www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(7): 101-104

www.thepharmajournal.com
Received: 01-05-2020
Accepted: 03-06-2020

Dr. Raghunath BV
Ph.D. Scholar, Department of Livestock Products Technology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Dr. G Kumaresan
Professor and Head, Department of Livestock Products Technology, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

Dr. A Elango
Professor and Head, Department of Livestock Products Technology (Dairy Science), Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Dr. S Suresh Kumar
Associate Professor and Head, Department of Livestock Products Technology (Meat Science), Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Dr. Chandrashekara N
Assistant Professor, Department of Microbiology Veterinary College Hassan, KVAFSU, Karnataka, India

Dr. Ramesh D
Assistant Professor, Department of Veterinary Physiology and Biochemistry, Veterinary College Hassan, KVAFSU, Karnataka, India

Corresponding Author:
Dr. Raghunath BV
Ph.D. Scholar, Department of Livestock Products Technology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Comparative analysis of the effect of herbs on diverse microbiological properties of yoghurt at different storage periods

Dr. Raghunath BV, Dr. G Kumaresan, Dr. A Elango, Dr. S Suresh Kumar, Dr. Chandrashekara N and Dr. Ramesh D

Abstract
Yoghurt is rich in nutrients like potassium, calcium, protein and vitamin B and has beneficial effects on human health by supplying prebiotic and probiotic bacteria. It helps to strengthen the immune system, improves lactose digestion and gastrointestinal conditions including lactose intolerance, constipation, diarrhoea, colon cancer, inflammatory bowel disease and allergies. Six batches of different herbal probiotic yoghurt were prepared using fresh milk by inoculated with 4 per cent of yoghurt cultures containing *Lactobacillus delbrueckii subsp. bulgaricus*, and *Streptococcus salivarius subsp. thermophilus.* In the viability study of probiotics organisms, it was revealed that there was significant (P<0.05) reduction in the viability of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (log10cfu/ml) were noticed in all the herbal yoghurt including plain yoghurt during storage period of 21days and wherein the reduction was high in AGY, CIY and HSY compared with PY and ABY and this may be attributed to slight acidic nature of herb. However the count of probiotic strains was maintained at 7 log units for all herb treated yoghurts throughout storage period of 21 days.

Keywords: Yoghurt, probiotic, *Streptococcus salivarius, Lactobacillus delbrueckii,* microbial growth

Introduction
Dairy products, particularly those containing prebiotics, probiotics and synbiotics are most popular in this category of foods. Probiosis can be defined as 'the positive effect of consumption of fermented dairy products with the culture of lactic acid bacteria (LAB) on the equilibrium maintainance of intestinal microflora' (Tomasik and Tomasik, 2003) [11]. Yoghurt is rich in nutrients like potassium, calcium, protein and vitamin B and has beneficial effects on human health by supplying prebiotic and probiotic bacteria. It helps to strengthen the immune system, improves lactose digestion and gastrointestinal conditions including lactose intolerance, constipation, diarrhoea, colon cancer, inflammatory bowel disease and allergies (Fitzgerald *et al*., 2004) [2]. Apart from this, yoghurt has also been associated with a number of health benefits including anti-carcinogenic properties, anti-mutagenic properties, reduction in serum cholesterol and management of hypertension, immune system stimulation and suppression of *Helicobacter pylori* infection (Shah, 2000b) [10].
The beneficial health effects of yoghurt have been partly linked to the proteolysis products produced during fermentation and storage. During fermentation, LAB produces a range of secondary metabolites, some of which have been associated with health promoting properties of which the notable ones are the B-vitamins and bioactive peptides.
The present study investigate infusion of the commonly used herbs Aloe barbadensis (Aloe vera), Anethum graveolence (dill), Costus igneus (insulin plant) and Hibiscus sabdariffa (roselle) on yoghurt formation and also to evaluate the Effect of herbs on microbiological properties of yoghurt at different storage periods..

Materials and Methods
Fresh cow milk obtained from the Department of ILFC, Veterinary College and Research Institute, Namakkal – 637 002 was used. Skim milk powder testing 5 and 95 per cent moisture and solubility, respectively was purchased locally (AAVIN). Commercially available good quality cane sugar was used in the current study. Freeze dried DVS cultures containing yoghurt bacteria *Lactobacillus delbirueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp.

thermophilus obtained from Chr. Hansen, Denmark, were used in this study. Herbal infusions like *Aloe barbadensis* collected from campus of Veterinary College and Research Institute, Namakkal. *Anethum graveolens* purchased from local markets of Kolar district, Karnataka. *Costus igneus* and *Hibiscus Sabdariffa* collected from Horticulture College, GKVK campus, Bangalore was employed in the study.

Preparation of probiotic yoghurt

Six batches of different herbal probiotic yoghurt were prepared using fresh milk. Skim milk powder at the rate of 2 per cent (w/v) and sugar at the rate of 6 per cent (w/v) were added to it and homogenized at 1000 psi. The contents were mixed well and pasteurized at 85 °C for 30 minutes, cooled to room temperature and inoculated with 4 per cent of yoghurt cultures containing *Lactobacillus delbrueckii subsp. bulgaricus*, and *Streptococcus salivarius subsp. thermophilus.* Different concentrations of herbs were added before incubation and mixed well and incubated at 42 °C for 4 to 5 hours and stored at 4 °C when the pH was reduced to 4.5. These yoghurts were then placed in the refrigerator for up to 21 days.

Herbal yoghurt

Five different concentrations of herbal yoghurt were prepared from each different herb for standardization and were denoted as

1. PY = Plain yoghurt without adding any herb as a standard yoghurt
2. ABY = *Aloe barbadensis* incorporated yoghurt
3. AGY = *Anethum graveolens* incorporated yoghurt
4. CIY = *Costus igneus* incorporated yoghurt
5. HSY = *Hibiscus sabdariffa* incorporated yoghurt

Different concentrations used for standardization of herbal yoghurt

Yoghurt	Concentration of herbs (per cent)				
PY	0	0	0	0	0
ABY	12	14	16	18	20
AGY	0.4	0.5	0.6	0.7	0.8
CIY	0.4	0.5	0.6	0.7	0.8
HSY	0.4	0.5	0.6	0.7	0.8

Thus prepared herbal yoghurt was subjected to evaluate the microbiological properties of yoghurt in detail at different storage periods.

Storage period used in the current study

Herbal yoghurt was subjected to evaluate microbiological properties of yoghurt in detail at different storage periods like zero, 7th, 14th and 21st day.

Results

The outcome of the current study is presented and depicted as tables 1-4 and figure 1-2.

Viability of *Streptococcus salivarius* subsp. *thermophilus* (log_{10} cfu/ml) in herbal yoghurt during storage period

Effect of herbs on viability of *Streptococcus salivarius* subsp. *thermophilus* (mean±SE) in herbal yoghurt during storage period of zero day, 7th day, 14th day and 21st day were represented as Tables 1, 2 and figure 1.

Table 1: Viability of probiotic *Streptococcus salivarius subsp. thermophilus* (log10 cfu/ml) in herbal yoghurt during storage period (mean±SE)

Days	PY	ABY	AGY	CIY	HSY	Storage mean±SE
zero day	8.93±0.01	8.85±0.01	8.81±0.01	8.81±0.01	8.82±0.01	8.84^{a}±0.002
7th day	8.95±0.01	8.90±0.01	8.83±0.01	8.83±0.01	8.84±0.01	8.87^{a}±0.002
14th day	8.81±0.01	8.76±0.01	8.43±0.03	8.40±0.03	8.60±0.01	8.60^{b}±0.01
21st day	8.14±0.03	7.75±0.06	7.87±0.02	7.86±0.02	7.50±0.06	7.82^{c}±0.01
Treatment mean±SE	8.71^{a}±0.01	8.56^{b}±0.02	8.48^{c}±0.02	8.47^{c}±0.01	8.44^{c}±0.02	-

[abcd] means bearing same superscript column wise and row wise was not significant

Table 2: ANOVA (Multivariate) of viability of probiotic *Streptococcus salivarius subsp. thermophilus* in herbal yoghurt during storage period

Source	df	MSS	F-value	P-value	Error	
					df	MSS
Treatment	4	0.282	13.541	0.00	100	0.021
Storage	3	7.209	345.669	0.00		
Storage*Treatment	12	0.097	4.677	0.00		

Reduction in the viability of *Streptococcus salivarius* subsp. *thermophilus* (log_{10} cfu/ml) was noticed in all the treated herbal yoghurts including plain yoghurt.

On zero day, the count for PY was 8.93±0.01 followed by ABY, HSY, CIY and AGY of 8.85±0.01, 8.82±0.01, 8.81±0.01 and 8.81±0.01, respectively which got reduced to 8.14±0.03, 7.75±0.06, 7.87±0.02, 7.86±0.02 and 7.50±0.06 for PY, ABY, AGY, CIY and HSY, respectively on 21st day.

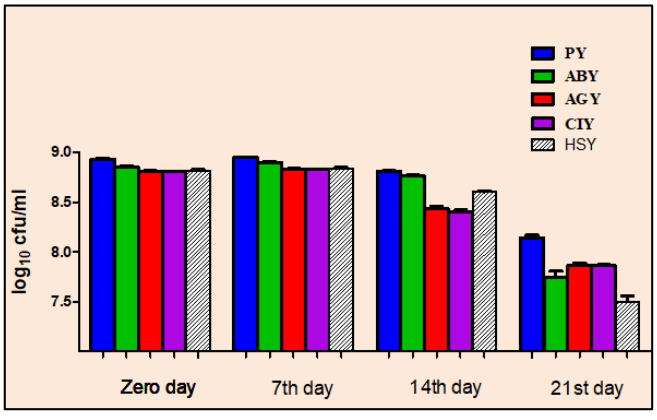

Fig 1: Viability of probiotic Streptococcus salivarius subsp. thermophilus (log10 cfu/ml) in herbal yoghurt during storage period

From the statistical analysis of the table 1, it was observed that there was a significant (P<0.05) reduction in the storage mean±SE value of viability from 8.84±0.002 to 7.82±0.01 during storage from day zero to 21, respectively. Overall treatments the count for PY and ABY were significantly (P<0.05) higher (8.71±0.01and 8.56±0.02, respectively) on comparison with AGY, CIY and HSY with the values of 8.48±0.02, 8.47±0.01and 8.44±0.02, respectively.

Viability of *Lactobacillus delbrueckii* subsp. *bulgaricus* (log_{10} cfu/ml) in herbal yoghurt during storage period

The effect of herbs on viability of *Lactobacillus delbrueckii* subsp. *bulgaricus* in herbal yoghurt during storage period (mean±SE) is presented in the Tables 3, 4 and in Figure 2.

Table 3: Viability of probiotic lactobacillus delbrueckii subsp. bulgaricus (log10 cfu/ml) in herbal yoghurt during storage period (mean±SE)

Days	PY	ABY	AGY	CIY	HSY	Storage mean±SE
zero day	8.31±0.01	8.31±0.02	8.36±0.02	8.34±0.02	8.31±0.01	8.33^{a}±0.003
7th day	8.35±0.01	8.47±0.01	8.43±0.02	8.37±0.03	8.35±0.01	8.39^{a}±0.004
14th day	8.14±0.02	8.39±0.01	7.96±0.02	7.95±0.02	8.14±0.02	8.14^{b}±0.01
21st day	7.07±0.05	7.22±0.05	6.95±0.07	6.69±0.05	7.07±0.07	7.00^{c}±0.01
Treatment mean±SE	7.96^{b}±0.02	8.10^{a}±0.02	7.92^{b}±0.03	7.84^{b}±0.03	7.96^{b}±0.02	-

^{abcd} means bearing same superscript column wise and row wise was not significant

Table 4: ANOVA (Multivariate) of viability of probiotic lactobacillus delbrueckii subsp. bulgaricus in herbal yoghurt during storage period

Source	df	MSS	F- value	P- value	Error	
					df	MSS
Treatment	4	0.207	4.714	0.00	100	0.044
Storage	3	12.685	288.780	0.00		
Storage*Treatment	12	0.097	4.677	0.00		

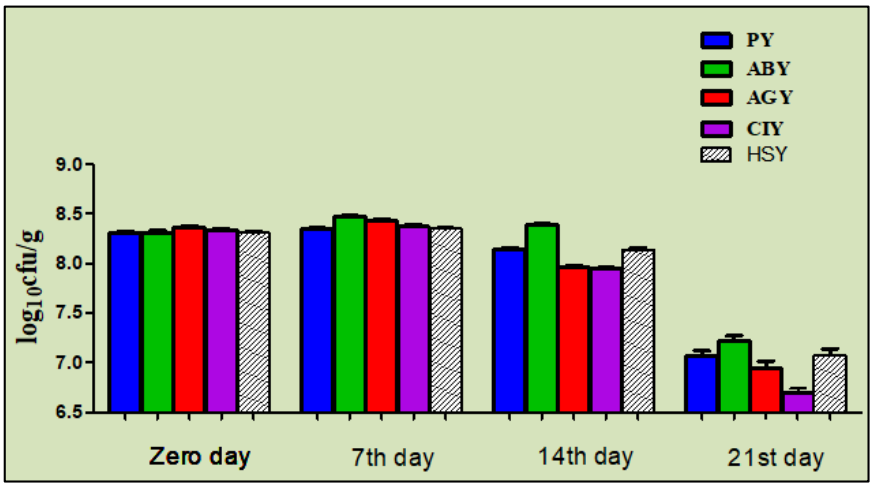

Fig 2: Viability of probiotic lactobacillus bulgaricus (log10 cfu/ml) in herbal yoghurt during storage period

A reduction in the viability of *Lactobacillus delbrueckii* subsp. *bulgaricus* count (log_{10} cfu/ml) was observed in all yoghurts during storage period of 21 days. There was increase in the count up to 7 days of storage and then the count was reduced gradually to 21 days storage.

Statistical analysis revealed significant (P<0.05) difference between the storage period with log_{10} values of 8.33±0.003, 8.39±0.004, 8.14±0.01 and 7.00±0.01 for zero, 7th, 14th and 21st day, respectively.

However, no significant differences were observed between PY, AGY, CIY and HSY treatments except ABY which showed lower reduction (8.10±0.02).

Discussion

There was significant (P<0.05) reduction in the viability of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (log_{10} cfu/ml) were noticed in all the herbal yoghurt including plain yoghurt with the values 8.84±0.002 and 7.82±0.01 for *S.thermophilus* and 8.33±0.003 and 7.00±0.01 for *L.bulgaricus* on zero day to 21st day, respectively (Table 1).

The present study is in agreement with previous studies of Lawin and Kongbangkerd (2010) [5] that the survival of *L. bulgaricus*, *S. thermophillus* and *L. casei* decreased throughout the storage period of 21days on addition of different levels of roselle (*Hibiscus sabdariffa*) syrup with probiotic count range of 8-9 log cfu/g and ensured that the yoghurt could be claimed for human health.

Panesar and Shinde (2011) [8] revealed that the addition of *Aloe vera* juice blend in to yoghurt decreases *Lactobacillus acidophilus* count from 39.7×10^{9} cfu/ml to 32.1×10^{9} cfu/ml and *Bifidobacterium bifidum* count from 16.9×10^{9} cfu/ml to 7.3×10^{9} cfu/ml during the storage period of 28 days.

Behrad *et al*. (2012) [1] noticed that addition of licorice resulted in significantly higher count of yoghurt cultures compared to plain yoghurt during the storage period of 28 days.

Marhamatizadeh *et al*. (2012) [6] determined that the shelf life of *Anethum graveolens* incorporated yoghurts was 21 days during which the bacterial count decreased but not less than 10^{9} and it was observed in the study that count of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was 6.75×10^{10} and

10.75×10^{10} on first day and 10×10^{10} and 9.5×10^{10} in 15th day, respectively on addition of 0.6 per cent *Anethum graveolens*.

Marhamatizadeh *et al*. (2013) [7] reported effect of green tea extract (0.9 per cent) on *Lactobacillus acidophilus* and olive leaf extract (0.9 per cent) on *Bifidobacterium bifidum* in yoghurt during storage and recorded *Lactobacillus acidophilus* count of 23.5×10^{10} cfu/ml on first day and 23.5×10^{10} cfu/ml on 21st day and *Bifidobacterium bifidum* count of 19.25×10^{10} cfu/ml on first day and 45.75×10^{10} cfu/ml on 21st day.

Houshang *et al*. (2014) [4] concluded that increasing the level of addition of *Aloe vera* has a positive influence on the growth and viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in probiotic milk and yoghurt.

The present study concluded that the addition of extracts of *Aloe barbadensis*, *Anethum graveolens*, *Costus igneus* and *Hibiscus sabdariffa* did not affect the growth and viability of the probiotic organism in the yoghurt during the storage period of

21 days and all the herbal yoghurts have been found to have the count above 6 $\log_{10}$.

In order to obtain the desired health effects, probiotic organisms should be present in a food at a minimum concentration of 10^5-10^6 cfu/g (Gomes and Malcata, 1999) [3]. Such high numbers have been recommended to compensate for possible losses in the numbers of the probiotic organisms during passage through the stomach and intestine. Therefore, in order to provide the therapeutic benefits, attempts to increase viability in dairy products have drawn the attention of researchers in recent years. On the other hand, there is much evidence to show that *S. thermophilus* and *L. bulgaricus* survive at high concentration (>10^7 cfu/g) in yoghurts after manufacture until the time of consumption (Rohm *et al*., 1990) [9].

Conclusion

In the viability study of probiotics organisms, it was revealed that there was significant (P<0.05) reduction in the viability of *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (log10cfu/ml) were noticed in all the herbal yoghurt including plain yoghurt during storage period of 21days and wherein the reduction was high in AGY, CIY and HSY compared with PY and ABY and this may be attributed to slight acidic nature of herb. But, the count of probiotic strains was maintained at 7 log units for all herb treated yoghurts throughout storage period of 21 days.

Acknowledgement

Authors are thankful to Vice chancellor TANUVASU, Dean VCRI Namakkal, Head Department of LPT VCRI Namakkal. For providing necessary facility to accomplish the work in time.

References

1. Behrad S, Yusof MY, Goh KL, Baba AS. Manipulation of probiotics fermentation of yogurt by *Cinnamon* and *Licorice*: Effects on yogurt formation and inhibition of *Helicobacter pylori* growth *in vitro* world academy of science. *Eng. Technol.*, 2012; 60:590-594.
2. Fitzgerald RJ, Murray BA, Walsh DJ. Hypotensive peptides from milk proteins. J Nutr. 2004; 134:980 -988.
3. Gomes AMP, Malcata FX. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends Food Sci. Technol. 1999; 10:139-157.
4. Houshang J, Mohammad Hossein M, Mohaddese Y, Zahra KJ, Elham R. Studying the influence of Aloe vera on the growth of the *Lactobacillus acidophilus* and Bifidobacterium bifidum probiotic bacterias in producing probiotic milk and yoghurt. Unique J pharma. boil. sci., 2014; 2(02):16-20.
5. Lawin P, Kongbangkerd T. Development of probiotic yoghurt mixed with roselle syrup. KKU Res. J. 2010; 15(9):803-808.
6. Marhamatizadeh MH, Jaafari F, Rezazadeh S, Ehsandoost E, Mohammadi M. Effects of dill extract (*Anethum graveolens*.) on growth and survival of lactobacillus acidophilus and Bifidobacterium bifidum in probiotic milk and yoghurt. Global Veterinaria. 2012; 9(3):252-257.
7. Marhamatizadeh M, Ehsandoost E, Gholami P. The influence of green tea (*Camellia sinensis* L.) extract on characteristic of probiotic bacteria in milk and yoghurt during fermentation and refrigerated storage, Int. J Farm Alli. Sci. 2013; 2(17):599-606.
8. Panesar PS, Shinde C. Effect of storage on syneresis, pH, Lactobacillus acidophilus count, *Bifidobacterium bifidum* count of Aloe vera fortified probiotic yoghurt. Curr. Res. Dairy Sci. 2011, 1-7.
9. Rohm H, Lechnerand F, Lehner M. Microflora of Australian natural-set yoghurt. J Food Prot., 1990; 53:478-480.
10. Shah NP. Effect of milk-derived bioactives: an overview. Brit. J. Nutr. 2000b; 84(1):3-10.
11. Tomasik PJ, Tomasik P. Probiotics and prebiotics. Cereal Chem. 2003; 80:113-117.