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Research Article

OPTIMIZATION OF PROBIOTIC INOCULUM LEVELS IN BIO-YOGHURT BASED ON PHYSICO-CHEMICAL AND SENSORY ATTRIBUTES

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A B S T R A C T

The objective of this study was to analyze the physico-chemical and sensory attributes of bio-yoghurt at different inoculum levels (2, 3, 4 and 5 %) of Bifidobacteriumbifidum (BB12), *Lactobacillus acidophilus* (LA) and combination of these two cultures (1:1 ratio) in short set yoghurt. The milk setting time of bio-yoghurt was decreased by 15 min at 5 % inoculum levels irrespective of the type of probiotic cultures used as compared to control (yoghurt without probiotic cultures). The titratable acidity and syneresis were increased with the inoculum levels for BB12 and combination cultures (BB12 and LA,1:1) but, LA culture has shown decrease in acidity and controlled syneresis as compared to control, irrespective of inoculum levels. The sensory attributes of bio-yoghurt on 9 point Hedonic scale as evaluated by a panel of 5 judges showed maximum scores at inoculum levels of 4 % for BB12 culture and 3 % for LA culture and 4 % for combination cultures of BB12 and LA (1:1). The sensory attributes such as flavor, sourness and overall acceptability of the BB12 culture was higher at 4 % inoculum level, whereas, 3 % inoculum level LA culture showed higher sensory scores for colour and appearance, body and texture and overall acceptability as compared to control. The combination culture (BB12 and LA, 1:1) bio-yoghurt at 4 % inoculum levels showed higher scores for all the sensory attributes as compared to control yoghurt.

INTRODUCTION

The word 'Probiotic' is derived from Greek words pro and biotos and translated as 'for life'[1]. In October, 2001, an expert consultation meeting convened by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) defined probiotics as "live microorganisms which when administered in adequate amount confer ahealth benefit on host" [2,3].

Lactic acid bacteria (LAB) play a major role in determining the positive health effects of fermented milks and related products which possess various nutritional and therapeutic properties. Yoghurt can be redefined as a probiotic carrier food. Therapeutic benefits have led to an increase in the incorporation of probiotic bacteria such as bifidobacteria and lactobacilli in dairy products, especially fermented dairy products. Bio-yoghurt containing these probiotic cultures with established and potential health benefits include; improvement of the immune system, reduction in the side effects of antibiotics, prevention of intestinal infections, improvement in lactose digestion, treatment of infant diarrhoea (rotavirus), cholesterol-lowering effects. In order to claim and to ensure maximum health benefits, yoghurt should meet the suggested minimum number of 10^6cfu viable probiotic bacteria cells per gram[4,5]. The cultures most often mentioned as probiotics for humans include *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium* species. All these species can survive and grow in the intestinal tract and thus have the potential to provide benefits[6,7].

A probiotic yoghurt must elicit not only the minimum number of viable cells to confer health effects but also sensory acceptability by consumers. Thus, sensory evaluation of such probiotic added products must be evaluated before commercialization to avoid any off-flavours developed during the manufacture. In general, all probiotic foods must be safe and should have good sensory properties. The success of sensory evaluations regarding probiotic dairy products depends on the methodology applied and the inclusion of similar non-probiotic products in the test to obtain scientific sound results and also to analyze the main positive/negative points of the food product[8].

In the present study, individual cultures of *Bifidobacterium bifidum* (BB12), *Lactobacillus acidophilus* and combination of

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these two cultures (1:1 ratio) were used to optimize for different probiotic culture levels in set yoghurt prepared using 2 % *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp*. *Bulgaricus* in the ratio of 1:1. The product was evaluated based on the physico- chemical and sensory qualities of the bio-yoghurt.

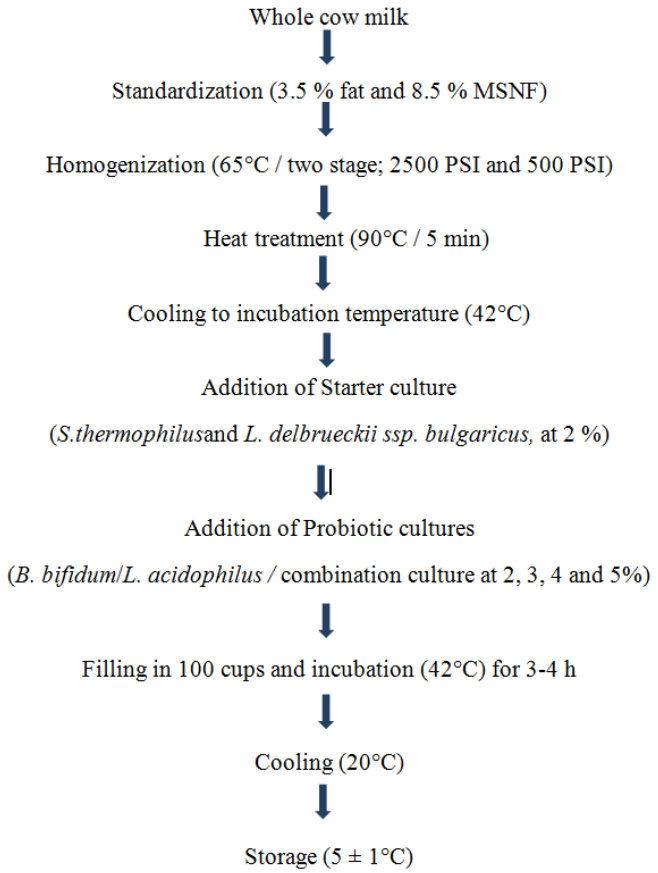
MATERIALS AND METHODS

***Whole milk*:** Cow milk was procured from Dairy farm under ILFC department, Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), Hebbal, Bangaluru.

***Yoghurt Starter cultures*:** Mixed yoghurt culture of S*treptococcus thermophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus* (2500 U) were obtained in the form of freeze dried - direct vat set (FD-DVS) from Chr. Hansen Laboratories, Copenhagen, Denmark.

***Probiotic cultures*:** Probiotic cultures such as *Bifidobacterium bifidum* (Nutrish® BB12) and *Lactobacillus acidophilus* (Nutrish®) were obtained in the form of freeze dried - direct vat set (FD-DVS) from Chr. Hansen Laboratories, Copenhagen, Denmark.

***Preparation of bio-yoghurt*:** Short set bio-yoghurt was prepared as given in the following flow chart using individual cultures of BB12 and LA and combination of these two cultures in the ratio of 1:1[9].



Flow chart for preparation of bio-yoghurt incorporated with GMP

Packaging material used was polypropylene 100 ml cup with air tight lids for sensory evaluation of bio-yoghurt.Total Solids (TS), fat, titratable acidity of milk, bio-yoghurt samples were determined as per procedure given in ISI: SP 18 (Part XI) 1981[10].

***Syneresis*:** The susceptibility of bio-yoghurt to syneresis was determined using drainage method, performed at 6°C. Bio-yoghurt was transferred into a funnel fitted with a 120 mesh stainless steel screen. The volume of the collected whey over 2 h was measured and expressed in ml/100g (%, v/w) of yoghurt [11].

***Sensory evaluation*:** The bio - yoghurt was served to panel of 5 judges to analyze for sensory characteristics *viz*.colour and appearances, body and texture, flavour sourness and over all acceptability. The sensory scores were awarded using 9 point Hedonic scale[12].

RESULTS AND DISCUSSION

The effect of various inoculum levels of probiotic cultures (2, 3, 4 and 5 % of *B. bifidum, L. acidophilus* and combination of these two cultures) on milk setting time, titratable acidity and syneresis in bio-yoghurt was studied. Development of acidity depends on the inoculum levels and the type of probiotic cultures used for product preparation, which in turn decides the setting time of milk, syneresis and sensory attributes of bio-yoghurt.

Effect of inoculum levels of probiotic cultures on physico-chemical characteristics of bio-yoghurt

Inoculum levels of probiotic cultures such as *B.bifidum*, *L.acidophillus* individually and in combination (1:1 ratio) at 2, 3, 4 and 5 % levels were used in preparation of short set bio-yoghurt along with yoghurt cultures *S.thermophilus* and *L. delbrueckii ssp. Bulgaricus* (2 %, 1:1). The results pertaining to the effect of these probiotic cultures on milk setting time, titratable acidity and syneresis are depicted and discussed below.

Milk setting time

As could be observed from the results (Figure 1) that the type of cultures used for inoculum had no significant effect on milk setting time. The setting time for control, 2, 3, 4 and 5 % level of inoculum was observed to be 240, 235, 235, 230 and 225 min, respectively for all types of cultures viz. individual *B.bifidum, L.acidophilus* and combination culture. The statistical analysis has shown significant difference in milk setting time with the levels of inoculum used and control but type of cultures has no effect. Increase in the levels of probiotic cultures, resulted in decrease in milk setting time by 15 min less than control (240 min) at 5 % culture levels. The decrease in milk setting time may be due to faster development of acidity as the inoculum levels increased. El-Dieb *et al* (2012)[13] also reported that yoghurt culture, YC Fast 1 developed acidity faster than YC-380 and YC-180 and had taken 3, 3.5 and 4 h of milk setting time, respectively in presence of *L.casei and B.bifidum*.

Titratable acidity

Titratable acidity (% lactic acid) recorded (Table 1) for bio-yoghurt incorporated with *B.bifidum* was 0.78, 0.81, 0.86 and 0.89 % lactic acid (LA) where as it was 0.81, 0.83, 0.85 and 0.86 % LA for combination culture at 2, 3, 4 and 5 % level of inoculum, respectively. However, yoghurt prepared with *L. acidophilus* showed a lesser acidity with 0.73, 0.71, 0.72 and 0.72 % LA, respectively at the above levels of inoculum as against control (0.77 % LA).

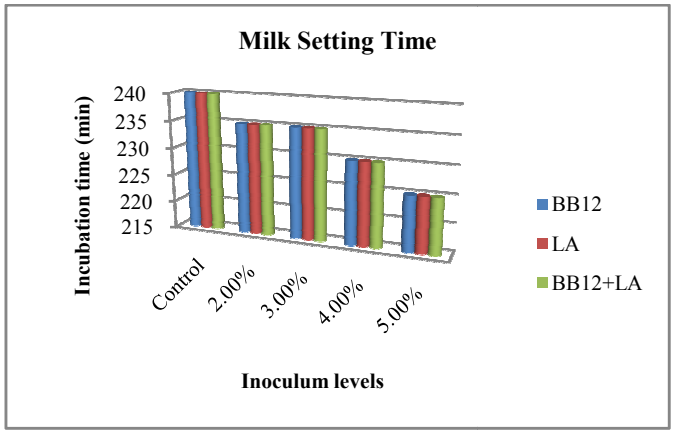


Figure 1 Milk setting time of probiotic cultures at various inoculum levels in short setbio-yoghurt

Maximum acidity was observed incombination culture at 5 % level (0.86 % LA) as against control (0.77 % LA) and yoghurt with individual cultures of *B. bifidum and L. acidophilus* (0.89 and 0.72%, respectively). Titratable acidity was found to be statistically significant for different types of cultures used and the inoculum levels. Combination culture was effective in producing bio-yoghurt with optimum acidity as compared to individual cultures which showed too high and too low acidity which might affect the sensory properties, syneresis and wheying off on the surface of the product. Probiotic cultures *B.bifidum* and *L. acidophilus* are slow acid producers hence, the symbiotic relationship between *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* cultures and influence of probiotic cultures on the growth behavior may influence on the acid production. The results of the present study are in agreement with El-Dieb*et al.* (2012) [13] who reported that the titratable acidity increased rapidly with yoghurt samples made with YC-Fast 1 and for bio- yoghurt made with YC Fast 1, YC-380 and YC-180 in presence of *L.casei and B bifidum*.

Syneresis

Syneresis (ml /100g) increased with the increasing in the inoculum levels of probiotic culture except in the case of *L. acidophilus* culture, which showed a constant value at all the levels (42 %) with a meagre increase compared to control (40 %). Syneresis was observed to be maximum for *B. bifidum* culture (50 %) at both 4 and 5 % inoculum levels as against control (40 %), *L. acidophilus* culture (42 %) and combination of cultures (43 %) at same inoculum levels (Table 1). The statistical analysis showed significant differences indicating both inoculum levels and the type of cultures used had an impact on syneresis (***P***≤0.05). The maximumsyneresis in *B.bifidum* culture at 4 and 5 % inoculum levels may be due to higher activity of *S. thermophilus and L. delbrueckii ssp. bulgaricus* cultures in the presence of *B. bifidum* which produced higher acidity in the product.

Combination culture could control the syneresis effectively even at higher levels of inoculum whereas, *L. acidophilus* has shown controlled syneresis at all levels of inoculum. So combination culture has produced best results with development of optimum acidity with controlled syneresis.

Table 1 Effect of probiotic cultures on titratable acidity and syneresis in short set bio- yoghurt

Inoculum levels (%)	Type of probiotics cultures							
	BB12	LA	BB + LA	CD (***P***≤0.05)	BB12	LA	BB + LA	CD (***P***≤0.05)
	Titratable Acidity (% lactic acid)				Syneresis (%, v/w)			
Control	0.77^{dA}	0.77^{aA}	0.77^{cA}		40^{cA}	40^{aA}	40^{bA}	
2	0.78^{dC}	0.73^{bB}	0.81^{bA}		42^{bcA}	42^{aA}	40^{bA}	
3	0.81^{cB}	0.71^{bC}	0.83^{bA}	0.020	44^{bA}	42^{aA}	42^{aA}	2.46
4	0.86^{bA}	0.72^{bB}	0.85^{aA}		50^{aA}	42^{aB}	43^{aB}	
5	0.89^{aA}	0.72^{bC}	0.86^{aB}		50^{aA}	42^{aB}	43^{aB}	
CD (***P***≤0.05)		0.028				2.58		

Note
All the values are average of 5 trials
Control: Yoghurt without probiotic culture (*S. thermophillus and L. bulgaricus* (2 %, 1:1)
BB12- *B. bifidum* ; LA - *L. acidophilus*; BB+LA - *B. bifidum and L. acidophilus* (1:1)
Similar superscripts indicate non significant at the corresponding CD
Small superscript: Inoculum Levels
Capital superscript: Type of cultures

Effect of probiotic culture inoculum levels on sensory attributes of bio-yoghurt

The effect of various probiotic cultures individually and in combination at different levels of inoculum on the sensory attributes of bio-yoghurt is discussed here under along with the results presented in Tables with statistical analysis.

Effect of levels of Bifidobacterium bifidumon sensory attributes of short set bio- yoghurt

B. bifidum probiotic culture was added to milk along with yoghurt culture at 2, 3, 4 and 5 % inoculum levels. The effect of *B. bifidum* culture at different inoculum levels on sensory attributes of yoghurt is presented in Table 2. The scores awarded to the bio-yoghurt at 4 % *B.bifidum* inoculum level was 7.50, 7.50, 8.05, 8.05 and 8.05 for colour and appearance, body and texture, flavour, sourness and overall acceptability attributes, respectively as compared to control (8.00, 7.66, 7.83, 7.75 and 7.75). The statistical analysis indicated that higher scores for *B. bifidum* bio-yoghurt has shown significant difference in flavour, sourness and overall acceptability attributes whereas control sample showed better score with respect to colour and appearance and body and texture attributes at all levels of inoculum was found to be statistically non significant (***P*** ≤ 0.05). Bifidobacteria produce acetic acid and lactic acid in the proportion of 3: 2 ratio. An advantage in using *Bifidobacterium sp*. yoghurt is that they are slow acid producers and over acidification will not occur during production and storage[13]. The results obtained in the present study was on contrary with the above findings, *B.bifidum* culture has shown a higher acidity product with higher syneresis compared to control and other two types of cultures used.

Table 2 Effect of levels of *Bifidobacterium bifidum* on sensory attributes of short set bio-yoghurt

Inoculum levels (%)	Sensory attributes				
	Colour and Appearance	Body and Texture	Flavour	Sourness	Overall acceptability
	Sensory scores on 9 point Hedonic scale				
Control	8.00 ± 0.60^{a}	7.66 ± 0.65^{a}	7.83 ± 0.53^{ab}	7.75 ± 0.62^{ab}	7.75 ± 0.58^{ab}
2	7.50 ± 0.79^{a}	7.50 ± 0.79^{a}	7.16 ± 0.48^{b}	7.08 ± 0.67^{b}	7.20 ± 0.54^{b}
3	7.50 ± 0.79^{a}	7.50 ± 0.79^{a}	7.25 ± 0.58^{b}	7.16 ± 0.56^{b}	7.29 ± 0.51^{b}
4	7.50 ± 0.20^{a}	7.50 ± 0.40^{a}	8.16 ± 0.27^{a}	8.05 ± 0.41^{a}	8.00 ± 0.44^{a}
5	7.25 ± 0.51^{a}	7.66 ± 0.51^{a}	7.75 ± 0.41^{ab}	7.58 ± 0.49^{ab}	7.58 ± 0.49^{a}
CD (***P***≤ 0.05)	0.74	0.75	0.73	0.65	0.55

Note
All the values are average of 5 trials
Control : Yoghurt with 2 % *S.thermophilus* and *L. delbrueckii ssp. bulgaricus*
Similar superscripts indicate NS at the corresponding CD

Effect of levels of Lactobacillus acidophilus on sensory attributes of short set bio-yoghurt

The effect of *L. acidophilus* culture at various inoculum levels of 2, 3, 4 and 5 % on sensory attributes of bio-yoghurt is presented in Table 3. The scores awarded for sensory attributes viz. colour and appearance, body and texture, flavour, sourness and overall acceptability for control was 8.00, 7.66, 7.83, 7.75 and 7.75, respectively. Among the treated samples, 3 % inoculum level of *L.acidophilus* secured higher scores viz. 8.25, 7.86, 7.66, 7.66 and 7.86, respectively for the above said sensory attributes. However, the scores were not significantly different between control and 3 % inoculum level bio-yoghurt. The scores of colour and appearance, body and texture and overall acceptability were higher at 3 % inoculum levels of as compared to other levels of *L.acidophilus* and control. The reason for lower scores for flavour and sourness may be due to the fact that lower lactic acid and flavour production but higher scores for body and texture may be due to firm and smooth body with no wheying off on the surface and also demonstrated with less syneresis as compared to control as evaluated by a panel of judges.

The development of titratable acidity of yoghurt containing *L. acidophilus* was lower as yoghurt culture association may not have stimulated its growth and less acceptability of the product with respect to flavour, sourness and overall acceptability was because of less lactic acid (%) production. The results of the present study are in agreement with the findings of Maragkoudakisa *et al*. (2006)[15], who evaluated the sensory properties of probiotic Greek yoghurt with *L.paracasei* exhibited a rich, smooth and traditional taste, not too acidic and showing good sensory acceptance among all the *Lactobacillus* strains tested.

Table 3: Effect of levels of *Lactobacillus acidophilus* on sensory attributes of short set bio-yoghurt

Inoculum levels (%)	Sensory attributes				
	Colour and Appearance	Body and Texture	Flavour	Sourness	Overall acceptability
	Sensory scores on 9 point Hedonic scale				
Control	8.00±0.60^{a}	7.66±0.65^{a}	7.83±0.63^{a}	7.75±0.62^{a}	7.75±0.68^{a}
2	8.25±0.75^{a}	7.66±0.63^{a}	7.58±0.70^{a}	7.58±0.56^{a}	7.00±0.63^{b}
3	8.25±0.64^{a}	7.86±0.76^{a}	7.66±0.65^{a}	7.66±0.78^{a}	7.86±0.75^{a}
4	8.25±0.74^{a}	7.66±0.78^{a}	7.16±0.78^{ab}	7.00±0.65^{ab}	7.41±0.81^{ab}
5	8.00±0.56^{a}	7.66±0.68^{a}	6.83±0.78^{b}	6.83±0.78^{b}	7.00±6.73^{b}
CD ($P \leq 0.05$)	0.77	0.76	0.77	0.79	0.65

Note
All the values are average of 5 trials
Control : Yoghurt with 2 % *S.thermophilus* and *L. delbrueckii ssp. bulgaricus*
Similar superscripts indicate NS at the corresponding CD

Effect of B. bifidum and L. acidophilus (1:1) combination cultures on sensory attributes of short set bio-yoghurt

The effect of *B.bifidum and L.acidophilus* culture at various inoculum levels 2, 3, 4 and 5 % on sensory attributes of bio-yoghurt is presented in Table 4. The scores awarded to 4 % inoculum level of combination culture in yoghurt was 8.00, 8.00, 7.83, 7.80 and 8.00 for colour and appearance, body and texture, flavour, sourness and overall acceptability sensory attributes, respectively as compared to control which secured respective scores of 8.00, 7.66, 7.83, 7.75 and 7.75. The scores were higher for all the sensory attributes at 4 % inoculum level of combination culture as compared to other levels and were found to be statistically significant. However, the increase in scores for 4 %treated samples was found to be statistically similar to cont rol as it was a short set bio-yoghurt whose probiotic culture incorporated just get activated within short time (4 h) in association with yoghurt culture.

The results of the present study is in agreement with Champagne *et al*. (2005)[16], Hekmat and Reid (2006)[17], Hussain *et al*. (2009) [18] who concluded that probiotic cultures did not tend to strongly modify the sensory properties of the products such as body and texture, flavour and appearance. The results are also in correlation with the studies of Kailasapathy (2006)[19], who reported that the addition of probiotic cultures either in free or encapsulated form did not significantly affect the appearance and colour, acidity, flavour and after taste of the bio-yoghurt (*B. lactis* and *L.acidophilus*) only significant textural difference was observed.

Table 4 Effect of levels of *B.bifidum and .acidophilus*on sensory attributes of short set bio-yoghurt

Inoculum levels (%)	Sensory attributes				
	Colour and Appearance	Body and Texture	Flavour	Sourness	Overall acceptability
	Sensory scores on 9 point Hedonic scale				
Control	8.00±0.00^{a}	7.66±0.65^{ab}	7.83±0.63^{a}	7.75±0.62^{ab}	7.75±0.58^{ab}
2	8.00±0.25^{a}	7.75±0.75^{ab}	7.25±0.76^{ab}	7.16±0.70^{bc}	7.50±0.78^{ab}
3	8.00±0.00^{a}	7.75±0.64^{ab}	7.33±0.64^{ab}	7.16±0.73^{bc}	7.25±0.75^{b}
4	8.00±0.25^{a}	8.00±0.34^{a}	7.83±0.70^{a}	7.80±0.61^{a}	8.00±0.68^{a}
5	8.00±0.25^{a}	7.25±0.72^{b}	6.91±0.76^{b}	7.00±0.72^{c}	7.08±0.69^{b}
CD ($P \leq 0.05$)	0.34	0.54	0.62	0.70	0.69

Note:
All the values are average of 5 trials
Control : Yoghurt with 2 % *S.thermophilus*and *L. delbrueckii ssp. bulgaricus*
Similar superscripts indicate NS at the corresponding CD

In the present study, high acid production and high syneresisbut higher scores for flavor and sourness were observed in bio-yoghurt with *B.bifidum* culture. But low acid production and low sensory scores for flavor and sourness with better colour & appearance and body & texture and controlled syneresis were observed in *L. acidophilus* bio-yoghurt. But, combination of cultures was beneficial in terms of milk setting time, controlled syneresis and optimum acidity so that all the sensory attributes of the bio-yoghurt was improved as compared to individual cultures. Hence, it can be concluded that combination of probiotic cultures has a positive impact on all the sensory attributes and physico-chemical properties compared to individual cultures of *B.bifidum and L.acidophilus* for the particular stains used in the present study. Hence, selection of strains of probiotic cultures is very much essential, which influence the overall acceptability of the product.

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