Int.J.Curr.Microbiol.App.Sci (2020) *9*(1): 527-533

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 1 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.901.058

Standardization of Synbiotic Drinkable Fruit based Yoghurt using *Lactobacillus brevis*

Ayyavoo Preamnath Manoharan*, J. Jayapratha and C. Ashokkumar

Department of Food Science and Technology, College of Food and Dairy Technology, Chennai –52, India

**Corresponding author*

Keywords

Yoghurt, Synbiotic, Mango, *Lactobacillus brevis*

Article Info

Accepted:
15 December 2019
Available Online:
20January 2020

A B S T R A C T

The demand for foods with specific functional characteristics and nutritional balance is increasing. This study was investigated for the feasibility of symbiotic yoghurt with *Lactobacillus brevis* as probiotics. This yoghurt was prepared by addition of natural fruits (mango or banana). Hereby the drinkable yoghurt might act as a good source of calcium, Vitamin D and other nutrients. The incorporation of fruits also enhances the flavour of yoghurt establishing the need to optimize the level of inclusion of these fruits in yoghurt. This study was carried out to analyze the effect of inclusion of different levels of fruits and prebiotic into yoghurt thus optimizing the composition of fortified synbiotic drinkable yoghurt. Sensory evaluation was conducted to assess the optimum inclusion levels of the above ingredients in the fortified synbiotic drinkable yoghurt. One way analysis of variance (ANOVA) was conducted to study the significance of difference among the fortified synbiotic drinkable yoghurt.

Introduction

Yoghurt is one of the health promoting delicious dairy products liked by all age groups of people throughout the world. Yoghurt production is increasing every year tremendously. According to the Code of Federal Regulations of the FDA (CFR, 2013): Yogurt is the food produced by culturing one or more of the optional dairy ingredients with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Probiotics are defined as "living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Guarner and Schaafsma, 1998).

A similar definition was proposed by a United Nations and World Health Organization Expert Panel: "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). Younus *et al.,* (2002) reported that the word "yoghurt" is derived from Turkish "jugurt", used to describe any fermented food with an acidic in taste.

Karagul *et al.,* (2004) stated that yoghurt was made by fermenting milk with indigenous microorganisms. Yoghurt having high nutritional and therapeutic properties is being highly produced and consumed worldwide. Goldin *et al., (*1992) reported that *Lactobacillus* can survive and temporarily colonize the human gastrointestinal tract and can affect the metabolic activity of the resident microflora.

Samona and Robinson(1994) reported that yogurt organisms tended to suppress the growth of the *bifido bacteria*, subsequent storage in the presence of the yogurt cultures did not lead to any significant decline in numbers. Axelsson, (1998) stated that lactic acid bacteria (LAB) family is composed of a heterogeneous group of Gram positive, non-spore-forming, catalase- and cytochrome-negative, anaerobic or aero tolerant bacteria. Ayad *et al.,* (2004) observed that *Lactobacillus brevis* (B23) also showed moderate acidification activity at low pH with an increase in cell number found to be necessary for the production of high levels of abacteriocins like substance. Stanton, (2005) stated that the most important prebiotics are glucans, fructans and mannans. The consumption of fermented products has long been associated with good health given that they can contain probiotics, prebiotics or both.

Materials and Methods

Fresh Cow milk, Sugar, Skimmed milk powder, mango and banana pulp was purchased from the nearby super market was used to in the preparation of fortified synbiotic drinkable yoghurt. Drinkable yoghurt mix was prepared to contain a final composition of 3.5% fat, 8.5% SNF and 5% sugar in the drinkable yoghurt, as per BIS (IS: 12898, 1989) specification (Sukumar De, 2015). In each treatment, mix was preheated to 60°C and ingredients were homogenized as described in Arbuckle (1986) and then heated to 85°C for 30mins as suggested by Lee and Lucy (2010).

Mixes were cooled to 42°C and inoculated with yoghurt culture and *Lactobacillus brevis* at the rate of 1 per cent each and incubated for 3 to 5 hours in the incubator or till the titrable acidity reaches 0.75 per cent and cooled to 20 to 25°C.

Microbial analysis of fruit synbiotic drinkable yoghurts during storage

Total Viable Count, Coliform, Yeast and Mold in the fortified synbiotic drinkable yoghurt were determined as per the method described by Bureau of Indian Standards described in IS:12898 - 1989.

Sensory evaluation of developed product

The sensory assessments were conducted in the Department of Food Science and Technology laboratory with sensory panelist at College of Food and Dairy Technology, Koduvalli, Chennai. A sensory score card to evaluate colour and appearance, flavor, body and texture, sweetness, sourness and overall acceptability using 9 point hedonic scale was prepared and given to the sensory panelist.

Statistical analysis

The data collected on various parameters were analyzed as per the standard method of described by Snedecor and Cochran (1989).

Analysis of variance (one way ANOVA) was used to study the significant difference by post hoc Duncan, Tamhane's T2 procedure.

Results and Discussion

Different level of mango pulp added drinkable yoghurt Sensory scores

The average overall sensory score card for drinkable yoghurt with different levels of mango pulp is represented in Table 1.The inclusion of mango pulp at 10 per cent level had maximum sensory score of 8.11 and the samples with 5, 10 and 15 per cent inclusion had the overall average scores of 7.47,8.11 and 7.61, respectively; while the control had the average of 6.91. The inclusion of mango pulp at 10 per cent level has scored the maximum value for all the sensory characters and hence considered as optimum and chosen for further analysis.

In the present study as seen in Table 1, significant difference was observed in the sensory perception between control and mango drinkable yoghurt. The inclusion level of mango pulp into yoghurt was optimized at an acceptable level of tenper cent in the preparation of drinkable yoghurt. Studies by Venkateshaiah (1995) also showed that among pineapple, mango, banana and sapota fruit pulps tried with different levels in frozen yoghurt, mango fruit pulp at 10 per cent level was found to be highly acceptable product as compared to other types of fruit yoghurts.

Different level of banana pulp added drinkable yoghurt Sensory scores

The respective mean sensory scores for drinkable yoghurt prepared by inclusion of different levels of banana pulp. The inclusion level of banana pulp at 10 per cent level resulted in maximum score of 7.88. The samples with 5, 10 and 15 per cent inclusion had the overall average scores of 7.58, 7.88 and 7.13 respectively; while the control had the average of 6.91 shown in table 2. Since the yoghurt drink with 10 per cent banana pulp showed significant difference in sensory scores than the rest it was considered for further analysis. Tenper cent inclusion level of banana pulp for the preparation of drinkable yoghurt was found to be optimum since it scored maximum on sensory attribute as seen in table 2. In a similar study by Amna Mahmood *et al.,* (2008), it was revealed that 8 per cent inclusion of banana pulp scored maximum followed by 10 per cent. This might be due to the amount of reducing sugar(carbohydrate) present in banana fruit.

Probiotic properties of *Lactobacillus brevis* by bile salts tolerance test

The bile tolerance of *L.brevis* to varying levels of bile salts. The respective mean viable count (log_{10}cfu/ml) of *L.brevis* for control, 0.2, 0.4 and 0.6 per cent of bile were 10.57±0.005, 8.36±0.049, 7.24±0.051 and 6.58±0.005 shown in table 3.Significant difference was observed in the viable counts between 0.2 per cent of bile treated culture and control. There was a decreasing trend in viability of *L. brevis* with increase in bile concentration. *L.brevis* showed tolerance to maximum bile concentration (0.6%) with a viability of 6.58±0.005 (log_{10}cfu/ml).

From Table 3,it may be seen that the *L. brevis* had tolerance towards varying levels of bile salts. *L. brevis* showed good tolerance to 0.4 and 0.6 per cent of bile. The mean viable count (log_{10}cfu/ml) at 0.4 and 0.6 per cent of bile were 7.24±0.051 and 6.58±0.005, respectively. Thus, the prerequisite of 10^6cfu/ml recommended for a probiotic food as suggested by Samona and Robinson (1994) was met in this study.

Table.1 List of various proportions of synbiotic yoghurt preparations

TC	**Control drinkable yoghurt (yoghurt culture + *Lactobacillus brevis*)**
T1	Drinkable yoghurt + Mango (5% pulp)
T2	Drinkable yoghurt + Mango (10% pulp)
Daa1T3	Drinkable yoghurt + Mango (15% pulp)
T4	Drinkable yoghurt + Banana (5% pulp)
T5	Drinkable yoghurt + Banana (10% pulp)
T6	Drinkable yoghurt + Banana (15% pulp)

Table.2 Sensory scores for the drinkable yoghurt prepared with different level of mango pulp (Mean± SE)[@]

Inclusion level of Mango pulp in yoghurt(%)	Sensory attributes (9-point hedonic scale)					
	Colour and Appearance	Flavor	Body and Texture	Sweetness	Sourness	Overall acceptability
C	6.88±0.237^{a}	7.18±0.102^{ab}	6.96±0.137^{a}	7.03±0.090^{a}	7.18±0.236^{a}	6.91±0.133^{a}
5%	7.60±0.165 ^{bc}	6.95±0.194^{a}	7.08±0.079^{a}	6.91±0.169^{a}	6.88±0.219^{a}	7.47±0.126^{ab}
10%	7.87±0.144 ^{c}	8.32±0.328^{c}	8.04±0.084^{b}	8.378±0.187^{b}	7.59±0.180^{a}	8.11±0.232^{c}
15%	7.07±0.065^{ab}	7.90±0.280^{bc}	7.03±0.216^{a}	8.01±0.210^{b}	7.27±0.124^{a}	7.61±0.124^{bc}

[@]*Average of eight trials*

Means bearing different superscripts in a column differ significantly (P<0.05)

Table.3 Sensory scores for the drinkable yoghurt prepared with different level of banana pulp (Mean± SE) [@]

Inclusion level of banana pulp in yoghurt (%)	Sensory attributes (9-point hedonic scale)					
	Colour and Appearance	Flavor	Body and Texture	Sweetness	Sourness	Overall acceptability
Control	6.88±0.237^{a}	7.18±0.102^{a}	6.96±0.137^{a}	7.03±0.090^{a}	7.18±0.236^{a}	6.91±0.133^{a}
5%	7.27±0.276^{a}	7.30±0.248^{a}	7.09±0.225^{ab}	7.28±0.244^{ab}	7.12±0.243^{a}	7.58±0.168^{ab}
10%	7.62±0.275^{a}	8.06±0.377^{a}	7.88±0.165^{b}	8.15±0.261^{b}	7.76±0.251^{a}	7.88±0.264^{b}
15%	7.05±0.22^{a}	7.24±0.431^{a}	6.71±0.321^{a}	6.94±0.361^{a}	6.81±0.300^{a}	7.13±0.259^{ab}

[@]*Average of eight trials*

Means bearing different superscripts in a column differ significantly (P<0.05)

Table.4 Probiotic properties of *Lactobacillus brevis*by bile saltstolerance test (Mean± SE)[@]

Name of the culture	Control	Percentage of bile salt		
		0.2%	0.4%	0.6%
Lactobacillus brevis	10.57±0.005^{c}	8.36±0.049^{b}	7.24±0.051^{a}	6.58±0.005^{a}

[@]*Average of eight trials*

[#]$\log_{10}$cfu/ml

Means bearing different superscripts in a row differ significantly (P<0.05)

Table.5 *Lactobacillus brevis*[#] tolerance to acidity at pH 3(Mean± SE) [@]

Name of the culture	Incubation period in minutes		
	0	90	180
Lactobacillus brevis	7.57 ± 0.007^{c}	4.00 ± 0.015^{b}	3.43 ± 0.007^{a}

[@]*Average of eight trials;* [#]$\log_{10}$cfu/ml^{-1}

Means bearing different superscripts in a row differ significantly (P<0.05)

Chart.1 Preparation flow chart for Fortified synbiotic drinkable yoghurt

Cow milk (3.5 per cent Fat and 8.5 per cent SNF)

⇩

Addition of sugar (5 per cent) and skim milk powder (4 per cent)

⇩

Preheating at 60°C

⇩

Homogenization (2000 psi)

⇩

Pasteurization at 85ºC for 30mins

⇩

Cooling to 42ºC

⇩

Inoculation of culture (Yoghurt culture 1 per cent; *L.brevis* culture 1 per cent)

⇩

Incubation at 42ºC for 3 -5 hours (0.75% titrable acidity)

⇩

Cooling (20 – 25^{0} C)

⇩

Stirring

⇩

Addition of compounds (as per treatment combination)

⇩

Mixing and Straining

⇩

Cooling (5 – 7^{0} C)

⇩

Packing and Storage @ 5^{0}C

This was further affirmed by Prasad *et al.,* (1999) who reported that one of the criteria for lactic acid bacteria to be called probiotic is its tolerance to bile salt. Jin *et al.,* (1998) also reported that *L.brevis* showed tolerance to 0.3 per cent bile. In the present study the tolerance level of 0.6 per cent w/v bile concentration was observed for *L. brevis*.

Probiotic properties of *Lactobacillus brevis*to acidity at pH 3

The tolerance of *L.brevis* to acidity at pH 3. The respective mean values for tolerance of *L.brevis* to acidity at pH 3 in (log_{10}cfu/ml) at 0, 90 and 180 minutes were 7.57±0.007, 4.00±0.015 and 3.43±0.007. The lowest tolerance with 3.43±0.007log_{10}cfu/ml was observed in 180 minutes of incubation at pH 3 shown in Table 4. The findings are in agreement to the observations of *L. brevis* young–wood Kim *et al.,* (2014) who stated that strains should be strongly tolerant to acidic condition as acid tolerance is an important quality for a probiotic.

The tolerance of *L. brevis* to acidity at pH 3 as in most in vitro assays, pH 3 has been preferred (Jin *et al.,* 1994).This was in agreement with the findings of Pushkaraj Sawant *et al.,* (2015) who reported that the fresh yoghurt contained 3.21 per cent protein and 3.35 per cent fat. As the mango pulp contains lower fat than milk, the decrease is very apparent and understandable. These results were in accordance with findings of Sengupta *et al.,* (2014).

The addition of fruit has caused the increase in protein content of yoghurt as proved by Amna Mahmood *et al.,* (2008).As per the Codex standards (2003) for fermented milk, there should be a minimum of 2.7 per cent of milk protein and less than 10 per cent of milk fat. The fortified synbiotic yoghurt in the present study had met the Codex requirement.

A study was conducted for the development of fortified synbiotic drinkable yoghurt. Skeletal disease of bone thinning and compromised bone strength, osteoporosis, continues to be a major public health issue as the population ages. This disease is characterized by bone fragility and an increased susceptibility to fractures, especially of the spine and hip, although any bone can be affected. Hence the drinkable yoghurt was developed by fortification with Vitamin D and calcium. This yoghurt was prepared by addition of natural fruits (mango or banana) in the presence of a prebiotic (honey or fructo-oligosaccharides). Hereby the drinkable yoghurt might act as a good source of calcium, Vitamin D and other nutrients. The incorporation of fruits also enhances the flavour of yoghurt establishing the need to optimize the level of inclusion of these fruits in yoghurt. This study was carried out to analyze the effect of inclusion of different levels of fruits and prebiotic into yoghurt thus optimizing the composition of fortified synbiotic drinkable yoghurt.

References

AmnaMahmood, N. Abbas, and A.H.Gilani, 2008. Quality of stirred buffalo milk yogurt blended with apple and banana fruits. Pak. J. Agric. Sci, 45(2):275-279.

Axelsson, L., 1998. Lactic acid bacteria: classification and physiology, In Salminen S, von Wright A (ed), Lactic acid bacteria. *Microbiology and functional aspects*.1 – 72

Ayad, E.H.E., S.Nashat, N.El-Sadek, H.Metwaly, and M.El-Soda, 2004. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food microbiology, 21(6):715-725.

FAO/WHO Experts Report. 2002. Guidelines for the evaluation of probiotics in food.

Goldin, B., S.Gorbach, M.Saxelin, S.Barakat, L.Gualtieri and S.Salminen, 1992.Survival of Lactobacillus GG in human gastrointestinal tract.*Digestive Diseases and Sciences*.37(1):121-128.

Guarner, F., and G.J. Schaafsma, 1998. Probiotics. International journal of food microbiology, 39(3): 237-238.

Indian Standard: 12898. 1989. Dairy products-Yoghurt Specification. Bureau of Indian Standards, New Delhi.

Institute of Medicine.2012 DRI – Dietary reference intakes – Calcium and vitamin D. J Nutr Food Sci; 42:131-1.

Karagul, Y.,C.Wilson, and H.White, 2004. Formulation and processing of yoghurt. *J Dairy Sci*, *87*:543-550.

Lee, W. J., and J.A.Lucey, 2010.Formation and Physical Properties of Yoghurt. Asian-Australian Journal of Animal Science 23(9): 1127 – 1130.

Prasad, J., H. Gill, J. Smart and P. K. Gopal, 1999.Selection and characterization of Lactobacillus and Bifidobacterium strains for use as probiotics. International Dairy Journal, 8(12):993-1002.

Samona, A., and R.K.Robinson,1994. Effect of yogurt cultures on the survival of bifidobacteria in fermented milks. *International Journal of Dairy Technology*, 47(2): 58-60.

Sawant, P., D.Kumar, V. Patil, Yy.Ingale, Y., and Sawant, D. 2015. Physico-chemical, Sensory and Microbial Quality of Yoghurt Drink Fortified with Pineapple Pulp.International Journal of Food and Fermentation Technology, 5(1), 59.

Snedecor, G.W., and W. G. Cochran, 1989. Statistical methods. 9thEdn. The lowa State University Press, Ames, lowa.

Stanton, C., R.P.Ross,G.F. Fitzgerald,and D.V.Sinderen,2005. Fermented functionalfoods based on probiotics and their biogenic metabolites. Current Opinionin Biotechnology, 16:198–203.

Sukumar De., 2008. Outlines of dairy technology.Published by Oxford University Press, New Delhi. 183 - 219.

Venkateshaiah, B. V., 1995. Utilization of whey solids in the preparation of frozen yoghurt.University of Agricultural Sciences, GKVK.

Younus, S., T.Masud, and T.Aziz, 2002. Quality evaluation of market yoghurt/dahi. *Pakistan Journal of Nutrition*, 1(5): 226-230.

How to cite this article:

Ayyavoo Preamnath Manoharan, J. Jayapratha and Ashokkumar, C. 2020. Standardization of Synbiotic Drinkable Fruit based Yoghurt using *Lactobacillus brevis*. *Int.J.Curr.Microbiol.App.Sci.* 9(01): 527-533. doi: https://doi.org/10.20546/ijcmas.2020.901.058