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Propagation of *Bifidobacterium bifidum NCDC 232* in various mediums

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The work was under taken to optimize the propagation of *Bifidobacterium bifidum NCDC 232* in different mediums for blending into the functional weaning food. Sterilized cheese whey was used to propagate *Bifidobacterium bifidum NCDC 232* **(**Bb-N) along with supplementation of honey, carrot and tomato (CT) juice and WPH. With the increase in the level of inoculum from 0.5 to 1.5 per cent, there was significant decrease in pH at all duration of incubations. There was significant increase in the viable counts when the level of inoculum increased from 0.5 to 1.5 per cent level. 1.5 per cent inoculum and incubation for a period of 15 h is optimum for obtaining the maximum effect in decreasing pH of whey medium. Addition of honey has significant effect on the growth and development of Bb-N and addition of honey at 2 per cent and incubating a period of 15 h is optimum. It is noteworthy that supplementation of CT juice has further significant effect on the growth of probiotics in addition to honey supplementation. The extent of viable counts attained was significantly higher with 4 per cent supplementation of CT juice as compared to without supplementation.

Key Words : Whey, Bifidobacterium, Prebiotics, Probiotics, Carrot juice, Comato juice

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INTRODUCTION

Probiotics are gaining popularity in view of their role in imparting health benefits beyond inherent basic nutrition which are more online with the functional foods. Weaning foods containing probiotics may provide numerous benefits as compared to their number of non-cultured counterparts. Probiotics based foods are helpful in controlling most of the gastrointestinal and related disorders besides other diseases by restoring normal lactic intestinal flora and inhibiting undesirable putrefactive organisms. Functional food ingredients such as prebiotics and probiotics could effect a beneficial modification in the composition and activities of gut microflora of infants by increasing positive flora components. The prebiotic approach aims to increase resident bacteria that are considered to be beneficial for human health, e.g. bifidobacteria and lactobacilli (Parracho *et al.*, 2007). The probiotic currently available in the market are based on lactic acid bacteria, lactobacilli, bifidobacteria and streptococci which have been shown to be important components of gastrointestinal microflora and are relatively harmless (Jayaprakasha *et al.*, 2005). Various health benefits such as controlling colon cancer, antiatherogenic properties have been attributed to probiotics (Singh *et al.*, 2007 and Vibha Sinha and Yadav, 2007). Probiotics can combat traveler's diarrhoea and atopic diarrhoea (Mulder, 2004). Probiotics are able to

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improve immunological factors that are related to overweight health problems. In addition, probiotics have been shown to be able to prevent the development of diabetes which is highly linked to obesity (Sofia *et al.,* 2010). Probiotic foods affect the health of consumers by reducing the risk of heart attacks by improving the microflora of the intestinal tract. Yoghurt enriched with either *Bifidobacterium bifidum* or *Lactobacillus acidophilus* was known to reduce cholesterol level (Niazmand *et al.,* 2005). It also has been shown to reduce the incidence of allergy at-risk infants through administration of *L. rhamnosus GG* to infants during the first half-year of life (Ouwehand, 2007) The importance of bifidobacteria should not be under estimated especially in small children and breast fed infant as it is found to promote better bifidobacterial growth in the intestine as compared to standard formula feeding (Harmsen *et al.,* 2000). The bifidobacterial growth in protein adjusted UF milk with lactose and honey showed improved colony count. The counts were 8.95 $\log_{10}$ cfu/g after 20th h (Suresh *et al.,* 2005). Bifidobacteria are predominant in the newborn and have been known as one of the major groups of saccharolytic bacteria in the human colon, constituting upto 25 per cent of the total population in the adult colon (Wang *et al.,* 1999). Supplementation of bifidobacteria has been shown to influence immunoparameters such as stimulation of local Iga-production (Amster *et al.,* 1994) as well as other beneficial effects such as synthesis of folate (Crittenden *et al.,* 2003) are well known. Whey proteins which possibly have too high a molecular weight to be rendered available for direct bacterial uptake but could be enzymatically cleaved, leading to formation of bifidobacterial growth factors. The highest bacterial growth of 9.1 log cfu/ml was obtained when milk was supplemented with 2 per cent WPC (Janer *et al.,* 2004). Dave and Shah (1998), observed that the addition of cysteine, whey protein concentrate, acid casein hydrolysate or tryptone improved the viability of *bifidobacteria* to variable extent. Keeping in view the benefits rendered by probiotics the present work was under taken to optimize the propagation of *Bifidobacterium bifidum NCDC 232* in different mediums for blending into the functional weaning food.

METHODOLOGY

Bifidobacteria agar M1396-500G was procured from HiMedia to enumerate *Bifidobacterium bifidum NCDC 232.* Good quality carrot procured from the local market was cleaned and properly washed with potable water. Carrot were cut in to small pieces and grinded in wet grinder followed by filtering and squeezing to get juice. The resultant juice was used for blending with the whey medium. Similarly fresh tomatoes procured from local market were washed with tap water. The washed tomatoes were cut in to small pieces and grinded in wet grinder, the juice obtained was filtered to remove fibre material. Resultant juice was used for blending with whey medium for propagating probiotics. The probiotic culture was propagated in whey medium enriched with different prebiotics at various levels in order to obtain higher viable count. Probiotic culture *Bifidobacterium bifidum NCDC 232* (Bb-N) was inoculated separately to the sterilized cheese whey medium at 0.5, 1.0, 1.5 and 2.0 per cent levels and incubated at 37^{o}C for a period of 24 h. The growth of probiotics as measured in terms of pH, acidity and viable counts was monitored at a regular interval of 3 h. Sterilized whey medium was fortified with various prebiotic namely honey, blend of carrot and tomato juice (1:1) and whey protein hydrolysate. The fortified whey medium was inoculated with probiotic culture and incubated at 37^{o}C for a known period to study the effect of prebiotics. Cheese whey medium was added with honey at 1, 2 and 3 per cent levels and subjected to sterilization. Sterilized medium was inoculated separately with Bb-N at their optimum level and incubated for an optimum period at 37^{o}C. The effect of honey on the activity of probiotics was measured in terms of pH, acidity and viable counts to adjudge the optimum level of honey to be added. Cheese whey medium was added with an optimum level of honey and further added with 3, 4 and 5 per cent levels of carrot and tomato juice blend (1:1) and subjected to sterilization. Sterilized medium was inoculated separately with Bb-N at their optimum concentration and incubated at 37^{o}C. At a regular interval, the growth of probiotics in terms of pH, acidity and viable counts was monitored to select an appropriate level of carrot and tomato juice to be blended. Cheese whey medium was added with an optimum level of honey and a blend of carrot and tomato juice. Whey medium was further added with 0.5, 1.0, 1.5 and 2.0 per cent levels of whey protein hydrolysate (WPH) and subjected to sterilization. Sterilized whey medium was inoculated with Bb-N at their optimum level and incubated at 37^{o}C. At a regular

interval, the growth of probiotics was monitored in terms of pH, acidity and viable counts to elicit the effect of whey protein hydrolysate on the growth of probiotics. The stock solution was prepared by dissolving 3.4 g of Potassium dihydrogen phosphate in 100 ml of distilled water and pH was adjusted to 7.0 with 0.5N NaoH. 1.25ml of stock phosphate buffer solution was taken and the volume was made upto 1000ml with distilled water and distributed in 9ml or 99ml quantities in tubes or bottles and sterilized by autoclaving at 121°C/15 min. 49.3 g of Bifidobacteria agar was suspended in 1000 ml distilled water, mixed well and heated till it was boiling to dissolve the medium completely and filling in to flasks followed by sterilization by autoclaving for 15 lb pressure at 121 °C for 15 min, cooled to 50°C and used for plating. The plates were incubated in anaerobic condition for 72-96 h in an anaerobic jar adopting candle method to enumerate *Bfidobactrium bifidum NCDC 232*. The pH of reconstituted samples was measured using digital pH meter (Elico make). Acidity was measured by titrating against 0.1N NaoH using phenolphthalein indicator and expressed in terms of per cent lactic acid as per the method described in IS:SP:18 (Part XI) 1981. The results were analyzed statistically for test of significance by using statistical packages for social sciences (SPSS) version 8 software programme.

OBSERVATIONS AND ASSESSMENT

Sterilized cheese whey was used to propagate *Bifidobacterium bifidum NCDC 232* **(**Bb-N) along with supplementation of honey, carrot and tomato (CT) juice and WPH. The effect of various level of inoculum, duration of incubation and supplementation of various ingredients on growth and activity of Bb-N was studied in order to optimize the right conditions for the growth of probiotics. Sterilized cheese whey was inoculated with *Bifidobacterium bifidum NCDC 232* (Bb-N) at 0.5, 1.0, 1.5 and 2.0 per cent and incubated upto a period of 24 h. The effect of level of inoculum and duration of incubation on pH, acidity and viable counts was monitored at an interval of 3 h. Strain of probiotics *Bifidobacterium Bifidum NCDC 232* was propagated in whey medium using different prebiotics for further incorporation into weaning food. In order to optimize the process to have rich harvest of probiotics, cheese whey was sterilized and cooled to 37°C and inoculated with *Bifidobacterium bifidum NCDC 232* (Bb-N) at 0.5, 1.0, 1.5 and 2.0 per cent levels and incubated for a period of 24 h. The activity of probiotics as measured in terms of acidity, pH and viable counts was monitored at a regular interval of 3 h. The results are presented in Table 1.

With the increase in the level of inoculum from 0.5 to 1.5 per cent, there was significant decrease in pH at all duration of incubations. However, at 2.0 per cent level, the extent of decrease in pH was not significant as compared to 1.5 per cent inoculum. At this level of inoculum, the extent of decrease in pH was significant upto 15 h of incubation. Similar trend was also observed with respect to increase in acidity of whey medium at 15 h of incubation. The pH was found to be 5.42, 5.34, 5.24 and 5.22 whereas the corresponding acidity was 0.34, 0.37, 0.40, and 0.41 per cent lactic acid, respectively at 0.5, 1.0, 1.5 and 2.0 per cent level of inoculum.

The viable counts of Bb-N at 0.5, 1.0, 1.5 and 2.0 per cent inoculum was found to be 6.61, 6.76, 6.93 and 6.89 log cfu/ml, respectively at 15 h of incubation. It is evident from the result that there was significant increase in the viable counts when the level of inoculum increased from 0.5 to 1.5 per cent level. However, further increase in the level of inoculum had no significant effect on increase in the viable count. Similarly, further increase in the duration of incubation from 15 to 24 h, there was no significant increase in the viable count of Bb-N. It is evident from the result presented in Table 1 that an inoculum of 1.5 per cent and incubation for the period of 15 h is optimum for propagation of Bb-N. It is observed from the result that increasing in the level of inoculum from 0.5 to 1.5 per cent, there was significant decrease in pH at all durations of incubation. However, above 1.5 per cent, the extent of decrease in pH was not significant. The extent of decrease in pH was observed upto 15 h of incubation. Further incubation upto 18 h had no significant effect in decreasing the pH at any level of inoculum. From the results it is evident that both level of inoculum and duration of incubation have significant effect in decreasing the pH of whey medium. From the study it is evident that 1.5 per cent inoculum and incubation for a period of 15 h is optimum for obtaining the maximum effect in decreasing pH of whey medium. Many of the earlier workers observed better activity of probiotics in terms of decreasing pH even upto 18 h. Many have suggested an incubation of 18 h for the maximum reduction of pH (Bordignon *et al.,* 2004; Borpujari *et al.,*

2007). The activity of probiotic as measured in terms of acidity (Table 1) depicted that with increasing in the level of inoculum there is significant increase in the acidity attained in the whey medium irrespective of the type of culture and duration of incubations. A significant increase in acidity was observed from 3 h of incubation upto 15 h of incubation at all levels of inoculum. The extent increase in acidity was found to be non-significant after 15 h of incubation for Bb-N indicating that 15 h is optimum enough to get the maximum acidity. It is confirmed from the results that 1.5 per cent inoculum and 15 h duration of incubation is optimum to attain a maximum acidity. Further increase in incubation did not increase the acidity which could be probably due to depletion of inherent nutrients present in the whey system. The acidity development followed the similar pattern as that of pH with an inverse relationship. Several earlier workers reported a maximum acidity of around 0.5 per cent lactic acid after 18 h of incubation (Borpujari *et al.*, 2007). The increased acidity, decreased pH were also reflected with respect to viable counts of probiotics. The extent of increase in viable count with the increase in the duration of incubation followed the similar trend as that of acidity and pH. A maximum viable count of La-N was 6.95 log cfu/ml after 15 h of incubation at 1.5 per cent inoculum whereas it was 6.93 log cfu/ml for Bb-N. The viable counts of Bb-N were found to be lesser as compared to La-N. Better activity and higher viable counts of *Lactobacillus* as compared to *Bifidobacterium* species has been reported by several earlier workers (Kusuma Rani, 2006 and Belkaaloul *et al.*, 2010).

Cheese whey was added with 1, 2 and 3 per cent honey and subjected to sterilization. The sterilized whey medium was inoculated with 1.5 per cent *Bifidobacterium bifidum NCDC 232* (Bb-N) and incubated upto a period of 18 h. The growth was

Table 1: Effect of level of inoculum of *Bifidobacterium bifidum NCDC 232* on pH, acidity and viable counts in whey medium

Level of inoculum (%)	Period of incubation (h)								Mean
	3	6	9	12	15	18	21	24	
					pH				
0.5	5.69	5.63	5.58	5.48	5.42	5.39	5.37	5.34	5.487
1.0	5.67	5.58	5.49	5.40	5.34	5.31	5.28	5.25	5.415
1.5	5.61	5.52	5.40	5.32	5.24	5.21	5.19	5.17	5.332
2.0	5.57	5.49	5.38	5.29	5.22	5.19	5.17	5.14	5.306
		Levels			Period			Levels x Period	
F test		*			*			NS	
C.D. (P=0.05)		0.050			0.048			---	
					Acidity (% LA)				
0.5	0.25	0.27	0.29	0.32	0.34	0.35	0.36	0.37	0.318
1.0	0.26	0.29	0.32	0.35	0.37	0.38	0.39	0.40	0.345
1.5	0.29	0.31	0.35	0.38	0.40	0.41	0.42	0.43	0.373
2.0	0.30	0.32	0.36	0.39	0.41	0.42	0.43	0.44	0.383
		Levels			Period			Levels x Period	
F test		*			*			NS	
C.D. (P=0.05)		0.017			0.014			---	
					Viable count (log cfu/ml)				
0.5				5.36	6.61	6.47	5.99	5.66	6.018
1.0				5.54	6.76	6.65	6.23	5.72	6.180
1.5				5.81	6.93	6.84	6.36	5.86	6.360
2.0				5.85	6.89	6.80	6.29	5.79	6.324
		Levels			Period			Levels x Period	
F test		*			*			NS	
C.D. (P=0.05)		0.081			0.602			---	

All values are average of three trials * indicate significance of value at P=0.05 NS= Non-significant

monitored in terms of pH, acidity and viable counts. The results are presented in Table 2.

It is observed from the results that increase in the level of honey from 0 to 2.0 per cent, there was significant decrease in pH of the medium. At 15 h of incubation the pH was found to 5.24, 5.19, 5.07 and 5.05 at 0, 1.0, 2.0 and 3.0 per cent level of inoculum. Increase in incubation period from 15 to 18 h had no significant effect in decreasing pH at all levels of honey addition. Similarly, with increase in the level of honey from 1.0 to 2.0 per cent there was significant increase in acidity upto15 h of incubation. The corresponding acidity was 0.40, 0.42, 0.46 and 0.47 per cent lactic acid, respectively at 0, 1.0, 2.0 and 3.0 per cent supplementation of honey. The viable counts as affected by level of honey supplementation depicted that the extent of honey addition upto 2.0 per cent has significant effect on the viable counts of Bb-N. The viable counts without addition of honey was only 6.93 log cfu/ml whereas it increased to 7.25, 7.51 and 7.51 log cfu/ml at 1.0, 2.0, and 3.0 per cent addition of honey. Whey medium was enriched with different levels of honey and subjected to sterilization. The sterilized whey medium was inoculated separately with 1.5 per cent *Bifidobacterium bifidum NCDC 232* (Bb-N) and incubated upto a period of 18 h. The effect of honey in enhancing the activity of probiotic was monitored. It was interesting to note the addition of honey had a stimulating effect on both the probiotics as measured in terms of pH, acidity and viable counts. Addition of honey upto a level of 2 per cent had a significant effect in increasing acidity, decreasing pH and increasing the viable count**.** The maximum acidity attained without honey supplementation was 0.40 per cent for Bb-N, whereas it was 0.46 per cent lactic acid with supplementation of 2 per cent honey with respect to Bb-N. A minimum pH attained was 5.24 with respect to Bb-N after 15 h of incubation with 1.5

Table 2 : Effect of supplementation of honey on the activity of of *Bifidobacterium bifidum NCDC 232* in whey medium

Level of enrichment (%)	Period of incubation (h)						Mean
	3	6	9	12	15	18	
	pH						
0	5.61	5.52	5.40	5.32	5.24	5.21	5.383
1	5.55	5.46	5.34	5.25	5.19	5.16	5.325
2	5.45	5.34	5.22	5.13	5.07	5.04	5.208
3	5.41	5.28	5.20	5.10	5.05	5.01	5.175
		Levels		Period		Levels x Period	
F test		*		*		NS	
C.D. (P=0.05)		0.041		0.048		---	
	Acidity (% LA)						
0	0.29	0.31	0.35	0.38	0.40	0.41	0.356
1	0.30	0.33	0.37	0.40	0.42	0.43	0.375
2	0.33	0.37	0.41	0.44	0.46	0.47	0.413
3	0.34	0.38	0.42	0.45	0.47	0.48	0.423
		Levels		Period		Levels x Period	
F test		*		*		NS	
C.D. (P=0.05)		0.016		0.013		---	
	Viable count (log cfu/ml)						
0				5.81	6.93	6.84	6.526
1				6.35	7.25	6.90	6.833
2				6.67	7.51	6.98	7.053
3				6.73	7.51	6.94	7.060
		Levels		Period		Levels x Period	
F test		*		*		NS	
C.D. (P=0.05)		0.039		0.601		0.031	

All values are average of three trials * indicate significance of value at P=0.05 NS= Non-significant

per cent inoculum without supplementation of honey. The respective values of pH with supplementation of 2 per cent honey was 5.07 for Bb-N indicating the effect of honey supplementation in decreasing the pH of whey medium. The enhanced activity of probiotic could be attributed to the fructose present in the honey which acts as a prebiotic for the growth of these probiotic organisms. The effect of prebiotic especially fructose has demonstrated a triggering effect on the activity of probiotic. Effect of honey on the growth of probiotics has been reported by some of the earlier workers (Bordignon *et al*., 2004 and Mendoz *et al.*, 2007). From the results it is evident that, addition of honey has significant effect on the growth and development of Bb-N and addition of honey at 2 per cent and incubating a period of 15 h is optimum.

Cheese whey was supplemented with 2.0 per cent honey and further added with carrot and tomato (CT) juice at 3.0, 4.0, and 5.0 per cent level and subjected to sterilization. The sterilized whey medium was inoculated with 1.5 per cent *Bifidobacterium bifidum NCDC 232* (Bb-N) culture and incubated for a period of 18 h. The pH, acidity and viable counts were monitored at a regular interval to optimize the condition for propagation. The effect of addition of CT juice on the activity of Bb-N culture is depicted in Table 3.

The results revealed that supplementation of CT juice upto a level of 4.0 per cent has significant effect on both pH and acidity of whey medium. The pH at 15 h incubation was found to be 5.07, 5.01, 4.94 and 4.91 at 0, 3.0, 4.0 and 5.0 per cent supplementation of CT juice. The corresponding acidity at this level was 0.46, 0.48, 0.50 and 0.51 per cent lactic acid, respectively. The viable counts of Bb-N also significantly increased with the supplementation of CT juice upto 4.0 per cent level. The viable counts after 15 h incubation was 7.51, 8.21, 8.39

Table 3: Effect of supplementation of carrot and tomato juice on the activity of *Bifidobacterium bifidum NCDC 232* in whey medium

Level of enrichment (%)	Period of incubation (h)						Mean
	3	6	9	12	15	18	
				pH			
0	5.45	5.34	5.22	5.13	5.07	5.04	5.208
3	5.42	5.31	5.19	5.07	5.01	4.98	5.163
4	5.37	5.21	5.07	4.98	4.94	4.92	5.081
5	5.34	5.18	5.04	4.96	4.91	4.90	5.055
		Levels			Period	Levels x Period	
F test		*			*	NS	
C.D. (P=0.05)		0.046			0.038	---	
				Acidity (% LA)			
0	0.33	0.37	0.41	0.44	0.46	0.47	0.413
3	0.34	0.38	0.42	0.46	0.48	0.49	0.428
4	0.36	0.41	0.46	0.49	0.50	0.51	0.455
5	0.37	0.42	0.47	0.50	0.51	0.52	0.465
		Levels			Period	Levels x Period	
F test		*			*	NS	
C.D. (P=0.05)		0.019			0.011	---	
				Viable count (log cfu/ml)			
0				6.67	7.51	6.98	7.076
3				7.32	8.21	7.94	7.823
4				7.58	8.39	8.00	7.990
5				7.66	8.35	7.95	7.976
		Levels			Period	Levels x Period	
F test		*			*	*	
C.D. (P=0.05)		0.051			0.650	0.062	

All values are average of three trials * indicate significance of value at P=0.05 NS=Non-significant

and 8.35 log cfu/ml, respectively at 0, 3.0, 4.0 and 5.0 per cent supplementation of CT juice.

Supplementation of a combination of carrot and tomato (CT) juice to whey medium and its effect on growth and development of probiotics is demonstrated in Table 4. It is noteworthy that supplementation of CT juice has further significant effect on the growth of probiotics in addition to honey supplementation. The maximum activity attained in whey medium with 2 per cent honey supplementation with respect to Bb-N was 0.46 per cent LA after 15 h of incubation whereas the acidity with supplementation of CT juice was 0.50 per cent LA for Bb-N, under similar condition. The pH of whey medium as affected by the supplementation of CT juice followed the similar pattern as that of acidity indicating the positive effect of CT juice. CT juice supplementation had significant effect on both pH and acidity upto a level of 4 per cent. Further enhancing the level of supplementation to 5 per cent had no positive effect. Supplementation of CT juice also had positive effect on extent of viable count attained. The extent of viable counts attained was significantly higher with 4 per cent supplementation of CT juice as compared to without supplementation. The counts of Bb-N with supplementation of CT juice was 8.39 log cfu/ml whereas the respective count without CT juice supplementation was 7.51 log cfu/ml. The results demonstrated that CT juice has significant effect on the activity of both probiotics. The tomato juice and carrot

Table 4 : Effect of supplementation of whey protein hydrolysate (WPH) on the activity of *Bifidobacterium bifidum NCDC 232*

Level of enrichment (%)	Period of incubation (h)						Mean
	3	6	9	12	15	18	
	pH						
0	5.37	5.21	5.07	4.98	4.94	4.92	5.081
0.5	5.35	5.19	5.05	4.95	4.90	4.87	5.051
1.0	5.28	5.13	4.98	4.89	4.83	4.80	4.985
1.5	5.18	5.03	4.88	4.79	4.73	4.70	4.885
2.0	5.16	5.01	4.86	4.77	4.71	4.68	4.865
		Levels		Period		Levels x Period	
F test		*		*		*	
C.D. (P=0.05)		0.025		0.039		0.027	
	Acidity (% LA)						
0	0.36	0.41	0.46	0.49	0.50	0.51	0.455
0.5	0.37	0.42	0.47	0.50	0.52	0.53	0.468
1.0	0.39	0.44	0.49	0.52	0.54	0.55	0.488
1.5	0.42	0.47	0.52	0.55	0.57	0.58	0.518
2.0	0.43	0.48	0.53	0.56	0.58	0.59	0.528
		Levels		Period		Levels x Period	
F test		*		*		NS	
C.D. (P=0.05)		0.011		0.018		---	
	Viable count (log cfu/ml)						
0				7.58	8.39	8.00	7.990
0.5				8.28	8.76	8.43	8.490
1.0				8.49	8.85	8.72	8.686
1.5				8.75	9.04	8.85	8.880
2.0				8.82	9.00	8.79	8.870
		Levels		Period		Levels x Period	
F test		*		*		*	
C.D. (P=0.05)		0.0112		0.301		0.047	

All values are average of three trials * indicate significane of value at P=0.05 NS= Non-significant

juice are known to carry certain trace elements and nutrients which probably enhance the activity of probiotic by serving as growth promoters. The effect of CT juice on growth and activity of probiotic has been demonstrated by some of the earlier workers (Yoon *et al.,* 2004 and Tsen *et al.,* 2008). Kun *et al.* (2008) reported that, Bifidobacteria strains are capable of growing well on pure carrot juice without nutrient supplementation from 10^7cfu/ml to 10^8 cfu/ml after 6 h of incubation.

Cheese whey was supplemented with 2.0 per cent honey and 4.0 per cent CT juice followed by supplementation with various levels of WPH (0.5, 1.0 1.5 and 2 %) and sterilization. The sterilized whey medium was inoculated with 1.5 per cent Bb-N culture and incubated upto18 h. The extent of growth of Bb-N culture was monitored at a regular interval in terms of pH, acidity and viable counts. The results are represented in Table 4.

Addition of WPH at 0, 0.5, 1.0, 1.5 and 2.0 per cent led to a pH of 4.94, 4.90, 4.83, 4.73 and 4.71 after 15 h of incubation. The corresponding acidity was observed to be 0.50, 0.52, 0.54, 0.57 and 0.58 per cent lactic acid, respectively indicating that supplementation of WPH has significant effect on changes in both pH and acidity of whey medium. It is further observed that supplementation of WPH has significant effect on viable counts of Bb-N upto a level of 1.5 per cent. The viable counts at 0, 0.5, 1.0, 1.5 and 2.0 per cent levels of supplementation were found to be 8.39, 8.76, 8.85, 9.04 and 9.00 log cfu/ml. From the result presented, it is evident that WPH supplementation has significant effect on pH, acidity and viable counts of both probiotics. The minimum pH attained without WPH supplementation was 4.94 with respect to Bb-N whereas it was 4.73 pH when whey medium was supplemented with 1.5 per cent WPH indicating that WPH is a potential substrate for enhancing the activity of probiotic. Similar observations were also made with respect to acidity of whey medium. Increased level of WPH in whey medium led to increase in acidity upto 1.5 per cent levels at all durations. Further increased level of supplementation had no significant effect on the acidity. The enhanced activity of probiotic as observed with respect to pH and acidity was also depicted in terms of viable count**.** With the increasing in the level of WPCH, there was significant increase in viable counts of Bb-N upto 1.5 per cent supplementation. Viable counts for Bb-N without supplementation of WPH was 8.39 log cfu/ml whereas it was 9.04 log cfu/ml with 1.5 per cent WPH supplementation. The enhanced activity of probiotic could be attributed to the amino acids and peptides which were released as a result of enzymatic hydrolysis of whey proteins. Enzymatic hydrolysis known to release several amino acids and bioactive peptides. Some of these peptides are known to influence the growth of probiotics. It is reported by some of the earlier workers that whey protein hydrolysates enhances the activity of probiotics (Gomes *et al.,* 1998). It was observed by Janer *et al.* (2004) that the probiotic counts as high as 9.1 log cfu/ml could be obtained by supplementing milk with 2 per cent WPC. It is confirmed from our result that WPH supplementation enhances the viable count of Bb-N by one log as compare to without supplementation.

The optimized conditions for the growth of *Bifidobacterium* were 1.5 per cent inoculums, incubation for a period of 15 h, addition of honey at 2 per cent and supplementation of CT juice at 4 per cent (Gomes and Malcatta, 1999 and Swamy, 2003).

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