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Growth of *Bifidobacterium pseudocatenulatum* G4 and changes in organic acid profile in peanut milk and skim milk supplemented with fructooligosaccharides

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ABSTRACT

Bifidobacterium pseudocatenulatum G4 was evaluated for its potential use in peanut milk (PM) based product. The study examined the growth and subsequent organic acids production compared with that on reconstituted skim milk (SM). The initial pH was relatively set at 6.5. Inoculums of 0.67% were monitored and samples were incubated anaerobically for a period of 27 h under control conditions using 1L volume bioreactor. The maximum growth was attained at incubation period of 24h. The growth was inspired by means of fructooligosaccharides (FOS) supplementation. High growths of 8.35 log CFU/ ml PM + FOS and 8.39 log CFU/ml SM + FOS were recorded as compared with 8.35 log CFU/ ml TPY (Trypticase Phytone Yeast Extract) media. During incubations, the pH reductions were explained by increases in lactic, acetic, probionic and butyric acids. Profiles of probionic and butyric acids in fermented PM did not significantly varied from accepted levels as demonstrated in consumable fermented SM. The maximum growth obtained in fermented samples was high, exceeding (> 7 log CFU/ml) the recommended level of ten million viable cells per ml product. Therefore, PM medium could be developed beside SM as a potential carrier for strain G4.

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INTRODUCTION

Bifidobacterium pseudocatenulatum G4 has been isolated from infants (Shuhaimi *et al*., 2001a; Shuhaimi *et al*., 2001b; Shuhaimi *et al*., 2002). Aaccording to Tuohy *et al*., (2003), human bifidobacteria and lactobacilli are the most important probiotic candidates for application into food industry. Residing on the same interest, the potential

of synbiotics formulation of *B. pseudocatenulatum* G4 with FOS has been developed (Shuhaimi *et al*., 2009) to enhance growth and facilitate delivery of this strain via foods. This is because probiotic foods should contain a sufficient number of viable microorganisms, which are able to alter the microflora composition of the host through colonization to generate more beneficial effects (Schrezenmeir and de Vrese, 2001).

Nowadays, in the area of functional foods, probiotic products have become the best choice for health conscious people. Scientifically approved probiotics have been successfully promoted to develop foods for specific health purposes. Probiotics incorporation into fermented dairy products adds values to several health aspects, which include prevention of gastrointestinal disorders, mucosal immunity, allergy, cardiovascular disease, and urogenital tract disorders (Huis in't Veld & Have-naar, 1991). Currently, the mechanisms exerting these benefits are well documented (Shah, 2000).

Bifidobacteria can grow and survive in the lower buffering capacity of dairy, as a conventional carrier (FAO/ WHO, 2001; Frece *et al*., 2005). However, probiotic dairy products are expensive and are not consumed by the rural population where both malnutrition and gastrointestinal diseases are widely spread. Therefore, probiotics growth and viability in none dairy products has received much attention (Prado N.F *et al*., 2008). This has paved the way for researchers to introduce affordable carriers such as soy milk to reach a larger population, the benefits of probiotics foods (Chou & Hou, 2000; Wang *et al.,* 2002). However, developing of an alternative nutritious cheap medium for the delivery of *Bifidobacterium* is still at its infancy. Recent interest to develop cereal and soymilk bases as a means for strains of this genus has emerged (Kaur *et al*., 2002; Kabeir *et al*., 2004). In this respect, peanut milk (PM) could be a potential carrier. It is characterized as a source of high vegetable proteins, low in cholesterol, thus has attracted much attention from consumers. It is also extremely rich in minerals, and essential fatty acids, such as linoleic and oleic, which are highly valuable in human nutrition (Diarra *et al*., 2005). In addition, peanut milk can also provide basic nutrients for bacteria growth. Growth of Lactobacillus in peanut medium was reported by Wang *et al.* (2007).

Nevertheless, the growth of bifidobacteria in peanut milk has not been explored, particularly, for *B. pseudocatenulatum* G4. Therefore, in this study, the growth of strain G4 and its subsequent acid production during incubation of peanut milk (PM) as compared with that on skim milk (SM) was elucidated.

MATERIALS AND METHODS

Preparation of fermentation media

Peanut beans were cleaned, washed with distilled water and soaked for 8 hours at 45 °C using a temperature controlled water bath. The beans were then hand dehulled and blended with 1L stainless steel Warning Commercial Blender at medium speed for 3 min. Next, the obtained slurry was filtered through double-layered cheese cloth to prepare the peanut milk (PM), which was used directly or supplemented with fructooligosacharide (FOS) (Orafti Pty.Ltd, Tienen, Belgium) and human grade yeast extract (Biospringer, Maissonc-Alfort Cedex, France) to a final concentration of 10% w/v. the resultant peanut milk was autoclaved for 15 min at 121 °C prior to fermentation.

A 10% reconstituted skim milk (SM) was prepared using skim milk (NZMP skim milk powder, Auckland, New Zealand) and the slurry was then sterilized as was done for PM.

Starter culture and Bifidobacterium inoculum

Probiotic Bifidobacterium pseudocatenulatum G4 is a human-derived strain obtained from the stock culture collection of Biotechnology and Functional Food Laboratory (Faculty of Food Science and Technology, UPM, Malaysia).

The strain was maintained at –20 ºC in 20% (v/v) glycerol and skim milk supplemented with yeast extract. A working culture was prepared by activating the frozen bacteria. It was grown in skim milk (10%, w/v) supplemented with yeast extract (0.05%), incubated under anaerobic conditions using Anaerocut A GasPac system (Merck, Darmstadt, Germany) for 48h. Active cells were transferred twice at 1% inoculation rate (v/v) in skim milk yeast extract medium followed by anaerobic incubation at 37 ºC for 24h prior to use.

Batch cultivation conditions

Fermentation was carried out in batches using a four vessels bioreactor with a temperature controlled water bath (Jeio Tech Desk Top, Korea) and an electronic stirrer (Gas-Col Ltd, USA). The media used for fermentation were PM and SM. They were placed in clean vessels and autoclaved for 15 min at 121 °C. The contents were then mixed to equilibrate the temperature to 37 °C. Inoculations were next carried out with 0.67% (v/v) *Bifidobacterium* strain G4 starter culture. The initial pH was set at 6.7 using human grade sodium bicarbonate and the fermentation was run for 27h. Oxygen free nitrogen was used during fermentation to create the anaerobic environment. The stirring speed was set at 200 rpm/min.

Microbiological analysis

Samples were collected aseptically at 0h and every three hours post incubation up to 27h. Growth of *Bifidobacterium* was enumerated in colony forming units (CFU) per ml using the plate count method. De Manan Rogosa Agar MRS agar containing 0.05% L-cysteine was used for enumeration.

Organic acid profiles

One ml of liquid fermented medium was centrifuged at 10000 rpm for 15 min. The supernatant was filtered through a 0.22 μm membrane filter and stored at -20 °C for analysis of organic acids, reducing sugars and total soluble solids.

Profiles of organic acids were analyzed by high- performance liquid chromatography (HPLC) [Shimadzu LC-10AS Liquid Chromatography, Japan] with a Shimadzu SPD-10AV UV-VIS detector. An organic column, packed with nine µm of polystyrene divinylbenzene ion exchange resin (Aminex HPX-87H; 300 mm x 7.8 mm, Bio-Rad Laboratories, USA) and maintained at 65 °C was used. The UV detector was set at 220 nm and the mobile phase was 0.009 N sulphuric acid with a flow rate of 0.7 ml/min.

RESULTS AND DISCUSSION

Growth of *Bifidobacterium pseudocatenulatum* G4 during incubation of PM and SM

As shown in Table 1, the growth of strain G4 in PM and SM based meda had increased from the initial seeding to a maximum after 24h incubation. Thereafter, no increase in population was noted except for the SM with FOS supplementation. The maximum growths obtained in PM and SM are 7.12 log and 7.28 log CFU/ml, respectively. However, the growth gain of the strain was higher in PM. Both PM and SM had achieved 2.38 and 2.03 log CFU/ml increases, respectively. Moreover, the respective 47.02 and 59.89% growth increases in PM and SM was attained in the first 12h of incubation.

Similar growths for other *Bifidobacterium* strains in soy milk and oat based medium have amounted to about 7 log CFU/ml after 24h incubation as reported by Wang *et al*.

(2002) and Laine *et al*. (2003). Therefore, the growth of strain G4 in PM is a testimony that this medium has potential as a *Bifidobacterium* carrier, like the well-developed skim milk, soymilk, and oat based media. Nevertheless, to exert beneficial effects to the host, a probiotic food should contain a sufficient amount of a live probiotic at the time of consumption. The suitable amount recommended for a good culture milk to provide a dietetic and therapeutic benefit is 7 log CFU a live probiotic per a ml of the product (Schuler-Malyoth *et al*., 1968). With regard to this, enhancing the growth of strain G4 in SM medium using prebiotics, as a growth stimulant had become a useful approach for this strain (Shuhaimi *et al*., 2009).

Table 1: Growth of *Bifidobacterium pseudocatenulatum* G4 (Log CFU/ml) during fermentation of peanut milk (PM) and skim milk (SM) with different supplements

Time (h)	PM	PM + FOS	PM + FOS +YE	SM	SM + FOS	TPY
0	4.74±0.08	4.97±0.36	4.66±0.49	5.01±0.10	4.74±0.30	4.88±0.39
3	5.25±0.17	5.95±0.30	5.93±0.71	5.19±0.35	5.00±0.41	5.51±0.26
6	5.38±0.08	5.97±0.56	6.58±0.47	5.81±0.30	5.52±0.05	6.69±0.72
9	5.41±0.02	6.69±0.61	6.88±0.06	5.77±0.40	6.38±0.40	7.51±0.23
12	5.86±0.05	7.06±1.04	7.53±0.66	6.49±0.80	7.70±0.85	7.62±0.94
15	6.26±0.18	7.61±0.57	7.83±0.50	6.55±0.06	7.73±0.10	7.99±0.60
18	6.42±0.36	8.29±0.41	7.67±0.60	7.03±0.44	7.95±0.13	7.87±0.40
21	6.50±0.31	8.35±0.36	8.12±0.43	6.96±0.67	8.39±0.27	8.25±0.23
24	7.12±0.07	8.31±0.17	8.04±0.28	7.28±0.29	8.37±0.57	7.96±0.59
27	7.08±0.11	8.25±0.10	8.02±0.24	7.25±0.43	8.34±0.69	7.97±0.47

[a] Valuse are mean ± std of triplicate independent runs

PM = peanut milk; FOS = fructooligosaccharide (0.67% w/v); YE = yeast extract (0.5% w/v); SM = reconstituted skim milk, TPY= Trypticase Phytone Yeast Extract.

Most human origin probiotics are fastidious. When used alone, they are characterized with low growth capability in food media including the dairy as a recommended carrier to human (FAO/WHO, 2001). Their formulation with prebiotics could be a promising approach. Prebiotics selectively stimulate the growth and/or activity of indigenous probiotic bifidobacteria and lactobacillus in the intestinal tract (Gibson & Roberfroid, 1995). This concept is known as synbiotics (presence of probiotics and prebiotics at the same time in a product) which effectively enhances their complementary technological and beneficial qualities (Shin *et al*., 2000; Bielecka *et al*., 2002).

Prebiotics supplementation is a means, to improve growth of probiotics; enhance safety and extend storage of probiotics fermented products; facilitate digestion, and improve nutritional and therapeutic quality of probiotic foods (Caplic & Filr, 1999). This synbiotics formulation could positively stimulate the growth of strain G4 in the delivery medium besides enhancing quality of the fermented product.

Table (1) shows that the growth of strain G4 in PM and SM were higher in the presence of FOS, as compared with samples without the supplementation. The increased growth in SM due to supplementation with FOS was 2.189 fold higher than in SM without FOS. However, the amount of increase in PM due to FOS addition was lower, with only 0.37 log CFU fold higher than in PM without FOS. This finding is supported by a recent study, which observed that the strain G4 was

able to ferment FOS supplemented with skim milk medium (Shuhaimi *et al*., 2009). Also consistent with our findings, the stimulation of different *Bifidobacterium* spp. by the use of oligosaccharides, inulin, and Fructooligosaccharides (FOS) was made possible in a study by Shin *et al*. (2000). Another evaluation conducted by Bielecka et al. (2002) revealed the incapability of eighteen *Bifidobacterium* species out of thirty in utilizing FOS, clearly indicates that fermentation of a prebiotic is a probiotic strain dependent.

The results presented in Table 1 also shows that yeast extract further stimulate the growth of strain G4, when supplemented with FOS. The attained growth is higher than that of PM+FOS without yeast extract. This finding did not contradicts the report of Stephenie *et al*. (2007), which showed that yeast extract supplementation significantly enhanced the growth of strain G4 in skim milk medium. Supporting this, Ibrahim and Bezkorovaniny (1994) and Lee *et al*. (2008) have also found that the growth of bifidobacteria in soymilk and rice medium were enhanced with yeast extract and L-cysteine supplementation to the growth medium.

For further elucidation on this issue, calculated the impact of yeast extract supplementation during incubation of PM+FOS. The result showed growth improvement in the first 12h of incubation, where a growth increase to 63.57% of the maximum growth at 21h incubation was induced by the yeast extract. At the same incubation period, the growth increase in PM+FOS without the yeast extract was 56.41% of the growth, while the maximum growth was attained at 24h of incubation. Therefore, yeast extract has a positive impact on growth of strain G4 as reported earlier by Stephenie *et al*., (2007). However, the effects of protein supplementation to the growth medium of Bifidobacterium strains were found to be dependent on bacterial species, besides, the type of medium (Kim *et al*., 2000; Chou and Hou, 2000).

Referring to Table 1, TPY medium showed the highest growth gain of 3.47 log CFU/ml. Some 64.483%, of this growth occurred in the first 12h of incubation, which comparable with that of PM+FOS+yeast extract. The final growth recorded at 24h incubation of TPY was 8.25 log CFU/ml. Supplementation of FOS to PM and SM had also resulted in a similar growth, where the respective final populations were 8.35 log CFU/ml and 8.39 log CFU/ml.

pH changes and organic acid profiles during incubation of PM and SM with *Bifidobacterium pseudocatenulatum* G4

As presented in Table (2), the pH had decreased through out the incubation period due to acids accumulations. The decreases were higher in TPY and FOS supplements as compared with PM and SM without supplementation. FOS supplementation has caused more pH decrease in both PM and SM. With the presence of yeast extract in PM+FOS more pH decrease was recorded. The respective decreases in pH for PM, SM, PM+FOS, SM +FOS, and PM+FOS+yeast extract were 1.48, 1.54, 1.73, 1.72, 1.80 and 2.27, respectively.

The final pH of the fermented PM and SM samples was in the range from 4.64 to 4.99. This range of pH is the favorable in fermented foods destined for human consumption. In a study by laine *et al*. (2003) half of the bifidobacteria strains tested for growth in oat based medium were able to cause acidification, reducing the pH to below five after a 24h of incubation. This result is similar to our observation with strain G4 in PM and SM mediums (Table 2).

Table 2: pH decline during fermentation of peanut milk and skim milk with *Bifidobacterium pseudocatenulatum* G4[a]

Time (h)	PM	PM + FOS	PM + FOS+YE	SM	SM + FOS	TPY
0	6.51±0.02	6.53±0.06	6.44±0.12	6.47±0.06	6.48±0.03	6.53±0.25
3	6.49±0.01	6.47±0.04	6.46±0.05	6.40±0.09	6.36±0.07	6.30±0.30
6	6.36±0.13	6.39±0.07	5.97±0.61	6.29±0.17	6.28±0.07	6.12±0.26
9	6.18±0.22	5.99±0.34	5.81±0.53	6.13±0.30	6.18±0.18	5.57±0.10
12	5.62±0.74	5.39±0.50	5.42±0.37	5.63±0.19	6.07±0.25	4.99±0.28
15	5.26±0.32	5.12±0.50	5.06±0.24	5.50±0.13	5.65±0.07	4.67±0.29
18	5.13±0.29	4.96±0.41	4.84±0.24	5.31±0.25	5.47±0.04	4.55±0.33
21	5.06±0.33	4.87±0.32	4.73±0.16	5.07±0.40	5.05±0.15	4.40±0.22
24	4.97±0.37	4.80±0.27	4.64±0.12	4.99±0.36	4.76±0.23	4.27±0.12
27	4.96±0.37	4.75±0.23	4.57±0.13	4.90±0.34	4.51±0.10	4.22±0.09

[a]Valuse are mean ± std of triplicate independent runs

PM = peanut milk, FOS = fructooligosaccharide, YE = yeast extract, SM = reconstituted skim milk, TPY= Trypticase Phytone Yeast Extract.

The concentration of lactic acid in fermented PM and SM was higher than those of probionic and butyric acids. However, fermented TPY has the highest lactic acid content at 24h incubation, followed by PM+FOS, PM+FOS+yeast extract, SM+FOS, SM in descending order (Table 3).

Respective increases of 49.86, 58.66, 44.12, 61.44, and 36.85%, were in the first 12h of incubation. These results indicate increase accumulation of lactic acid due to FOS supplementation. The acetic acid profile was also the highest in TPY, compared with fermented PM, SM and their supplements (Table 4). In fermented SM the acetic acid profile was higher than that of PM at similar incubation conditions. The results also showed that supplementation with FOS had caused slight increase in acetic profiles of PM and SM. However, many factors were reported to affect acetic accumulation during fermentation with probiotics. Bruno *et al*. (2002) found that acetic acid production in most mediums was dependent on type of the strain used, the type of supplementing prebiotics, and the type of growth medium used.

Table 3: Lactic acid (mmol/ml) production by *Bifidobacterium pseudocatenulatum* G4 during fermentation of peanut milk and skim milk with different supplements[a]

Time (h)	PM	PM + FOS	PM + FOS +YE	SM	SM + FOS	TPY
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.57±0.65	0.94±0.53	0.50±0.36	0.21±0.12	0.76±0.34	0.71±0.31
6	1.49±0.51	1.66±0.88	1.13±0.54	0.58±0.12	2.22±0.30	1.92±0.96
9	2.06±0.84	3.21±1.08	2.35±0.47	1.68±0.89	3.29±0.59	3.96±1.06
12	3.19±2.31	5.67±0.68	3.49±0.44	2.52±0.91	4.44±0.83	7.30±0.23
15	4.43±2.96	6.23±1.67	5.67±1.05	3.52±0.65	7.81±2.42	8.25±0.49
18	5.95±2.50	7.04±1.32	6.19±0.80	5.04±0.41	8.04±1.12	8.99±1.51
21	6.46±2.21	9.13±1.70	7.29±0.19	6.04±0.47	8.54±1.60	11.37±1.87
24	7.21±2.23	9.66±1.29	7.90±0.25	6.83±0.56	10.77±0.16	12.30±3.29
27	7.53±2.35	10.43±1.08	9.07±0.41	7.77±0.30	12.09±1.20	13.34±2.71

[a]Valuse are mean ± std of triplicate independent runs

PM = peanut milk, FOS = fructooligosaccharide, YE = yeast extract, SM = reconstituted skim milk, TPY= Trypticase Phytone Yeast Extract.

Table 4: Acetic acid (mmol/ml) production by *Bifidobacterium pseudocatenulatum* G4 during fermentation of peanut and skim milk with different supplements[a]

Time (h)	PM	PM + FOS	PM + FOS +YE	SM	SM + FOS	TPY
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.89±0.77	0.83±0.37	0.82±0.72	0.23±0.15	0.87±0.38	1.08±0.54
6	1.45±1.21	2.94±0.62	2.36±0.73	1.43±1.44	5.11±1.46	2.14±0.86
9	2.41±1.62	5.04±0.61	3.30±1.33	2.34±0.80	7.11±0.80	3.34±1.12
12	3.30±2.34	8.89±1.33	3.79±0.92	3.21±0.29	8.47±1.81	7.59±2.32
15	4.07±1.88	9.84±0.55	6.13±1.36	3.59±0.45	10.38±2.40	9.40±2.18
18	6.11±0.54	10.14±1.32	6.28±0.42	5.59±1.13	11.38±1.81	10.74±2.68
21	6.87±0.39	11.98±1.76	6.68±0.37	5.92±0.53	12.43±0.23	12.99±3.27
24	6.86±0.92	12.74±0.41	7.27±2.15	6.92±0.16	13.40±0.50	15.99±2.44
27	7.42±1.35	13.64±1.65	8.79±0.71	8.01±1.03	13.96±0.77	16.89±1.89

[a]Valuse are mean ± std of triplicate independent runs
PM = peanut milk, FOS = fructooligosaccharide, YE = yeast extract, SM = reconstituted skim milk, TPY= Trypticase Phytone Yeast Extract.

The profiles of probionic acid in PM and SM remained approximately the same at values of 4.33 and 4.34 mmol/ml, respectively. However, FOS supplementation has enhanced the production of acetic acid, whose content was further increased in PM+FOS supplemented with yeast extract (Table 5). Similar to lactic and acetic acid production, TPY was also the highest in probionic acid. In fermented PM, the concentration of probionic was 2.16 and 2.49 fold higher than acetic and lactic acid, respectively (Tables 3, and 4).

Table 5: Propionic acid (mmol/ml) productions by *B. pseudocatenulatum* G4 during fermentation of peanut and skim milk with different supplements[a]

Time (h)	PM	PM + FOS	PM + FOS +YE	SM	SM + FOS	TPY
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.22±0.16	1.06±0.68	1.31±0.74	0.64±0.46	0.58±0.30	4.89±2.46
6	0.89±0.24	1.67±0.67	1.88±0.38	1.11±0.69	1.79±0.50	12.95±1.37
9	1.34±0.48	3.67±1.01	2.94±0.20	1.92±0.65	2.45±0.43	17.12±2.54
12	1.96±0.58	4.95±0.72	4.75±0.37	2.41±0.29	3.90±0.20	16.14±5.98
15	2.54±0.49	5.99±1.52	5.25±0.018	2.39±0.46	33.46±48.10	21.20±4.14
18	3.04±0.58	6.43±1.47	6.41±0.91	2.87±0.48	5.83±1.42	27.02±4.48
21	3.53±0.67	6.16±1.80	8.26±1.30	4.10±0.93	7.84±1.11	32.67±0.83
24	4.33±0.87	6.86±1.37	8.89±1.47	4.34±0.91	8.81±1.02	36.41±1.44
27	4.99±1.38	7.08±2.19	9.93±2.29	4.70±1.03	10.92±0.46	40.95±3.81

[a]Valuse are mean ± std of triplicate independent runs
PM = peanut milk, FOS = fructooligosaccharide, YE = yeast extract, SM = reconstituted skim milk, TPY= Trypticase Phytone Yeast Extract.

The pattern of acid accumulation was differed for butyric acid, where the highest concentration was found in fermented PM, followed by fermented TPY, PM+FOS, PM+FOS+yeast extract, SM+FOS, and SM in descending order (Table 6). However, FOS and yeast extract supplementation to PM, negatively affected the accumulation of butyric acid during fermentation. On the contrary, the FOS addition had revealed positive effect in SM based medium.

Table 6: Butyric acid (mmol/ml) production by *Bifidobacterium pseudocatenulatum* G4 during fermentation of peanut and skim milk with different supplements[a]

Time (h)	PM	PM + FOS	PM + FOS +YE	SM	SM + FOS	TPY
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.36±0.35	0.52±0.69	0.04±0.04	0.07±0.06	0.11±0.06	0.28±0.054
6	0.93±0.50	0.72±0.77	0.17±0.09	0.14±0.10	0.15±0.04	0.52±0.16
9	1.29±0.54	1.12±1.02	0.24±0.09	0.17±0.13	0.31±0.11	0.87±0.23
12	1.52±0.67	1.30±1.13	0.56±0.43	0.32±0.14	0.40±0.06	1.01±0.17
15	1.68±0.68	1.44±1.33	0.74±0.38	0.36±0.17	0.65±0.35	1.29±0.32
18	2.21±0.72	1.57±1.38	0.95±0.59	0.38±0.17	1.04±0.73	1.49±0.37
21	2.37±0.61	1.63±1.40	1.27±0.94	0.44±0.13	1.04±0.79	1.67±0.28
24	2.70±0.41	1.80±1.44	1.45±0.98	0.48±0.10	1.22±0.92	1.96±0.41
27	2.92±0.36	2.01±1.36	1.54±1.16	0.52±0.16	1.41±0.79	1.95±0.42

[a]Valuse are mean ± std of triplicate independent runs
PM = peanut milk, FOS = fructooligosaccharide, YE = yeast extract, SM = reconstituted skim milk, TPY= Trypticase Phytone Yeast Extract.

This added value could promote the fermented PM as a potential probiotic product. The viable number of strain G4, obtained in fermented PM, was comparable to that in SM. Supplementation of PM with FOS showed potential high growth level of strain G4 in the fermented products, implying improvement in quality of the fermented product. Moreover, the viability obtained during fermentations was high, at a favorable level for consumer's health (Bergamini *et al*., 2005). Nowadays, the development of a new delivery medium for bifidobacteria is encouraged due to limitation or unavailability of the conventional dairy based carrier of the dairy based. The new media might be a superior to the dairy based. For instance, a formulated oat based medium has been claimed to be better than skim milk as a carrier for *Bifidobacterium* strains with respect to adhesion properties (Laine *et al*., 2003). Also with regard to the nutritional composition of a food medium, such as in case of strain G4 fermented PM, the new medium contributes positively to deliver the strain and confers additional nutritional value to the diet. The finding of this evaluation has elucidated the potential of using fermented PM with FOS for delivery of strain G4 to develop bifidus fermented none-dairy based product.

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