Effect of Probiotics and storage stability on quality of Bio-Fruit Yoghurt

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ABSTRACT

Freshly drawn buffalo milk after clarification, standardization (3% fat), addition of skim milk powder (SMP) (4 %), sugar (7 %) and sodium alginate (0.4%) was pasteurized (90^{0}C, 5 min), cooled (36^{0}C), mixed with papaya (10%) and mango (20%) pulp and then inoculated with 1.5 % Lactobacillus delbrukii spp. bulgaricus + 1.5 % Streptococcus thermophilus and Biofidobacterium (1 %) for preparing bio-fruit yoghurt. Incubation was carried out at 42 ± 1^{0}C for 3-3.5 h. The control and experimental samples were kept at 30 ± 1 and 4 ± 1^{0}C to study the effects of probiotics, fruit pulp and storage temperatures on the quality parameters of products. Results indicated that curd tension, viscosity, pH and acetaldehyde content registered a declining trend while syneresis, acidity and content of soluble nitrogen and free fatty acids (FFA) followed an increasing trend irrespective of storage temperature, fruit pulp and culture. Total plate count increased during storage at 30 ± 1^{0}C but an opposite trend was observed at 4 ± 1^{0}C. Yeast and mold, and coliforms were not detected during the entire period of storage at the either temperature. Overall acceptability of all the three samples was maintained only upto 4 h when stored at 30 ± 1^{0}C while control, papaya and mango yoghurt were acceptable upto 3, 3 and 6 days of storage, respectively at 4 ± 1^{0}C. Statistically, the effects of storage on the quality attributes was found significant.

Key words: *Probiotics, Bio-fruit yoghurt, Antagonistic activity, Pineapple and papaya Pulp, Storage stability.*

INTRODUCTION

Yoghurt is one of the widely used fermented milk products that was developed over thousands of years ago around the Mediterranean basin, Middle East and India(Marshall 1987) It is extremely successful in finding universal acceptance. According to FAO/WHO(1977). yoghurt is a coagulated milk product obtained by lactic acid fermentation, through the action of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk and milk products (pasteurized and concentrated milk) with and without addition of milk powder, skim milk powder,stabilizer and the microorganisms in the final product must be viable and abundant. Besides nutritional importance it also provides environment that facilitates growth and stability of indignant flora alone in the human intestine (Kurmann *et al.,* 1992). Bio-yoghurt with additional benefits is a type of yoghurt when probiotics like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are employed along with yoghurt culture. Incorporation of fruit also upgrades the nutritional and sensory characteristics of yoghurt and several reports are available in literature pertaining to fruit yoghurt (Desai *et al.,* 1994; Chandan 1982; Nile *et al.,* 1987; Balasubramanym 1991;Prasad and Geetha 2001).The present investigation was therefore, carried out with the purview of further improvement in the quality of fruit yoghurt by employing probiotics.

MATERIALS AND METHODS

Freshly drawn buffalo milk was obtained from Dairy Instructional Farm, G.B. Pant University of Agriculture and Technology, Pantnagar, India. Papaya, mango and sugar were purchased from Pantnagar Market Complex of this Univerisity and spray dried skimmed milk powder (SMP) manufactured by Hindustan Liver Ltd., Mumbai, India was used. Freeze dried cultures of *Lactobacillus delbrukii* spp. *bulgaricus* (NCDC-009), *Lactobacillus acidophilus* (NCDC-013), *Streptococcus thermophilus* (NCDC-074) and *Bifidobacterium bifidum*, (NCDC-229) were obtained from Culture Collection Centre, National Dairy Research Institute, Karnal (India). Pathogenic microbes *Salmonella typhimurium, E. coli and Staphylococcus aureus* were procured from the Department of Microbiology of G.B. Pant University of Agriculture and Technology, Pantnagar. Culture Media were purchased from HI-Media Laboratories (P) Ltd., Mumbai and Analytical Reagents (A.R.) grade chemicals were used.

Antagonistic Activity of Probiotics

To select an ideal probiotic and its level for producing bio-yoghurt their inhibitory activites against pathogens were determined by the modified Agar well Assay technique (British standard Institution 1968). Four lots of sterilized milk after inoculation with conventional yoghurt cultures (*Lactobacillus delbrukii* spp. *bulgaricus* 1.5% + *Streptococcus thermophilus* 1.5%) and both the probiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) at 0.5 and 1.0 % levels were incubated (48 h, 37 ± 1°C). Supernatant obtained by centrifugation (2000 g, 20 min, 4 °C) of curdled sample was passed through 0.45 filter (Millipore, U.S.A.) and cell free filtrates were employed to assess inhibitory activity. 0.2 and 20 ml of active pathogenic culture (OD 0.45-0.55) and nutrient agar, respectively

transferred to sterilized petri plate and mixed properly. The solidified layer was punched aseptically with a sterile well cutter (10 mm dia) and Cell free filtrate (0.2 ml) was poured in each sealed well. Each well was sealed by pouring small amount of melted agar. After incubation (24-26 h, 37 ± 1^{0}C), diameter of zone surrounding well was measured using a Vernier Caliper.

Extraction of Fruit Pulp

Ripe mango fruits were washed, peeled and destoned. Sliced flesh added with 10 % sugar was pulped in a mixi grinder and pulp following heating (80^{0}C for 1 min) was cooled. Similarly edible portion of ripe papaya fruit was sliced, added with 10 per cent sugar and 10 % water, pulped, heated (100^{0}C for 1 min) and cooled(Dauthy 1997). The extracted pulp samples were stored at 4 ± 1^{0}C.

Preparation of Yoghurt

Bio-fruit and control yoghurt samples were prepared as described by Amiri (2001).

STORAGE STUDIES

Yoghurt samples were stored at 30 ± 1 and 4 ± 1^{0}C and analysed at an interval of 4 h and 3 days, respectively for sensory, physico-chemical and microbiological characteristics.

Sensory Characteristics

Organoleptic quality of yoghurt samples was assessed by a semi trained panel of ten judges(Larmond 1997). The panel included the staff members and students of the Department of Food Science and Technology. The judges recorded their degree of liking on a 9 point hedonic scale wherein 1 and 9 represented “disliked extremely” and “liked extremely”, respectively. The samples were evaluated in a sensory lab maintained at 20^{0}C.

Physico-Chemical Analysis

Curd tension was determined by the method suggested by Curd Tension Committee of American Dairy Science Association as described elsewhere(Chandrakehara *et al.,* 1957). Brook Field Synchro Electric Viscosimeter was employed to measure viscosity. Spindle 4 was used to measure torque at speeds of 12 and 30 rpm. For estimation of syneresis, method described by (Modler *et al.,*1983) was used. The pH, acidity, soluble nitrogen , acetaldehyde and free fatty acids was determined in the samples as described in AOAC(1975)

TABLE 1 : ***Antagonistic Activity of Probiotics along with Yoghurt Culture****

Cell free filtrate	Inhibitory Zone Diameter (mm) towards Human Pathogens		
	E. coli	Staphylococcus aureus	Salmonella typhimurium
ST** (1.5%) + LB** (1.5%) + LA** (0.5%)	13.13	12.33	11.21
ST (1.5%) + LB (1.5%) + LA (1%)	15.10	19.00	17.00
ST (1.5%) + LB (1.5%) + BB** (0.5%)	14.30	14.20	13.35
ST (1.5%) + LB (1.5%) + BB (1%)	16.29	21.30	18.00

* Average of triplicate experiments
** ST,LB,LA and BB stand for *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidiobacterium bifidus*, respectively

Microbiological Quality

To enumerate microbial characteristics APHA (1992) methods were used. Tryptone dextrose extract agar, potato dextrose agar and violet red bile agar were employed to assess total plate, yeast and mold and coliform counts, respectively and the respective incubation conditions were 30 ± 1^{0}C, 48 h; 21 ± 1^{0}C, 3-5 days; and 30 ± 1^{0}C, 24 h.

Statistical Analysis

The data were subjected to statistical analysis by using ANOVA technique (Snedecor and Cochron 1997).

TABLE 2 : ***Sensory Characteristics of Bio-fruit Yoghurt during Storage***

Type of yoghurt	Storage period (h)	Organoleptic score at 30 ± 1^{0}C					Storage period (d)	Organoleptic score at 4 ± 1^{0}C				
		Colour	Appearance	Body and texture	Taste	OAA		Colour	Appearance	Body and texture	Taste	OAA
Control	0	7.3	7.2	7.8	7.3	7.8		7.3	7.2	7.8	7.3	7.8
	4	7.2	8.1	7.2	7.5	7.0	0	7.0	7.0	7.1	7.1	7.0
	8	6.8	7.8	6.5	7.5	6.4	3	7.0	5.2	6.0	6.6	5.5
	12	6.7	7.6	5.6	6.8	5.5	6	6.5	5.0	5.5	6.0	4.5
	16	2.2	4.2	4.5	2.5	5.0	9	5.5	4.6	4.0	5.5	4.0
CD at 5% level		**0.07**	**0.12**	**0.08**	**0.12**	**0.07**	**12**	**0.03**	**0.04**	**0.06**	**0.07**	**0.08**
Bio-papaya	0	8.3	8.3	8.2	8.8	8.8		8.3	8.3	8.8	8.8	8.8
	4	7.2	8.0	7.0	7.5	7.2	0	8.0	8.0	8.1	8.1	8.0
	8	6.9	7.8	6.5	7.0	6.2	3	8.0	6.2	7.0	7.6	6.5
	12	6.7	7.6	5.6	6.8	5.6	6	7.5	6.0	6.5	7.0	5.5
	16	2.6	5.5	2.5	4.5	4.5	9	6.5	5.6	5.0	6.5	5.0
CD at 5% level		**0.08**	**0.08**	**0.05**	**0.05**	**0.11**	**12**	**0.03**	**0.04**	**0.06**	**0.07**	**0.08**
Bio-mango	0	8.5	8.5	8.5	8.9	8.9		8.5	8.5	8.5	8.9	8.9
	4	7.3	8.2	7.5	7.5	7.5	0	8.5	8.5	8.5	8.3	8.3
	8	7.1	8.0	6.5	6.0	6.9	3	8.0	7.5	6.2	7.8	7.6
	12	6.0	7.6	6.2	6.0	6.8	6	7.5	6.1	6.1	6.8	6.3
	16	5.9	5.8	2.8	5.0	4.8	9	6.5	5.5	6.0	6.5	5.5
CD at		**0.08**	**0.08**	**0.06**	**0.06**	**0.11**	**12**	**0.04**	**0.05**	**0.06**	**0.07**	**0.07**

RESULTS AND DISCUSSION

Data pertaining to antagonistic activities of bacterial cultures against the human pathogens indicated largest inhibitory zone when *Biofidobacterium bifids* at a rate of 1% was used along with conventional yoghurt culture (Table 1). This combination of probiotic and yoghurt culture was therefore employed for the preparation of bio-fruit yoghurt in this study.

Sensory Characteristics

Results indicate that use of probiotics and either type of fruit pulp in the preparation of yoghurt samples did not alter the shelf life of products when stored at 30 ± 1^{0}C while appreciable increase in keeping quality was observed with low temperature storage (4 ± 1^{0}C) of the yoghurt samples. Overall acceptability of all the three types of yoghurt samples was maintained upto 4 h of storage at 30 ± 1^{0}C (Table 2) while control, papaya and mango yoghurt samples were found acceptable upto 3, 3 and 6 days respectively at 4 ± 1^{0}C (Table 2). Scores for all the sensory attributes reduced to varying degree with the progress of storage period but effect was much more with storage at higher temperature. Type of fruit pulp and storage temperature influenced all the sensory parameters significantly due to progress of storage period irrespective of use of probiotics. Reduction in body and texture score of yoghurt samples during storage at 7^{0}C was reported earlier (Amiri 2001). Flavour score of sucrose and sorbitol sweetened fruit yoghurt was however unaltered up to 28 days of storage at 7^{0}C (Keating and White 1990).

Physico-chemical Characteristics

Data furnished in Table 3 and Table 4 indicate that curd tension, viscosity, pH and acetaldehyde content exhibited a declining trend irrespective of storage temperature while syneresis, acidity and content of soluble nitrogen and FFA followed an increasing trend. Bio-mango yoghurt recorded maximum changes in viscosity, pH, acidity and soluble nitrogen content at the end of storage period at either temperature. Maximum reduction in curd tension was observed during storage of bio-mango yoghurt at refrigerated temperature. Similarly changes in syneresis and acetaldehyde content were highest with bio-papaya yoghurt. Variations in FFA content were maximum with bio-mango and bio-papaya yoghurt samples due to storage at 30 ± 1 and 4 ± 1^{0}C, respectively. Results reveal that storage of control samples either at refrigerated or higher temperature resulted into minimum changes in viscosity, syneresis, pH and content of acetaldehyde and FFA however papaya yoghurt recorded least degree of changes in acidity and acetaldehyde at 30 ± 1^{0}C.

In the present study effects of storage on all the physico-chemical parameters under investigation were statistically significant ($P < 0.05$). Increase in syneresis (Farooq and Haque 1992), soluble nitrogen (Amiri 2001)[1], volatile fatty acids (Formisano *et al.,* 1971) and acidity (Amiri 2001; Sarkar and Mishra 1998) during storage of yoghurt was reported earlier by workers. Similarly, reduction in pH (Amiri 2001; Keating *et al.,* 1990; Farooq and Haque 1992) and acetaldehyde content (Singh 1994) was observed by previous workers.

Microbial Quality

The initial total plate count in yoghurt samples varied from 3.6 $\times 10^8$ to 4.0 $\times$ 10^8 cfu/ml (Table5). Total plate count increased with the progress of storage period at 30 ± 1^{0}C and it was maximum (9.5 $\times$ 10^8 cfu/ml) with control and minimum (8.6 $\times$ 10^8 cfu/ml) with mango-yoghurt at the end of storage period. Storage at 4 ± 1^{0}C however resulted into reduction in the total plate count and there was slight

TABLE 3 : ***Physico-chemical characteristics of Bio-fruit Yoghurt during Storage at 30 ± 1^{0}C****

		Organoleptic score							
Type of Yoghurt	**Storage Period (h)**	**Curd tension (g)**	**Viscosity (cp)**	**Syneresis (ml/ 100g)**	**pH**	**Acidity (%, lactic acid)**	**Acetal dehyde (ppm)**	**Soluble Nitrogen (%)**	**FFA (meq/ ml)**
Control	0	42(-0.23)	6200(-)	20.0(-)	4.5(-)	0.95(-)	41.5(-)	0.10(-)	8.1(-)
	4	41.9(-4.76)	6160(-0.65)	20.2(+1.00)	4.3(-4.44)	1.02(+7.37)	40.0(-3.61)	0.11(+10)	8.2(+1.23)
	8	40(-13.10)	6100(-1.61)	22.1(+10.50)	4.2(-6.67)	1.26(+32.63)	39.5(-4.82)	0.13(+30)	8.4(+3.70)
	12	365	6056(-2.32)	24.0(+20.0)	4.1(-8.89)	1.31(+37.89)	37.0(-10.84)	0.15(+50)	8.6(+6.17)
	16	31.5(-25.00)	5936(-4.26)	25.5(+27.5)	3.9(-13.23)	1.39(+46.32)	33.5(-19.28)	0.19(+90)	9.2(+13.58)
CD at 5% level		**0.18**	**6.34**	**0.12**	**0.01**	**0.01**	**0.17**	**0.01**	**0.02**
Bio-papaya	0	36(-)	6103(-)	23.0(-)	4.3(-)	1.01(-)	38.3(-)	0.13(-)	8.6(-)
	4	35.9-(0.28)	6101(-0.03)	23.4(+1.74)	4.2(-2.33)	1.09(+7.92)	37.6(-1.82)	0.13(-)	8.7(+1.16)
	8	34.0(-5.56)	6095(-0.13)	25.0(+8.70)	4.1(-4.65)	1.30(+28.71)	36.9(-3.66)	0.14(+7.69)	9.0(+4.65)
	12	32.2(-10.56)	5820(-4.64)	28.5(+23.91)	3.8(-11.63)	1.37(+35.64)	34.0(-11.23)	0.17(+30.77)	9.2(+6.98)
	16	29.5(-18.06)	5210(-14.63)	31.0(+34.78)	3.5(-18.60)	1.42(+40.59)	29.5(-22.98)	0.23(+76.92)	10.0(+16.28)
CD at 5% level		**0.23**	**131.11**	**0.18**	**0.02**	**0.09**	**0.18**	**0.02**	**0.03**
Bio-mango	0	40.5(-)	5122(-)	25.0(-)	4.5(-)	0.92(-)	35.6(-)	0.11(-)	8.1(-)
	4	39.8(-1.73)	5120(-0.04)	25.0(+2.00)	4.3(-4.44)	0.99(+7.61)	35.4(-0.56)	0.12(+9.09)	8.2(+1.23)
	8	38.0(-6.17)	5110(-0.23)	27.0(+8.00)	4.2(-6.67)	1.12(+21.74)	34.9(-1.97)	0.13(+18.18)	8.3(+2.47)
	12	35.5(-12.35)	4900(-4.33)	29.0(+16.00)	3.8(-15.56)	1.20(+21.74)	32.0(-10.11)	0.17(+54.55)	8.9(+9.88)
	16	32.0(-20.99)	4200(-18.00)	33.0(+32.00)	3.5(-22.22)	1.40(+52.1)	28.0(-21.35)	0.23(+109.01)	10.1(+24.69)
CD at 5% level		**0.14**	**131.11**	**0.16**	**0.02**	**0.01**	**0.16**	**0.02**	**0.04**

Average of triplicate experiments
Figures in parenthesis indicate % change on fresh basis

TABLE 4 : ***Physico-chemical characteristics of Bio-fruit Yoghurt during storage at 4 ± 1^{0}C****

Organoleptic Score									
Type of Yoghurt	**Storage Period (days)**	**Curd tension (g)**	**Viscosity (cp)**	**Syneresis (ml/ 100g)**	**pH**	**Acidity (%, lactic acid)**	**Acetaldehyde (ppm)**	**Soluble Nitrogen (%)**	**FFA (meq/ ml)**
Control	0	42.0(-)	6200(-)	20.0(-)	4.5(-)	0.95(-)	41.5(-)	0.10(-)	8.1(-)
	3	39.5(-5.95)	6120(-1.29)	21.1(+5.5)	4.2(-6.67)	1.03(+8.42)	39.0(-6.02)	0.11(+30.0)	8.2(+3.21)
	6	38.0(-9.52)	6090(-1.77)	21.9(+9.5)	4.1(-8.89)	1.08(+13.68)	38.0(-8.43)	0.13(+40.0)	8.4(+4.81)
	9	37.2(-11.43)	6020(-2.90)	22.3(+11.5)	3.9(-13.33)	1.20(+26.32)	37.0(-10.84)	0.15(+60.0)	8.6(+5.31)
	12	36.8(-12.38)	6000(-3.23)	22.9(+14.5)	3.6(-20.0)	1.30(+36.84)	35.0(-15.66)	0.19(+70.0)	9.2(+7.41)
CD at 5% level		**0.79**	**2.20**	**0.06**	**0.87**	**0.01**	**0.17**	**0.02**	**0.02**
Bio-papaya	0	36.0(-)	6103(-)	23.0(-)	4.3(-)	1.01(-)	38.3(-)	0.13(-)	8.6(-)
	3	33.0(-8.33)	6002(-1.65)	25.5(+10.87)	4.0(-6.98)	1.09(+7.92)	35.9(-6.27)	0.13(+7.69)	8.7(+8.14)
	6	32.0(-11.11)	5920(-3.00)	27.0(+17.39)	3.8(-11.63)	1.20(+18.81)	34.8(-9.14)	0.14(+30.77)	9.0(+17.44)
	9	30.0(-16.67)	5700(-3.00)	29.5(+28.26)	3.6(-16.28)	1.30(+28.71)	31.0(-19.06)	0.17(+69.23)	9.2(+43.02)
	12	28.0(-22.22)	5100(-16.43)	31.0(+34.78)	3.4(-20.93)	1.45(+43.56)	27.0(-29.78)	0.23(+92.31)	10.0(+61.63)
CD at 5% level		**0.27**	**18.96**	**0.16**	**0.02**	**0.01**	**0.12**	**0.01**	**0.11**
Bio-mango	0	40.5(-)	5122(-)	25.0(-)	4.5(-)	0.92(-)	35.6(-)	0.11(-)	8.1(-)
	3	39.2(-3.21)	5101(-0.41)	28.0(+12.0)	3.9(-13.33)	1.10(+19.57)	33.5(-5.90)	0.12(+18.18)	8.2(+9.88)
	6	34.8(-14.07)	4800(-6.29)	29.9(+19.6)	3.7(-17.78)	1.26(+36.96)	32.4(-8.99)	0.13(+45.45)	8.3(+14.81)
	9	31.0(-23.46)	4650(-9.22)	30.5(+22.0)	3.6(-20.0)	1.30(+41.3)	29.0(-18.54)	0.17(+81.82)	8.9(+27.16)
	12	27.0(-33.33)	4200(-18.0)	33.4(+33.6)	3.5(-22.22)	1.42(+54.35)	25.0(-29.50)	0.23(+118.18)	10.1(+53.09)
CD at 5% level		**0.15**	**18.35**	**0.16**	**0.02**	**0.01**	**0.20**	**0.02**	**0.08**

**Average of triplicate experiments*
Figures in parenthesis indicate % change on fresh basis

TABLE 5 : ***Microbial profile of Bio-fruit Yoghurt during storage***

Type of Yoghurt	**Storage Period (h)**	**Total Plate Count (cfu/ml) at 30 ±1C**	**Storage Period (days)**	**Total Plate Count(cfu/ml) at 4 ±1^{0}C**
Control	0	3.9×10^8	0	3.9×10^8
	4	4.9×10^8	3	3.5×10^8
	8	6.6×10^8	6	3.35×10^8
	12	8.8×10^8	9	3.1×10^8
	16	9.5×10^8	12	3.1×10^8
CD at 5% level		0.12	-	0.35
Bio-papaya	0	3.6×10^8	0	3.6×10^8
	4	4.2×10^8	3	3.5×10^8
	8	6.0×10^8	6	3.0×10^8
	12	8.3×10^8	9	2.8×10^8
	16	9.0×10^8	12	2.7×10^8
CD at 5% level		0.12	-	0.35
Bio-mango	0	4.0×10^8	0	4.0×10^8
	4	4.8×10^8	3	3.7×10^8
	8	5.7×10^8	6	3.6×10^8
	12	8.0×10^8	9	2.9×10^8
	16	8.6×10^8	12	2.8×10^8
CD at 5% level		0.10	-	0.35

** Average of triplicate experiments*
Yeast & Mould and coliforms counts not detected in any bio-yogurt samples irrespective of storage temp

variation of 2.7×10^8 to 3.1×10^8 cfu/ml at the end of 12 days of storage. Statistical analysis of data revealed that effect of storage temperature on the total plate count was significant. Results also show that yeast and mold and coliforms were not detected during the entire period of storage at either temperature indicating optimum sanitary conditions during manufacture of bio yoghurt samples. Similarly reduction in total plate count of yoghurt samples was observed earlier (Amiri 2001;Singh 1994;Khandelwal 2002). Absence of yeast and mold and coliforms in refrigerated stored yoghurt have also been reported (Amiri 2001; Khandelwal 2002).

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