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Research Article

OPTIMIZATION OF INOCULATION LEVEL OF PROBIOTICS (*L. ACIDOPHILUS* LA5 & *B. BIFIDUM* BB12) ON THE QUALITY PARAMETERS OF WHEY PROTEIN ENRICHED PROBIOTIC CONCENTRATED YOGHURT

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ABSTRACT

The objective of this study was to standardize the rate of addition of probiotics in whey protein enriched concentrated (WPEC) yoghurt and its effect on various quality parameters viz., sensory, physico-chemical, and microbiological characteristics. Among the different levels of probiotics tried for production of WPEC probiotic yoghurt, 1.5% each of *L. acidophilus* and *B. bifidum* (along with 3% starters) secured non-significantly (P≤0.05) higher sensory scores of 7.45, 7.01, 7.10 and 8.00 for colour and appearance, flavour, body and texture and overall acceptability, respectively; whereas for control, same scores were 8.00, 7.00, 6.90 and 7.80, respectively. As the level of probiotics increased from 0 to 1.5%, all the physico-chemical properties decreased viz., pH: (4.85 to 4.72), acidity (1.69 to 1.26% LA), synersis (1.50 to 0.85 ml), penetration value (125 to 122 mm) and time of setting (360 to 330 min.); whereas the respective log counts of *L. acidophilus* LA5 and *B. bifidum* BB12 increased significantly (Pd"0.05) from 9.36 to 9.78 and 9.04 to 9.67, respectively.

Keywords: Probiotics, Starters, Whey protein enriched concentrated (WPEC), WPC70, Yoghurt

Yoghurt is one amongst the most consumed fermented milk products in the world due to its therapeutic value, hence, projected its image as 'healthy product'. The production and consumption of probiotics is expected to increase by a compounded annual growth rate (CAGR) of 5.8% between 2017 to 2022 (www.statista.com). It has been suggested that probiotic food shall have a probiotic count of 108–109 cfu/g to make sure that adequate therapeutic dose of 106–107 cfu/g possibly will reach the colon to claim the health benefits (Garcia *et al.*, 2012). This could be achieved by using different methods of encapsulation of probiotic organisms with varied encapsulating materials like combination of different stabilizers and whey protein concentrates (WPC) etc. Generally, skim milk powder (SMP) is used to increase the total solid content of yoghurt. WPC70 due to various functional properties could be used to replace SMP in yoghurt (Sharanagouda *et al.*, 2014). However, studies associated to the qualitative changes occurred due to the addition of different levels of probiotic bacteria in the yoghurt having high total solid content (concentrated yoghurt having WPC70) have not been described. In this context, the present research aimed to evaluate the effect of adding increasing amounts of probiotic inoculum, particularly *Lactobacillus acidophilus* LA5 and *Bifidobacterium bifidum* BB12 on the physicochemical characteristics and sensory attributes of WPEC Probiotic Yoghurt.

MATERIALS AND METHODS

Fresh cow milk and cream (60% fat) was procured from Student Experimental Dairy Plant of Dairy Science Collage, KVAFSU, Bangalore and that was used for the preparation of yoghurt. Whey proteins concentrate (PROCON 3700 WPC70), procured from M/s. Mahaan Protein Ltd, New Delhi, was used for the protein enrichment of yoghurt. The yoghurt culture of *Streptococcus thermophilus* NCDC 074 and *Lactobacillus delbrueckii* ssp. *bulgaricus* NCDC 009 are mixed in 1:1 ratio containing 7x109 and 5x109 cfu/g active cells, respectively.

Bacterial strains and growth conditions

Freeze dried probiotic cultures of *B. bifidum* BB12 NCDC255 and *L. acidophilus* LA5 NCDC015 were obtained from National Dairy Research Institute, Karnal. Yoghurt starter culture of *S. thermophilus* NCDC074 and *L. bulgaricus* NCDC009 were propagated and maintained individually in reconstituted skim-milk and product preparation was done in the Postgraduate Laboratory, Department of Dairy Technology, Dairy Science college, KVAFSU, Bangalore. The two probiotic strains were mixed in the ratio of 1:1 just before use, for the preparation of probioic yoghurt.

Preparation of wpec probiotic yoghurt

The procedure given by Sharanagouda *et. al.* (2014) was followed for preparation of WPEC yoghurt (Fig. 1).

Optimization of level of probiotics in WPEC yoghurt: To optimize the level of probiotics in the preparation of protein enriched concentrated probiotic yoghurt, two probiotic organisms *viz*., *B. bifidum* BB12 and *L.*

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acidophilus LA5 having an initial count of 6x109 and 8x109 cfu/g (1:1 ratio, which were grown separately before adding to yoghurt mix) were used along with yoghurt starters at levels like 1.0, 1.5 and 2.0% each by weight of mix, in combination. The WPEC yoghurt contained 70.8% moisture, 6.43% fat, 12.16 % protein, 8.31 % lactose and 2.30% minerals. The level of probiotics was decided based on their count in the final product as well as on the basis of sensory evaluation.

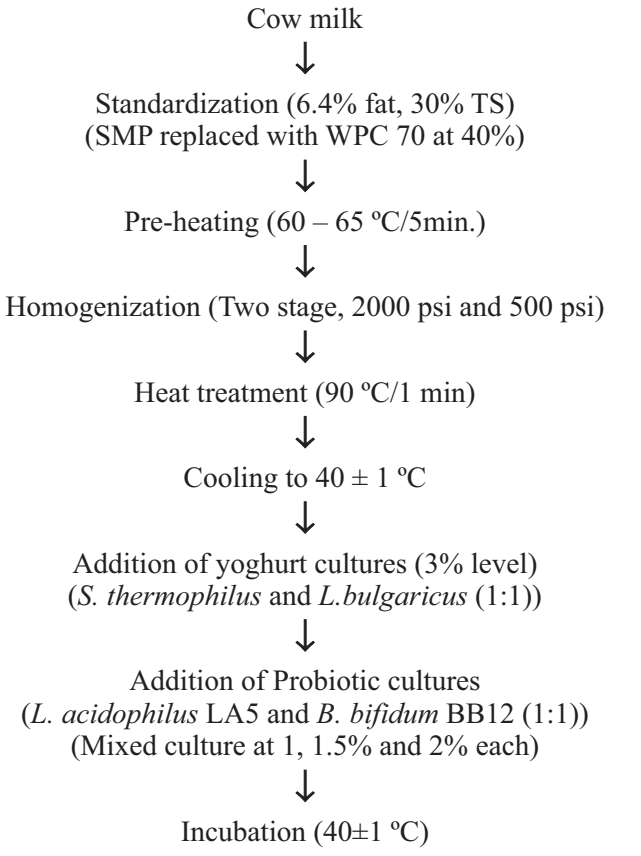

Fig. 1. Flow diagram for the preparation of WPEC probiotic yoghurt

Methods of estimation of physico-chemical parameters of WPEC probiotic yoghurt: Sensory evaluation of the products was conducted by applying 9-point hedonic scale (Peryam and Pilgrim, 1957). Evaluation was carried out by a panel of five expert judges.

Fat content in Yoghurt was estimated as per the procedure of AOAC (1980). pH was measured using digital pH meter (Elico make). Titratable acidity, protein, lactose, minerals and total solids of all the samples were estimated as per the method described in IS:SP:18 (Part XI) 1981. The penetration value of yoghurt was measured as per the procedure of Shihata and Shah (2002). Syneresis of yoghurt samples were analyzed as per Yeganehzad *et al.* (2007). The time for setting was noted by noting the time of inoculation to the end of yoghurt setting.

Enumeration of yoghurt starters and probiotic cultures: Enriched yoghurt containing mixed cultures stored at 4±10 ºC was examined for their viability. Bifidobacterium agar with 0.3% lithium chloride was added (by the dry weight of media, for selective growth) and Rogosa SL agar (with 1.32 ml/l glacial acetic acid for selective enumeration) were used for enumeration of *B. bifidum* BB12 and *L. acidophilus* LA5, respectively. Dave and Shah (1996) procedure was followed for the estimation of same; whereas yoghurt starters were estimated as per Kim *et al.* (1997). The plates were inverted and incubated in anaerobic jars under CO_2 atmosphere at 40±1 ºC for 72 h. The colonies developed were counted; the average counts of duplicate plates were taken and tabulated.

Statistical analysis: The data was analyzed using one way ANOVA, Two factor ANOVA depending on the experiment and the number of treatments in question. The results were analyzed statistically for test of significance by using three factorial ANOVA as per the procedure of Sundarraj *et al.* (1972) in SAS 9.2 Version.

RESULTS AND DISCUSSION

Effect of different levels of probiotics on sensory quality of WPEC probiotic yoghurt: It was observed from Table 1 that as the level of probiotics increased from 0 to 1.5% each, the flavour and overall acceptability increased non-significantly ($P \leq 0.05$), this could be attributed to the higher acetaldehyde and other flavouring compounds produced by probiotics and yoghurt starters in WPEC yoghurt utilizing non-protein nitrogenous compounds required for flavour production (Chatterjee and Kanawajia, 2010). Whereas probiotics above 1.5% level, the scores decreased significantly ($P \leq 0.05$), may be due to over flavour and acid production which might have imparted harsh flavour and that might have affected the overall acceptability. The colour and appearance scores decreased though non-significantly ($P \leq 0.05$) with the increase in the level of probiotics up to 1.5%. This may be due to the slighter dull appearance with little wheying off on the surface of yoghurt; but above that level, wheying off was significantly (($P \leq 0.05$); Table 2) more which was observed in the present study. The body and texture scores at all levels of probiotics was not significantly ($P \leq 0.05$) affected. Based on the sensory scores, good quality protein enriched probiotic yoghurt with 1.5% each of probiotics can be prepared.

Table 1

Effect of levels of probiotics (*L. acidophilus LA5 and B. bifidum BB12*) *on* sensory quality of WPEC probiotic yoghurt

Sensory attributes	Percentage of Probiotics				CD
	0	1.0	1.5	2.0	(P ≤ 0.05)
	--- Sensory Scores on 9.0 point hedonic scale ---				
Colour & Appearance	8.00 ± 0.17[a]	7.50 ± 0.19[a]	7.45 ± 0.20[a]	7.00 ± 0.18[b]	0.632
Flavour	7.00 ± 0.38[a]	6.85 ± 0.18[a]	7.01 ± 0.09[a]	6.50 ± 0.50[b]	0.487
Body & Texture	6.90 ± 0.39[a]	7.05 ± 0.08[a]	7.10 ± 0.06[a]	7.00 ± 0.15[a]	0.709
Overall acceptability	7.80 ± 0.25[a]	7.95 ± 0.10[a]	8.00 ± 0.20[a]	7.44 ± 0.30[b]	0.324

n=5.Treatments bearing different superscripts in row are statistically different (p ≤ 0.05)

Table 2

Effect of different level of probiotics (*L. acidophilus LA$_5$and B. bifidum BB$_{12}$*) on the physico-chemical properties of WPEC probiotic yoghurt

Physico-chemical attributes	Percentage of Probiotics				CD
	0	1.0	1.5	2.0	(P ≤ 0.05)
Ph	4.85 ± 0.06[a]	4.78 ±0.09[ab]	4.72 ±0.05[bc]	4.70 ± 0.04[c]	0.071
Acidity (% TA)	1.69 ± 0.10[a]	1.12 ± 0.10[b]	1.26 ± 0.08[c]	1.32 ± 0.05[d]	0.013
Syneresis (ml)	1.50 ± 0.1[a]	0.96 ± 0.05[b]	0.85 ± 0.10[c]	0.75 ± 0.05[d]	0.001
Penetration value (mm)	125 ± 10[a]	124 ± 15[a]	122 ± 15[b]	120 ± 10[c]	0.951
Fat (%)	6.4±0.10[a]	6.4±0.20[a]	6.4 ± 0.10[a]	6.4±0.20[a]	NS
Total solids (%)	30.0 ± 1.35[a]	30.0 ± 1.51[a]	30.0 ± 1.05[a]	30.0 ± 1.39[a]	NS
Time for setting (min) at 40±1°C	360 ± 15[a]	330 ± 15[b]	330 ± 15[b]	300 ± 15[c]	0.009

n=3.Treatments bearing different superscripts in row are statistically different (p ≤ 0.05)

Effect of different levels of Probiotics on the physico-chemical quality of WPEC probiotic yoghurt: The pH of protein enriched concentrated yoghurts decreased slowly and significantly (P ≤ 0.05) from 4.85 to 4.70 (Table 2) as the level of probiotic from 0% to 2% each, this may be due to small quantity of acids produced by probiotics. There could have been more decrease in pH, but the added WPC70 might have imparted the buffering capacity to yoghurt (Kailasapathy *et. al.*, 1996). After inoculation of starter cultures, the yoghurt was kept for incubation, the time required for setting of protein enriched concentrated probiotic yoghurt decreased from 360 to 300 min (Table 2) may be due increased level of probiotics which might have set the probiotic yoghurt faster than control (Bury *et al.*, 2003).

It was also noticed from the Table 2 that there was a gradual significant decrease (P ≤ 0.05) in the acidity of samples from 1.69% to 1.32% TA as the level of probiotics increased from 0 to 2.0%. This could be attributed to the increase in the concentration of probiotic cultures that decreased the setting time, which might have affected the acid production due to less time available for the yoghurt starters to produce acid in probiotic yoghurt than control (Sady *et al.*, 2009). Syneresis (wheying-off) is defined as the expulsion of whey from the network which then becomes visible as surface whey. Wheying-off negatively affects consumer perception of yoghurt as consumers think there is something microbiologically wrong with the product. Yoghurt manufacturers use stabilizers, such as, pectin, gelatin, starch and WPC and try to prevent wheying-off. Another approach is to increase the total solids content of yoghurt milk, especially the protein content, to reduce wheying-off. There was significant decrease (P≤ 0.05) in syneresis and penetration value from 1.50 to 0.75 ml and 125 to 120 mm with increase in level of probiotic from 0 to 2.0% (Table 2). This could be attributed to the binding of water by the whey protein gel matrix formed, whereas the decrease in penetration value may be due to the smoother body produced by whey proteins and also due to decrease in the level of casein content (Guzman-Gunzalez *et al.*, 1999). There was no effect on the fat and SNF content with increasing probiotics levels due to the same level of fat and SNF concentrations maintained at all the levels of probiotics.

Effect of different levels of probiotic on starter cultures and probiotics counts in WPE yoghurt: It was evident from the Table 3 that the viable log counts of *S. thermophilus* and *L. bulgaricus* decreased non-significantly (P ≤ 0.05)

Table 3

Effect of different levels of probiotic (*L. acidophilus LA*$_1$*and B. bifidum BB*$_{11}$*)* on the count of starter cultures and probiotics in WPEC yoghurt

Starter organisms	Percentage of Probiotics				CD
	0	1.0	1.5	2.0	(P ≤ 0.05)
S. thermophilus	10.11 ± 0.18^a	9.95 ± 0.13^a	9.93 ± 0.11^a	9.90 ± 0.17^a	0.908
L. bulgaricus	10.18 ± 0.14^a	9.91 ± 0.20^a	9.85 ± 0.18^a	9.78 ± 0.19^a	0.576
L. acidophilus	-	9.36 ± 0.15^a	9.78 ± 0.18^b	9.85 ± 0.13^b	0.075
B. bifidum	-	9.04 ± 0.14^a	9.67 ± 0.19^b	9.69 ± 0.16^b	0.023

n=3.Treatments bearing different superscripts in row are statistically different (p ≤ 0.05)

from 10.11 to 9.90 and 10.18 to 9.78, respectively; whereas the counts of *L. acidophilus* and *B. bifidum* increased from 9.36 to 9.85 and 9.04 to 9.69, respectively with increase in level of probiotics from 1.0 to 2.0%. But, the increase was non-significant (P ≤ 0.05) between 1.5% and 2.0% level of inoculation. This could be attributed to the WPC which might have acted as prebiotic for the growth of probiotics as reported by Christopher *et al.* (2006). The decrease in the viable log counts of starter cultures may be due to reduced incubation time from 360 min in control to 300 min in 2.0% probiotic yoghurt.

CONCLUSION

A good quality protein enriched concentrated probiotic yoghurt can be prepared with 1.5% of *L. acidophilus* and *B. bifidum* each along with yoghurt culture which has secured higher sensory scores than control without much effect on physico-chemical properties.

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