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Development of glycomacropeptide (GMP) fortified bio-yoghurt prepared from different probiotic cultures

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Abstract
The objective of this study was to analyze the physico-chemical and sensory attributes of glycomacropeptides (GMP) fortified bio-yoghurt prepared using *Bifidobacterium bifidum* (BB12), *Lactobacillus acidophilus* (LA) and combination of these two cultures (1:1 ratio) in short set yoghurt. The milk setting time of GMP fortified bio-yoghurt was decreased by 45 min at 1per cent GMP level when combination probiotic cultures were used as compared to control (without GMP). Addition of 0.25 per cent GMP to bio-yoghurt increased the titratable acidity significantly and controlled syneresis was observed at the same level of GMP. However, further increase in the levels of GMP upto 1 per cent did not show any significant effect on increase in acidity and syneresis. The sensory attributes of bio-yoghurt on 9 point Hedonic scale as evaluated by a panel of 7 judges showed maximum scores at 0.25 and 0.50 per cent levels of GMP in individual and combination probiotic cultures of *Bifidobacterium bifidum* (BB12), *Lactobacillus acidophilus*. The sensory attributes such as body and texture, flavour, sourness and overall acceptability of bio-yoghurt prepared from combination of probiotic cultures showed higher scores at 0.25 per cent GMP level as compared to individual cultures and control (without GMP). Addition of GMP to bio-yoghurt has brought significant difference in the physico-chemical properties of bio-yoghurt and improved sensory qualities at lower levels of fortification itself.

Keywords: Probiotic culture, bio-yoghurt, Glycomacropeptides, sensory quality

Introduction

The word ‘Probiotic’ is derived from Greek words pro and biotos and translated as ‘for life’ (Hamilton-Miller *et al*., 2003) [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 a health benefit on host” (Maurya *et al*., 2014 ; Singh *et al*.) [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. 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. Yoghurt is one such product acts as a probiotic carrier food, which is named by either probiotic or bio –yoghurt. 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 10^6 cfu viable probiotic bacteria cells per gram (Shiby and Mishra, 2012; Subrota *et al*.) [4, 5]. The cultures most often mentioned as probiotics for humans include *Bifidobacterium* species, *Lactobacillus acidophilus* and *Lactobacillus casei.* All these species can survive and grow well in the intestinal tract and thus have the potential to provide benefits (Bernardean and Vernoux, 2013; FAO/WHO, 2001) [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. 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 analysis to obtain scientific positive results (Cruz *et al.*, 2010) [8].

Glycomacropeptides (GMP) are bioactive peptides having 64 amino acid residues, which are found in cheese whey. They are the C-terminal part (f 106–169) of kappa-casein, released in whey during cheese making by the action of chymosin enzyme. GMP constitutes about 20–25 per cent of total proteins in whey products like whey powder, whey protein isolates (WPI), whey protein concentrates (WPC), manufactured from cheese whey (Farias *et al.*, 2010; Robitaille, *et al.*, 2012) [9, 10].

In the current years, several biological functions of GMP have been exploited viz. binding of enterotoxins, inhibit bacterial and viral adhesions, modulation in immune system and promoting growth of probiotic cultures (Brody, 2000; Thoma-Worringer *et al.*, 2006) [11, 12]. The unique amino acid profile and techno-functional along with biological properties encouraged its application as an ingredient for nutrition management, infant formulations, therapeutic and neutraceutical foods (Nakajima *et al.*, 2005; LaClair *et al.* 2009) [13, 14]. The enhanced growth of probiotic cultures in presence of GMP and their viability during storage at refrigeration temperature was demonstrated by many workers stating GMP and their hydrolyzates can act as a prebiotic (Tian *et al.*, 2015 and Robitaille, 2013) [15, 16]. The growth promoting effect of GMP on probiotic cultures might be related as well to its high content of glutamic acid, leucine and alanine and not due to the presence of sialic acid content in GMP (Tian *et al.*, 2015) [15].

In the present work, incorporation of GMP at various levels into bio-yoghurt containing different probiotic cultures were optimised based on the physico –chemical and sensory quality was studied.

Materials and methods

Whole milk: Cow milk was procured from Student's Experimental Dairy Plant, Dairy Science College, 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® BB-12) and *Lactobacillus acidophilus* (Nutrish®) were obtained in the form of freeze dried - direct vat set (FD-DVS) from Chr. Hansen Laboratories, Copenhagen, Denmark.

Glycomacropeptide (GMP): Commercially spray dried GMP (90% purity) isolated from cheese whey was a kind gift from AGROPUR Ingredients, Minnesota.

Preparation of GMP fortified short set bio- yoghurt: GMP was incorporated at different levels to homogenized whole milk (3.5% fat and 8.5% SNF) and was heated to 90°C /5 min, cooled to incubation temperature (42°C). Then bio – yoghurt cultures viz. *Bifidobacterium bifidum* (BB-12) and *Lactobacillus acidophilus* (1:1) were added individually and in combination to milk along with the yoghurt culture (2% *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp*. *bulgaricus*, 1:1). The milk mixture was filled into polypropylene 100 ml cup with air tight lids, incubated at 42°C for 3-4 h (short set) and then cooled to less than 20°C and stored at refrigerated temperature (5±1°C).

Chemical analysis: Total Solids (TS), fat, titratable acidity, free fatty acids and soluble nitrogen content of GMP fortified bio-yoghurt samples were determined as per the procedure given in ISI: SP 18 (Part XI) 1981 [17].

Syneresis: The susceptibility of bio-yoghurt to syneresis was determined using drainage method, performed at 6°C. A measured quantity of 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 (Hassan *et al.*, 1996) [18].

Sensory evaluation: The GMP fortified bio-yoghurt samples were subjected for sensory evaluation. The resultant bio-yoghurt was served to a panel of 7 judges along with the control to analyze for sensory attributes viz. colour and appearances, body and texture, flavour, sourness and overall acceptability. The sensory scores were awarded using 9 point Hedonic scale (McEwan and Lyon, 2003) [19] with a maximum score of 9 ('like extremely') and least score of 1('dislike extremely').

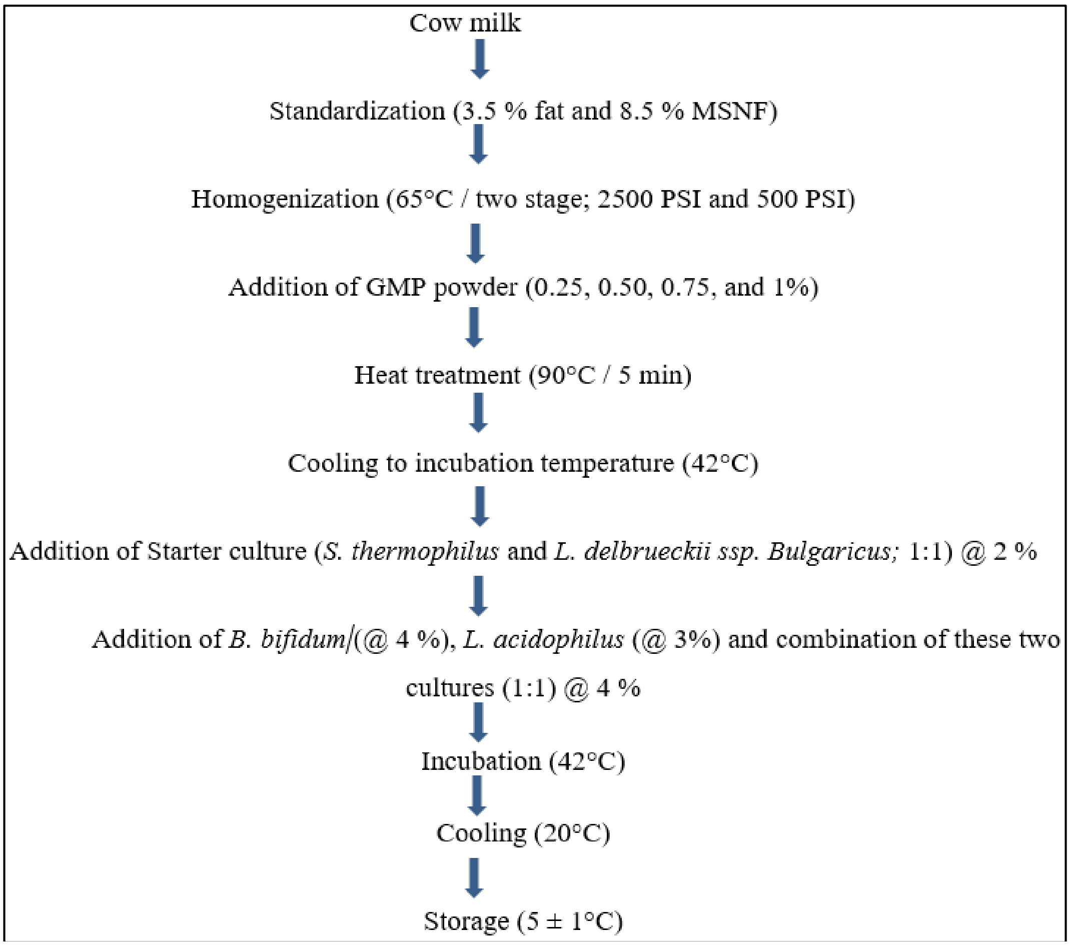
Statistical analysis: The results are the average of 5 replications and were statistically analyzed by subjecting to R Programme, R - Version 3.4.3.

Results and discussion

The effect of GMP levels (0.25, 0.50, 0.75 and 1%) in bio-yoghurt containing *B. bifidum*, *L. acidophilus* and combination of these two cultures on milk setting time, titratable acidity and syneresis was studied. Development of acidity and textural changes depends on the growth of yoghurt starter cultures and probiotic cultures, which in turn affects many physico - chemical and sensory qualities of bio-yoghurt.

Process optimization of bio-yoghurt fortified with GMP

The present investigation focuses on incorporation of GMP into bio-yoghurt to study the effect on different functionalities of the product such as physico - chemical and sensory attributes. The milk was fortified with GMP at 0.25, 0.50, 0.75 and 1 per cent levels along with *B. bifidum* (@ 4%), *L. acidophilus* (@ 3%) and combination of these two cultures (1:1; @ 4%) (Pushpa *et al.*, 2018) [19]. The flow chart for the preparation of GMP fortified bio-yoghurt prepared from cow milk is presented below.



Flow chart for preparation of bio-yoghurt incorporation with GMP

Effect of GMP fortification on physico-chemical properties of Bio-yoghurt

The effect of fortification of GMP at different levels (0.25, 0.50, 0.75 and 1%) on milk setting time, titratable acidity, syneresis in bio-yoghurt prepared with *B. bifidum* (4%), *L. acidophilus* (3%) and combination of these two cultures (1:1; 4%) is discussed below. The probiotic culture inoculum levels were optimized based on sensory and physico- chemical properties (Pushpa *et al*. 2018) [20].

Milk setting time (conversion of milk to set curd)

The milk setting time of bio-yoghurt decreased with the increasing levels of GMP as depicted in Figure 1. GMP fortification at 1 per cent level had taken least time of 195 min for setting in case of individual probiotic cultures and 180 min in combination cultures. The control sample (bio-yoghurt without GMP) had taken highest time of 230 min for individual *B. bifidum* culture and combination cultures and 235 min for *L. acidophilus* culture. The statistical analysis revealed that fortification with GMP has significant effect on milk setting time irrespective of probiotic cultures used ($P \leq 0.05$). Fortification of GMP to bio-yoghurt has helped the growth of probiotic cultures by providing nitrogenous materials and acting as a prebiotic factor.

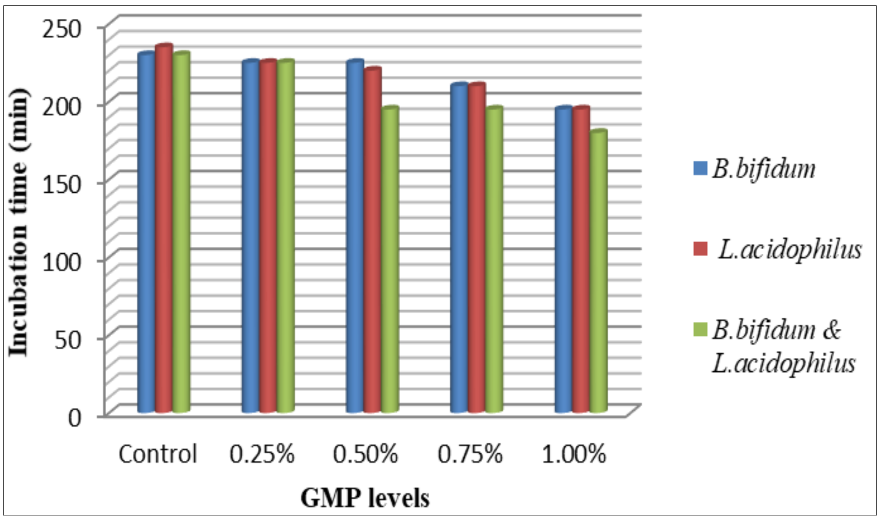


Fig 1: Effect of GMP levels on milk setting time in bio-yoghurt

Titratable acidity

As the GMP levels increased from 0.25 to 1 per cent, titratable acidity (% lactic acid) increased in all types of probiotic cultures used (Table 1). The maximum titratable acidity of 0.96, 0.84 and 0.89 per cent was observed at 0.5, 0.75 and 1 per cent levels of GMP for individual *B. bifidum, L. acidophilus* and combination culture, respectively as compared to control (0.86, 0.71 and 0.85, respectively). A significant increase in acidity (0.92, 0.82 and 0.87% LA) was observed at 0.25 per cent GMP level in the above mentioned cultures. Among all types of cultures used, *B. bifidum* showed highest acidity and the least being *L. acidophilus* in bio-yoghurt. The statistical analysis showed a significant difference in titratable acidity in all types of cultures used at 0.25 per cent GMP level ($P \leq 0.05$**)**. Rapid growth of cultures by fortification of GMP has contributed to the quick setting of milk with increased acidity. However, higher levels of GMP fortification have not favoured any increase in acidity is advantageous for product's optimum sensory quality.

Syneresis

With the increase in the levels of GMP, the extent of syneresis (ml /100g; v/m) decreased irrespective of the type of cultures used. As could be observed from the Table 1, the syneresis of control bio-yoghurt was 50, 42 and 43 per cent (v/m) for *B. bifidum, L. acidophilus* and combination cultures, respectively. Addition of GMP to bio-yoghurt decreased the syneresis to 36, 33 and 35 per cent, respectively for the above said respective cultures at all levels of GMP. Statistical analysis showed a significant difference in syneresis between the type of cultures used but not with the levels of GMP added ($P \leq 0.05$**)**. Hence, it is concluded that addition of GMP at lower level itself (0.25 per cent) can significantly reduce the syneresis in bio-yoghurt. The controlled syneresis could be attributed to the GMP participation in the micro textural development in the gel network during curdling.

Table 1: Effect of GMP fortification on titratable acidity and syneresis in bio- yoghurt

GMP levels (%)	**Type of probiotics cultures**							
	BB12	**LA**	**BB + LA**	**CD ($P \leq 0.05$)**	**BB12**	**LA**	**BB + LA**	**CD ($P \leq 0.05$)**
	Titratable Acidity (% lactic acid)				**Syneresis (%, v/w)**			
Control	0.86^{cA}	0.71^{bB}	0.85^{bA}	0.01	50^{aA}	42^{aA}	43^{aA}	1.20
0.25	0.92^{bA}	0.82^{aC}	0.87^{abB}		36^{bA}	33^{bC}	35^{bB}	
0.50	0.96^{aA}	0.83^{aB}	0.88^{aB}		36^{bA}	33^{bC}	35^{bB}	
0.75	0.96^{aA}	0.84^{aC}	0.89^{aB}		36^{bA}	33^{bC}	35^{bB}	
1.00	0.96^{aA}	0.84^{aC}	0.89^{aB}		36^{bA}	33^{bC}	35^{bB}	
CD ($P \leq 0.05$)	0.02				1.69			

Note:
- All the values are average of 5 trials
- Control: Bio-yoghurt without GMP (*S. thermophillus and L. bulgaricus* (2%, 1:1)
- BB- *B. bifidum* (@ 4%); LA - *L. acidophilus* (3%); BB+LA - *B. bifidum and L. acidophilus* (1:1; @ 4%)
- Similar superscripts indicate NS at the corresponding CD
- Small superscript: GMP levels
- Capital superscript: Type of cultures

Effect of fortification with GMP on sensory attributes of bio-yoghurt with *B. bifidum* culture

Effect of various levels of GMP (0.25, 0.50, 0.75 and 1%) on sensory attributes of bio-yoghurt containing 4 per cent *B. bifidum* is presented in Table 2. The results revealed that GMP addition of 0.25 per cent has significant effect in improving the sensory scores with respect to all the sensory attributes as compared to control (without GMP). The sensory scores of GMP fortification at 0.25 per cent were 8.00, 8.00, 8.25, 8.25 and 8.25 for colour and appearance, body and texture, flavour, sourness and overall acceptability as compared to control with scores of 7.50, 7.50, 8.05, 8.05 and 8.05, respectively. The scores of 0.50 per cent GMP were also similar to 0.25 per cent level for all the sensory attributes. However, further increase in levels of GMP to 0.75 and 1 per cent did not improve the sensory quality of the bio-yoghurt and were found to be significantly different with control and lower levels of GMP.

Statistical analysis revealed that all the attributes except colour and appearance were significantly different ($P \leq 0.05$) among treated samples. However, the sensory scores for control, 0.25 and 0.50 per cent levels of GMP in bio-yoghurt were not statistically significant revealing superiority of the GMP treated sample at lower levels. Lower scores for all the sensory attributes at 1 per cent GMP level was completely different from all the samples and was found to be statistically significant ($P \leq 0.05$).

Table 2: Effect of fortification with GMP on sensory attributes of bio-yoghurt prepared with *B. bifidum*

Levels of GMP (%)	**Sensory attributes**				
	Colour and Appearance	**Body and Texture**	**Flavour**	**Sourness**	**Overall acceptability**
	Sensory scores on 9 point Hedonic scale				
Control	7.50 ± 0.20^{a}	7.50 ± 0.40^{a}	8.05 ± 0.27^{a}	8.05 ± 0.40^{a}	8.05 ± 0.44^{b}
0.25	8.00 ± 0.00^{a}	8.00 ± 0.00^{a}	8.25 ± 0.27^{a}	8.25 ± 0.14^{a}	8.25 ± 0.37^{a}
0.50	8.00 ± 0.15^{a}	8.00 ± 0.45^{a}	8.25 ± 0.34^{a}	8.25 ± 0.37^{a}	8.25 ± 0.45^{a}
0.75	8.00 ± 0.27^{a}	7.75 ± 0.57^{a}	7.75 ± 0.54^{a}	7.50 ± 0.47^{a}	7.50 ± 0.57^{b}
1.00	7.75 ± 0.03^{a}	6.83 ± 0.67^{b}	7.00 ± 0.65^{b}	7.25 ± 0.55^{b}	7.00 ± 0.65^{c}
CD ($P \leq 0.05$)	0.65	0.68	0.52	0.55	0.49

Note:
- All the values are average of 5 trials
- Control: Probiotic Yoghurt with 4 per cent *B. bifidum*
- Similar superscripts indicate non-significant at the corresponding CD

Effect of GMP on sensory attributes of bio-yoghurt with *L. acidophilus* culture

Effect of various levels of GMP (0.25, 0.50, 0.75 and 1.0%) on sensory attributes of bio-yoghurt with *L. acidophilus* culture (3%) is presented in Table 3. The scores awarded for 0.25 per cent GMP level exhibited higher scores of 8.25, 8.25, 7.75, 7.75 and 8.25 for colour and appearance, body and texture, flavour, sourness and overall acceptability, respectively as against control (8.25, 7.86, 7.66, 7.66 and 7.86). The body and texture, and overall acceptability of the product was significantly improved by GMP addition. The sensory attributes of bio-yoghurt with 0.50 per cent GMP also obtained equal scores as compared to 0.25 per cent level. The lower sensory scores for bio-yogurt at 0.75 and 1 per cent GMP levels were found to be statistically significant.

Table 3: Effect of fortification with GMP on sensory attributes of bio – yoghurt prepared with *L. acidophilus*

Levels of GMP (%)	Sensory attributes				
	Colour and Appearance	Body and Texture	Flavour	Sourness	Overall acceptability
	Sensory scores on 9 point Hedonic scale				
Control	8.25±0.64^{a}	7.86±0.76^{b}	7.66±0.65^{a}	7.66±0.78^{a}	7.86±0.75^{a}
0.25	8.25±0.47^{a}	8.25±0.28^{a}	7.75±0.47^{a}	7.75±0.57^{a}	8.25±0.28^{a}
0.50	8.25±0.37^{a}	8.25±0.28^{a}	7.50±0.37^{a}	7.50±0.57^{a}	8.12±0.28^{a}
0.75	8.00±0.37^{a}	8.00±0.00^{a}	7.25±0.23^{b}	7.25±0.45^{b}	7.75±0.34^{b}
1.00	8.00±0.47^{a}	7.25±0.28^{c}	6.50±0.76^{c}	6.50±0.57^{c}	7.25±0.28^{c}
CD ($P \leq 0.05$)	0.34	0.27	0.34	0.26	0.34

Note:

- All the values are average of 5 trials
- Similar superscripts indicate non-significant at the corresponding CD
- Control: Probiotic Yoghurt with 3 per cent *L. acidophilus*

Effect of GMP on sensory attributes of Bio-yoghurt with *B. bifidum and L. acidophilus* culture

Bio-yoghurt optimized with *B. bifidum* and *L. acidophilus* (4%) was analyzed for influence of fortification of GMP at various levels on sensory attributes is presented in Table 4. The sensory scores revealed that 0.25 per cent GMP fortified bio-yoghurt had highest scores, 8.00, 8.50, 8.25, 8.25 and 8.25 with respect to all sensory attributes viz. colour and appearance, body and texture, flavour, sourness and overall acceptability, respectively against control (8.00, 7.75, 7.75, 7.25 and 8.00, respectively). However, statistical analysis indicated that there was significant difference between control and 0.25 per cent GMP fortified samples for body and texture, flavour and overall acceptability attributes. Higher levels of GMP 0.75 and 1 per cent were awarded with lower scores for all the attributes and were found to be statistically significant. Hence, bio-yoghurt prepared with combination of cultures has awarded with highest sensory scores for all the attributes as compared to individual probiotic cultures used in the present study.

The parameters such as optimum acidity or sourness, no wheying off, flavour production and smooth body and texture helped in securing higher scores in bio-yoghurt prepared with 0.25 per cent GMP. Reduction in milk setting time, controlled acidity and syneresis in combination probiotic cultures (*B. bifidum and L. acidophilus)* fortified with GMP has contributed to better acceptability of the product. Hence, based on the physico-chemical and sensory qualities of the bio-yoghurt (*B. bifidum and L. acidophilus* cultures) fortified with 0.25 per cent GMP was optimized and these qualities are equally matched for 0.5 per cent GMP level.

The results of the present study are comparable with Antunes *et al*. (2005) and Zhao and Zhang (2006) [21,22] who reported that the supplementation of products with nitrogen source derivatives such as hydrolyzed protein, whey proteins and amino acids had a positive impact on the viability of probiotic strains and on the product quality (syneresis and firmness) in yoghurt.

Table 4: Effect of fortification with GMP on sensory attributes of bio-yoghurt prepared with *B. bifidum and L. acidophilus* (1:1)

Levels of GMP (%)	Sensory attributes				
	Colour and Appearance	Body and Texture	Flavour	Sourness	Overall acceptability
	Sensory scores on 9 point Hedonic scale				
Control	8.00±0.65^{a}	8.00±0.34^{b}	7.86±0.70^{b}	7.80±0.61^{b}	8.00±0.68^{a}
0.25	8.00±0.81^{a}	8.50±0.57^{a}	8.25±0.28^{a}	8.25±0.75^{a}	8.12±1.18^{a}
0.50	8.00±0.81^{a}	8.00±1.41^{b}	8.00±0.50^{a}	8.00±0.50^{a}	7.75±1.19^{a}
0.75	8.00±0.81^{a}	7.50±1.00^{c}	7.25±0.81^{b}	7.25±0.81^{c}	7.25±0.50^{b}
1.00	7.50±1.00^{b}	7.50±1.00^{c}	7.00±0.00^{b}	7.00±0.00^{c}	7.00±0.00^{b}
CD ($P \leq 0.05$)	0.21	0.43	0.22	0.34	0.29

Note:

- All the values are average of 5 trials
- Control: Probiotic Yoghurt with 4% *B. bifidum and L. acidophilus*
- Similar superscripts indicate NS at the corresponding CD

Chemical and microbiological quality of optimized bio-yoghurt fortified with GMP

The gross chemical composition of the optimized bio-yoghurt fortified with 0.25 per cent GMP and control (without GMP) were analyzed for total solids, protein, fat, ash, lactose, lactic acid, soluble nitrogen and free fatty acids are delineated in Table 5.

The total solids, protein and ash content of bio-yoghurt with 0.25 per cent GMP were higher (13.06, 3.80 and 0.75%, respectively) as against control (12.80, 3.57 and 0.72%, respectively). Fat and lactose content of GMP treated bio-yoghurt was 3.59 and 3.29 per cent, respectively as compared to control (3.63 and 3.33%). Lactic acid, soluble nitrogen and free fatty acid content were higher in GMP fortified bio-yoghurt, the values being 0.87, 0.035 and 0.165 per cent, respectively as against control (0.85, 0.023 and 0.160%, respectively).

Statistical analysis revealed that total solid, protein, lactic acid and soluble nitrogen content were significantly different between control and GMP fortified sample. However, there was non-significant difference with respect to fat, ash, lactose and free fatty acid content. The chemical composition of bio-yoghurt fortified with GMP has met the chemical legal standards as prescribed by FSSAI, 2011.

The total solids and protein content was higher in treated sample due to addition of GMP. The increase in titratable acidity and soluble nitrogen may be due to enhanced growth of probiotic organisms in the GMP treated sample as compared to control.

In order to assess the safety of bio-yoghurt samples, coliforms and yeast & molds count were determined. The results showed no viable coliform counts, and yeast & molds counts were within the permissible limits (FSSAI standards, 2011) indicating that the final products prepared were safe for consumption.

Table 5: Effect of fortification of GMP on chemical composition of bio-yoghurt

Constituents (%)	Control	GMP fortified bio-yoghurt *	CD ($P\leq0.05$)
Total solids	12.80±0.03[b]	13.06±0.01[a]	0.055
Protein	3.57±0.04[b]	3.80±0.04[a]	0.056
Fat	3.63±0.03[a]	3.59±0.04[a]	0.092
Ash	0.72±0.02[a]	0.75±0.03[a]	0.064
Lactose	3.33±0.02[a]	3.29±0.04[a]	0.065
Lactic acid	0.85±0.00[b]	0.87±0.01[a]	0.022
Soluble nitrogen	0.023±0.00[b]	0.035±0.00[a]	0.007
Free fatty acid	0.160±0.01[a]	0.165±0.01[a]	0.011

Note:

- All values are average of 5 trials
- Control: Bio-yoghurt without GMP
- GMP at 0.25% level
- Similar superscripts indicate NS at the corresponding CD

Conclusion

The present study was unertaken to analyze the physico-chemical and sensory attributes of bio-yoghurt fortified with GMP at different levels. The results revealed that fortification of GMP has reduced the milk setting time, increase in acidity and reduced the syneresis in bio-yoghurt. The sensory attributes viz. body and texture, flavour, sourness and overall acceptability of the GMP fortified (0.25 and 0.50%) bio-yoghurt were improved. Combination cultures of *B. bifidum and L. acidophilus* in GMP fortified bio-yoghurt has showed better physico-chemical properties and higher scores for all the sensory attributes compared to individual cultures.

References

1. Hamilton-Miller JMT, Gibson GR, Bruck W. Some insight into the derivation and early uses of the word probiotic. British J Nutri. 2003; 90:845.
2. Maurya P, Mogra R, Bajpai P. Probiotics: An approach towards health and disease- Mini Review. Trends in Biosci. 2014; 7(20):3107-3113.
3. Singh K, Kallaali B, kumar A *et al*. Probiotics: a review. Asian Pac J Trop Biomed, 2011, 287-290.
4. Shiby VK, Mishra HN. Fermented milks and milk products as functional foods- A review. Critical Reviews in Food Sci. and Nutri. 2012; 53(5):482-496.
5. Subrota H, Surajit M, Prajapati JB. Methods for Improving Survival of Probiotics against Harsh Environments. Int. J Fermented Foods. 2015; 4(1-2):1-13.
6. Bernardeau M, Vernoux JP. Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion effects and health properties and consequences for extrapolation of farm animal results to humans. Clin Microbiol. Infect. 2013; 19:321-330.
7. FAO/WHO. Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Córdoba, Spain, 2001, 34.
8. Cruz AG, Cadena RS, Granato D *et al.* Sensory evaluation of probiotic, prebiotic and synbiotic foods: relevance for product development. Comp Rev Food Sci. Saf. Web of Science® Times Cited, 2010, 6.
9. Farías M, Martinez M, Piloso A. Casein glycomacropeptide pH- dependent self-assembly and cold gelation. Int. Dairy J. 2010; 20:79-88.
10. Robitaille G, Lapointe C, Leclerc D, Britten M. Effect of pepsin-treated bovine and goat caseinomacropeptide on Escherichia coli and Lactobacillus rhamnosus in acidic conditions. J. Dairy Sci. 2012; 95:1-8.
11. Brody EP. Biological activities of bovine glycomacropeptide. British J Nutri. 2000; 84(1):39-46.
12. Thoma-Worringer C, Krause I, Kulozik U. Precipitation behaviour of caseino macropeptides and their simultaneous determination with whey proteins by RP-HPLC. Int. Dairy J. 2006; 16:285-293.
13. Nakajima K, Tamura N, Kobayashi-Hattori K, Yoshida T, Hara-Kudo Y, Ikedo M *et al*. Prevention of intestinal infection by glycomacropeptide. Biosci. Biotechnol. Biochem. 2005; 69:2294-2301.
14. Laclair CE, Ney DM, Macleod EL, Etzel MR. Purification and use of glycomacropeptide for nutritional management of phenylketonuria. J. Food Sci. 2009; 74(4):199-206.
15. Tian Q, Wang TT, Tang X, Han MZ, Leng XJ, Mao XY. Developing a potential prebiotic of yogurt: Growth of *Bifidobacterium* and yoghurt cultures with addition of glycomacropeptide hydrolysate. Int. J Food Sci. Technol. 2015; 50(1):120-127.
16. Robitaille G. Growth-promoting effects of caseinomacropeptide from cow and goat milk on probiotics. J. Dairy Res. 2013; 80(1):58-63.
17. IS: SP18, Part XI. ISI hand book of food analysis, Dairy products. Bureau of Indian Standards. 1981. New Delhi.
18. Hassan AN, Frank JF, Schmidt KA *et al*. Textural properties of yogurt made with encapsulated nonropylactic cultures. J Dairy Sci. 1996; 79:2098-2103.
19. Mcewan, Lyon. Sensory evaluation. I Sensory rating and scoring methods. Caballero, B (Edn.) Encyclopedia of Food Sciences and nutrition. Elsevier, 2003, 5148-5152.
20. Pushpa BP, Jayaprakasha HM, Prabha R, Jayashree P Hiremath. Optimization of probiotic inoculum levels in bio-yoghurt based on physico- chemical and sensory attributes. Int. J Cur. Adv. Res, 2018, 17449-14753.
21. Antunes AEC, Cazetto TF, Bolini HMA. Viability of probiotic micro-organisms during storage, post acidification and sensory analysis of fat-free yogurts with

added whey protein concentrate. Int. J. Dairy Technol. 2005; 58(3):169-173.
22. Zhao H, Zhang L. Growth of probiotic bacteria in milk supplemented with protein hydrolysate. China Dairy Ind. 2006; 44:16-18.