering effects of its protein (Liu, 2004). Among the numerous health-promoting compounds of soy, isoflavones play a key role, including the prevention and treatment of cardiovascular disease, cancer, osteoporosis, premenstrual and postmenstrual symptoms, and many more (Liu, 2004; Messina, 2003; Zubik & Meydani, 2003). Fermentation and heat treatment are methods to increase the digestibility of soy proteins (Liu, 2004; Han, Rombouts, & Nout, 2001).

Almonds have been found to be a valuable source of many nutrients. Chen, Lapsley, & Blumberg (2006)found that a 28 gram serving of almonds provides 36.4% of the Daily Value of vitamin E, 36.0% of manganese, 19.5% of magnesium, 16.0% of copper, 13.4% of phosphorus, 13.2% of fibre, 13.5% of riboflavin, and 12.1% of protein(Chen, Lapsley, & Blumberg, 2006). Almonds are naturally high in monounsaturated fatty acid content, which is inversely associated with cardiovascular disease when consumed (Chen, Lapsley, & Blumberg, 2006). Additionally, almond proteins are high in arginine content, making them highly digestible (Ahrens, Venkatachalam, Mistry, Lapsley, & Sathe, 2005).

Peanuts contain many bioactive compounds, such as flavonoids, phenolic acids, plant sterols, and stilbenes (Francisco & Resurreccion, 2008). The flavonoid content of peanuts is well documented; Yang, Liu, and Halim (2009) determined that of ten nuts, including legume peanuts, peanuts had the third highest total flavonoid content (Yang, Liu, & Halim, 2009). Flavonoids confer many benefits. In foods, flavonoids are responsible for colour, taste, prevention of fat oxidation, and protection of vitamins and enzymes (Yao et al., 2004). In humans, flavonoids were reported to be protective against cardiovascular diseases, cancers, and age-related diseases (Rosenberg, Jenkins, & Diamandis, 2002;Prasain, Carlson, & Wyss, 2010).

To the authors' knowledge, there has been no research conducted on the survival of probiotic bacteria in non-dairy soy, almond, or peanut beverages nor were sensory evaluations conducted on these beverages. Isanga and Zhang (2007) conducted a study investigating the production and sensory properties of peanut milk stirred yogurt. Samples were prepared from a blend of 70% peanut milk with 30% reconstituted whole milk, 70% peanut milk and 30% reconstituted whole milk with yogurt flavouring, and pure reconstituted whole milk control (Isanga & Zhang, 2007). The results of this study indicate that peanut milk-based yogurt had a good sensory texture, appearance, and flavour (Isanga &Zhang, 2007).

The purpose of this study was to evaluate the survival of *Lactobacillus rhamnosus* GR-1 in soy, almond, and peanut milk over a 28-day period and to evaluate which sample was most preferred on measures of appearance, consistency, flavour, texture, and overall acceptability.

2. Method

2.1 Probiotic Stock Solution Preparation

Ten percent (wt/vol) of *L. rhamnosus* GR-1 inocula was added to sterilized DeMan-Rogosa-Sharpe (MRS) broth (EM Science, Gibbstown, NJ) and incubated in an anaerobic condition, using a gas pack (BBL GasPak^{TM} EZ, Becton Dickinson & Co., Sparks, BD), at 37 °C, for 24 hours.

2.2 Sample Production

2.2.1 Milk (Control)

6% (wt/vol) sucrose was added to 1% (milk fat) milk. The milk and sucrose mixture was autoclaved at 15psi for 15 minutes.

2.2.2 Soymilk

The different probiotic samples were prepared using the authors' adaption of conventional methods for preparing nut milks. Dried soybeans (*Glycine max*) were soaked overnight. The rehydrated soybeans wereblended with water in a 1:3 ratio, respectively, for 2 minutes. The resulting slurry was strained through a double-lined cheesecloth to render soymilk. 6% (wt/vol) sucrose was added to the soymilk, and the soymilk was autoclaved at 15psi for 15 minutes.

2.2.3 Almond Milk

Unblanched and unroasted almonds (*Prunus amygdalus*) were soaked overnight. After soaking, the skin of the almonds was manually removed. The hydrated almonds were blended with water in a 1:3 ratio, respectively, for 2 minutes. The resulting slurry was strained through a double-lined cheesecloth to render almond milk. 6% (wt/vol) sucrose was added to the almond milk, and the almond milk was autoclaved at 15psi for 15 minutes.

2.2.4 Peanut Milk

Unroasted, blanched, de-skinned peanuts (*Arachis hypogaea*) were soaked overnight. Although a 1:3 ratio was ideally preferred to be consistent with the other samples, this could not be achieved without undesirably over-thickening the resulting peanut milk. Therefore, after soaking, the hydrated peanuts were blended with water in a 1:5 ratio, respectively, for 2 minutes. The resulting slurry was strained through a double-lined cheesecloth to render peanut milk. 6% (wt/vol) sucrose was added to the peanut milk, and the peanut milk was autoclaved at 15psi for 15 minutes.

2.3 Probiotic Sample Production

After the milk (control), soymilk, almond milk, and peanut milk have been cooled to 37 °C, they were individually inoculated with 1% (wt/vol) *L. Rhamnosus* GR-1 stock solution and were incubated at 37 °C in an anaerobic environment for 24 hours. After incubation, the probiotic samples were stored at 4 °C.

2.4 Microbial Analysis

Enumeration of *L. rhamnosus* GR-1 from all samples was conducted on days 1, 14, and 28 of storage at 4 °C using serial dilution and subsequent plating. Two microbial analyses were performed using two different sets of the aforementioned samples. All four samples were diluted in sterile saline (0.85% wt/vol NaCl) to 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}, and 10^{-8} dilution factors. 0.1 mL of 10^{-5}, 10^{-7}, and 10^{-8} sample dilutions were plated, on two separate MRSagar plates for each sample, with a calibrated pipette. Agar plates were prepared using 1.5% (wt/vol) agar (EMD Laboratories), 5.22% (wt/vol) MRS, and 1.5×10^{-5} % (wt/vol) fusidic acid (Sigma Laboratories). The plates were inverted and incubated anaerobically at 37 °C for 48 hours. Viable numbers were determined and recorded as colony-forming units (CFU) per mL, based on colony counts. An average was taken from the two duplicate plates, for each replication, for analysis.

2.5 Sensory Panel

2.5.1 Panelists and Recruitment

For hedonic tests, typically 80-100 panelists are recommended (European Sensory Network, 2011); therefore, 110 participants were recruited from Western University and Brescia University College to fulfill both the study recommendations as well as to account for any potential drop-outs and/or unusable data. 93 lay panelists participated in this study, and the data of 90 participants was used. Participants were recruited via email, word-of-mouth, and posters. Participants 18 to 55 years old were invited to participate in this study. Individuals who are lactose intolerant, pregnant, under 18 or over 55, diabetic, and/or undergoing chemotherapy and individuals who have a tree-nut allergy, and/or peanut allergy, and/or soy allergy were not eligible to participate.

2.5.2 Sample Preparation for Sensory Panel

All four samples (milk, soy, almond, peanut) were produced on the same date and were stored at 4 °C. Each sample was distributed in 20 g portions in plastic cups and was presented in a balanced random order to reduce order bias. Three-digit codes were used to mask the identity of the samples.

2.5.3 Sensory Evaluation

Each panelist was seated in individual booths in the sensory testing area, separate from the preparation room. All panelists received a tray of four coded samples at 4 °C in balanced random order. Each tray also included a napkin, a glass of water, a $5 Tim Card (token of appreciation), and the accompanying questionnaire. Before evaluating, panelists were instructed to evaluate each sample from left to right and to cleanse their palate with water between each sample. Panelists were also instructed to not speak to other panelists as they completed their questionnaire.

2.6 Data Collection

Panelists were asked to rate each sample based on the characteristics of appearance, consistency, flavour, texture, overall acceptability using a nine-point hedonic scale. The hedonic scale ranged from one to nine, where one corresponded with “dislike extremely,” and nine corresponded with “like extremely.”

2.7 Statistical Analysis

Statistical analysis was conducted using SAS Version 9.3. A one-way repeated measures analysis of variance (ANOVA) and Tukey-Kramer adjustment ($P<0.05$) were used to analyze viable bacteria between samples and between days. A one-way repeated measures ANOVA and Tukey-Kramer adjustment were also used to compare differences amongst samples for each characteristics of appearance, consistency, flavour, texture, and overall acceptability.

3. Results

3.1 Bacterial Analysis

All three non-dairy milk samples (soy, almond, peanut) were successful at yielding a probiotic, fermented beverage. Table 1 shows the descriptive statistics for the enumeration of L. *rhamnosus* GR-1 in these samples and milk (control) over 28 days storage period. After 28 days of storage, the soymilk sample had the highest average mean count (22.1×10^7 CFU mL^{-1}) amongst the non-dairy samples. The almond milk sample was significantly different when compared to soy, almond, and peanut samples (p=0.023). The almond sample was significantly different on Days 1 and 14 (p=0.035) and Days 1 and 28 (p=0.025). When comparing between time, Day 1 (p<0.001) and Day 14 (p=0.002) were significantly different. For Day 1, there is a significant difference between almond and soy (p=0.032), almond and milk (p=0.016), soy and milk (p=0.001), almond and peanut (p=0.004), and milk and peanut (p<0.001). On Day 14, there is a significant difference between almond and milk (p=0.004), soy and milk (p=0.005), and milk and peanut (p=0.004).

Table 1. Bacteria count (CFU ml^{-1}) of each sample over time (days)

	Day 1	Day 14	Day 28
Soy	22.9×10^7	12.9×10^7	22.1×10^7
Almond	45.5×10^7	10.0×10^7	2.9×10^7
Peanut	9.4×10^7	10.8×10^7	7.6×10^7
Milk	72.5×10^7	92.0×10^7	27.5×10^7

Table 1. Bacteria counts for the three non-dairy probiotic samples and milk (control) sample over 28 days of storage.

3.2 Sensory Analysis

3.2.1 Appearance

There was a significant overall difference (p<0.001) amongst participants' perception of appearance for all the samples. Intra-sample comparisons reveal that there was a significant difference between all samples except between milk and almond (p=0.726). Mean score for the milk sample was the highest (5.9 ± 2.2), and soy scored the lowest (2.5 ± 1.4).

3.2.2 Consistency

There was a significant overall difference (p<0.001) amongst participants' perception of consistency for all the samples. Intra-sample comparisons reveal that there was a significant difference between all samples except between milk and almond (p=0.687). Mean scores for the consistency of milk was the highest (5.0 ± 2.6), and soy scored the lowest (2.4 ± 1.5).

3.2.3 Flavour

There was a significant overall difference (p<0.001) amongst participants' perception of flavour for all the samples. Intra-sample comparisons reveal that there was a significant difference between all samples except between milk and almond (p=0.067) and soy and peanut (p=0.523). Mean scores for the flavour of almond was the highest (5.1 ± 2.1), and soy scored the lowest (3.1 ± 1.9).

3.2.4 Texture

There was a significant overall difference (p<0.001) amongst participants' perception of texture for all the samples. Intra-sample comparisons reveal that there was a significant difference between all samples except between milk and almond (p=0.562). Mean scores for the texture of almond was the highest (5.4 ± 2.1), and soy scored the lowest (2.3 ± 1.3).

3.2.5 Overall Acceptability

There was a significant overall difference (p<0.001) amongst participants' perceived overall acceptability for all the samples. Intra-sample comparisons reveal that there was a significant difference between all samples except between milk and almond (p=0.434). Mean scores for the overall acceptability of almond was the highest (5.2 ± 2.1), and soy scored the lowest (2.7 ± 1.5).

Table 2. Scoring results of probiotic samples

Characteristic	Sample	Mean
Appearance	Milk (control)	5.9 ± 2.2
	Soy	2.5 ± 1.4
	Almond	5.6 ± 2.4
	Peanut	4.2 ± 2.1
Consistency	Milk (control)	5.0 ± 2.6
	Soy	2.4 ± 1.5
	Almond	5.3 ± 2.0
	Peanut	4.2 ± 1.9
Flavour	Milk (control)	4.3 ± 2.4
	Soy	3.1 ± 1.9
	Almond	5.1 ± 2.1
	Peanut	3.5 ± 2.0
Texture	Milk (control)	5.0 ± 2.5
	Soy	2.3 ± 1.3
	Almond	5.4 ± 2.1
	Peanut	4.2 ± 2.0
Overall Acceptability	Milk (control)	4.8 ± 2.4
	Soy	2.7 ± 1.5
	Almond	5.2 ± 2.1
	Peanut	3.8 ± 1.8

Table 2. Scoring results of the three non-dairy probiotic samples and milk (control) sample based on nine-point hedonic scale ratings, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely.

4. Discussion

The primary objective of this study was to measure the survival of L. *rhamnosus* GR-1 in non-dairy probiotic beverages over 28-day storage period. This study showed that almond, soy, and peanut milks are viable matrices that support L. *rhamnosus* GR-1 survival greater than 10^6 CFU mL^{-1} during 28 days of storage.

The secondary objective of this study was to determine which non-dairy probiotic beverage was most preferred on measure of appearance, consistency, flavour, texture, and overall acceptability. Probiotic almond beverage was the most similar to probiotic milk beverage (control) on measures of appearance, consistency, flavour, texture, and overall acceptability. Mean scores for almond was the highest for consistency, flavour, texture, and overall acceptability when compared to probiotic soy, peanut, and milk (control) beverages. The results of this study suggest that probiotic almond beverage is a feasible alternative and potentially a better sensory substitute to probiotic milk beverage. This study also shows the potential in the marketability of probiotic almond beverages for vegetarians and individuals with lactose intolerance.

A limitation to this research was the number of repetitions conducted (3 in total). If more repetitions were to be conducted, then statistical results for the bacterial enumeration component of this study would be less affected by outliers, as evidenced by the large standard deviation values in Table 1. Another limitation to this study was the inability to ensure that the bacteria grown on plates were exclusively L. *rhamnosus* GR-1, as it was impossible to ensure a 100% sterile storage and plating environment. Future research on these non-dairy samples may benefit from more repetitions.

5. Conclusion

Soy, almond, and peanut milk samples successfully support the growth of L. *rhamnosus* GR-1 beyond 10^6 CFU mL^{-1}, each yielding a probiotic beverage sample. Sensory analysis of these samples reveals that probiotic almond beverage was most liked on measures of consistency, flavour, texture, and overall acceptability. This study shows promise in the potential marketability of probiotic almond beverages for vegetarians and individuals with lactose intolerance.

References

Ahrens, S., Venkatachalam, M., Mistry, A. M., Lapsley, K., & Sathe, S. K. (2005). Almond (*Prunus dulcis* L.) Protein Quality. *Plant Foods for Human Nutrition, 60*(3), 123-8. http://dx.doi.org/10.1007/s11130-005-6840-2

American Dietetic Association, Dietitians of Canada. (2003). Position of the American Dietetic Association and Dietitians of Canada: Vegetarian diets. *Journal of the American Dietetic Association*, *103*(6), 748-65. http://dx.doi.org/10.1053/jada.2003.50142

Chen, C-Y., Lapsley, K., & Blumberg, J. (2006). A nutrition and health perspective on almonds. *Journal of the Science of Food and Agriculture, 86*(14), 2245-50. http://dx.doi.org/10.1002/jsfa.2659

European Sensory Network. (2011).*Guidelines for consumer testing - guidance from ESN members*. Retrieved from http://www.esn-network.com/990.html

Fox, N., & Ward, K. (2008). Health, ethics and environment: a qualitative study of vegetarian motivations. *Appetite, 50*(2-3), 422-9. http://dx.doi.org/10.1016/j.appet.2007.09.007

Francisco, M. L. D. L., & Resurreccion, A. V. A. (2008). Functional Components in Peanuts. *Critical Reviews in Food Science and Nutrition, 48*(8), 715-46. http://dx.doi.org/10.1080/10408390701640718

Gardiner, G. E., Heinemann, C., Baroja, M. L., Bruce, A. W., Beuerman, D., Madrenas, J., & Reid, G. (2002). Oral administration of the probiotic combination Lactobacillus rhamnosus GR-1 and L. fermentum RC-14 for human intestinal applications. *International Dairy Journal, 12*(2-3), 191-196. http://dx.doi.org/10.1016/S0958-6946(01)00138-8

Han, B-Z., Rombouts, F. M., & Nout, M. J. R. (2001). A Chinese fermented soybean food. *International Journal of Food Microbiology*, *65*(1-2), 1-10. http://dx.doi.org/10.1016/S0168-1605(00)00523-7

Hekmat, S., & Reid, G. (2006). Sensory properties of probiotic yogurt is comparable to standard yogurt. *Nutrition Research*, *26*(4), 163-6. http://dx.doi.org/10.1016/j.nutres.2006.04.004

Iposos-Reid. (2004). *Consumer preceptions of food safety and quality*. Retrieved from http://www4.agr.gc.ca/resources/prod/doc/agr/pdf/Canadian_Perceptions.pdf

Isanga, J. I., & Zhang, G-N. (2007). Preliminary investigation of the production and characterization of peanut milk based stirred yoghurt. *International Journal of Dairy Science, 2*(3), 207-216. http://dx.doi.org/10.3923/ijds.2007.207.216

Liu, K. (2004). *Soybeans as Functional Foods and Ingredients*. Champaign, Ill: AOCS Press. http://dx.doi.org/10.1201/9781439822203

Martins, E. M. F., Ramos, A. M., Vanzela, E. S. L., Stringheta, P. C., de Oliveira Pinto, C. L., & Martins, J. M. (2013). Products of vegetable origin: A new alternative for the consumption of probiotic bacteria. *Food Research International, 51*(2), 764-70. http://dx.doi.org/10.1016/j.foodres.2013.01.047

Messina, M. J. (2003). Potential public health implications of the hypocholesterolemic effects of soy protein. *Nutrition, 19*(3), 280-1. http://dx.doi.org/10.1016/S0899-9007(02)00995-4

Prasain, J. K., Carlson, S. H., & Wyss, J. M. (2010). Flavonoids and age-related disease: risk, benefits and critical windows. *Maturitas, 66*(2), 163-71. http://dx.doi.org/10.1016/j.maturitas.2010.01.010

Rosenberg Zand, R. S., Jenkins, D. J. A., & Diamandis, E. P. (2002). Flavonoids and steroid hormone-dependent cancers. *Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences, 777*(1-2), 219-32. http://dx.doi.org/10.1016/S1570-0232(02)00213-1

Rybka, S., & Kailasapathy, K. (1995). The survival of culture bacteria in fresh and freeze-dried AB yoghurts. *Australian Journal of Dairy Technology, 50*(2), 51-7.

Yang, J., Liu, R. H., & Halim, L. (2009). Antioxidant and antiproliferative activities of common edible nut seeds. *LWT - Food Science and Technology, 42*(1), 1-8. http://dx.doi.org/10.1016/j.lwt.2008.07.007

Yao, L. H., Jiang, Y. M., Shi, J., Tomás-Barberán, F. A., Datta, N., Singanusong, R., & Chen, S. S. (2004). Flavonoids in Food and Their Health Benefits. *Plant Foods for Human Nutrition, 59*(3), 113-22. http://dx.doi.org/10.1007/s11130-004-0049-7

Zubik, L., & Meydani, M. (2003). Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *The American Journal of Clinical Nutrition, 77*(6), 1459-65.