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Development and Quality Evaluation of Synbiotic Yoghurt Incorporated with Oat Flour and *Bifidobacterium bifidum* NCDC-255

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Abstract

Objectives: Development of synbiotic yoghurt using non-fat milk and by incorporating oat flour and probiotic strain of *Bifidobacterium bifidum*, evaluating the product for its physico-chemical, rheological and sensory characteristics and checking the viability of *Bifidobacterium bifidum* strain in final product during storage period. **Methods:** In the current study synbiotic yoghurt was prepared using skim milk and incorporating, probiotic strain of *Bifidobacterium bifidum* (NCDC 255) and oats as prebiotic at two different level (T1-1%) and (T2-2%). Plain yoghurt (C1) and probiotic yoghurt (C2) served as control. Physico-chemical properties of the synbiotic yoghurt and its comparison with control yoghurt were carried out during 1st, 3rd, 5th and 7th days of storage at 4°C. Quality parameters such as fat, total solids, acidity, pH, syneresis, water holding capacity, and probiotic count were assessed. Sensory evaluation was also carried out to check the acceptability of the product. **Findings:** The product T2 with two per cent oat flour was the best with right proportion of ingredients and lowest syneresis. There was a statistically significant ($p < 0.01$) increase in titratable acidity of yoghurt samples during storage. The water holding capacity of samples reduced gradually during storage. Test samples (T1 and T2) showed significantly higher *Bifidobacterium* count when compared with control. Minimum therapeutic requirement of probiotic organism was maintained within the product even after seven days of storage, in order to transfer the probiotic effect. Overall acceptability and keeping quality was observed to be high in developed product. **Novelty:** Synbiotic products have opened up novel perspectives for dairy based functional foods because of their health-benefits and worldwide popularity. Study emphasises development of synbiotic yoghurt that can provide benefits of oats and *Bifidobacterium bifidum*.

Keywords: Probiotic; Beta glucan; Synbiotic yoghurt; Bifidobacterium; Oats

1 Introduction

The rapidly changing elderly population has a big influence on national public health institutions, social services, and health-care systems. Because they suffer from chronic health disorders that damage their quality of life, senior people are becoming increasingly interested, resulting in a strong demand for health services in general. As a result, new possibilities for maintaining their health have been researched, with functional food being one of them. Probiotics, prebiotics, and synbiotics are all worth investigating in this context, since scientific proof of their positive effects on gut microbiota balance is growing all the time. While probiotics are defined as "living bacteria supplied in sufficient proportions to offer a health advantage," prebiotics are defined as "a substrate that is preferentially used by host microorganisms to confer a health benefit⁽¹⁾." Synbiotics is described as "a mixture of living microorganisms and substrate(s) preferentially used by host microorganisms that imparts a health advantage on the host" as a result of their combination⁽²⁾.

However, metabolic products of microorganism rely on the availability of substrate, which is created in part by prebiotics and the gut flora. Furthermore, prebiotics produce a decrease in intestinal pH and are necessary for sustaining osmotic water retention in the gut. These synbiotics are also known to induce and result in an increase in absorption surface area through bacterial fermentation-mediated proliferation of enterocytes. Absorption and release of vital minerals from the food (mostly calcium and phosphate), modulation of the immune system, maintenance of intestinal integrity and function, and the capacity to inhibit pathogenic invasion of dangerous bacteria or colonies are all actions of these microorganisms. Calcium absorption may also be influenced by the gut microbiota, notably Bifidobacteria and Lactobacillus, as well as fermented milk products, according to research⁽³⁾.

Increased public awareness of the need of leading a healthy lifestyle has resulted in a rise in demand for synbiotic dairy products. The idea behind synbiotic therapy is that when the appropriate probiotic and prebiotic are given together, they have a greater positive impact than if they were given separately⁽⁴⁾. Yogurt can be a good medium for delivering a probiotic-prebiotic combination. Oats, a cereal grain, are often taken as whole grains and are known to offer beneficial nutrients⁽⁵⁾. Oats are a good source of soluble fibre in the form of beta-glucans in terms of nutrition. Furthermore, beta-glucan has been found as a prebiotic, which promotes the growth of good intestinal microbes, giving them an edge over other bacteria in the gut. Beta-glucans have unique rheological properties, such as the capacity to gel and enhance viscosity of aqueous solution⁽⁶⁾.

Beta-glucans have been used to substitute fats in dairy products and can alter food texture. Whole oats or oat flour can be utilised as thickening agents or fat mimetics in the development of low-calorie meals due to their beta-glucan concentration. Furthermore, because of its potential to produce a structured and elastic casein-protein-glucan matrix, when β -glucan is introduced to milk, the fermentation is started⁽⁷⁾. In light of these considerations, research was designed to make synbiotic yoghurt using a probiotic strain of *Bifidobacterium bifidum* and oat flour, as well as to evaluate the product's chemical, microbiological, rheological, and sensory characteristics. It was also attempted to determine the viability of the probiotic strain in the final product during storage.

2 Materials and Methods

Fresh milk required for the study was procured from the University Dairy Plant, Mannuthy. Freeze dried yoghurt cultures (NCDC-264) and probiotic strain of *Bifidobacterium bifidum* (NCDC-255) was procured from National Dairy Research Institute, Karnal. All the bacteriological media required for the research was procured from Hi-media laboratories Ltd, Mumbai.

2.1 Standardization of yoghurt mix and preparation of yoghurt

The percentage of fat in skim milk and total solids (TS) content was estimated according to the procedure described by Bureau of Indian Standards (BIS) Skim milk with 0.3 per cent fat and 12.05 ± 0.04 per cent total solid was used for the development of new product. Yoghurt samples of different categories were prepared as per the method suggested by Pandurang et al.⁽⁸⁾ viz., (1) control plain yoghurt (C1), (2) control probiotic yoghurt (C2), (3) synbiotic yoghurt incorporating one per cent oats (T1) (4) synbiotic yoghurt with two per cent oats (T2) and (5) synbiotic yoghurt with three per cent oats. Sensory evaluation was carried out to check the acceptability of the product and the products which were found to be more acceptable were selected for further study. The yoghurt samples prepared were stored under refrigeration for further chemical and microbiological quality evaluations (Table 1).

Table 1. Composition of control and test samples

Sl.No	Experimental preparations	Composition
1.	C1	Skim milk + 3.5% sugar + Yoghurt culture (ST&LB)
2.	C2	Skim milk + 3.5 % sugar + Yoghurt culture (ST & LB) & <i>Bifidobacterium bifidum</i>
3.	T1	Skim milk + 3.5% sugar + 1% oats + Yoghurt culture (ST & LB) & <i>Bifidobacterium bifidum</i>
4.	T2	Skim milk+ 3.5% sugar+ 2% oats + Yoghurt culture (ST & LB) & <i>Bifidobacterium bifidum</i>

2.2 Analysis of physico-chemical properties of yoghurt

The procedure described by the Bureau of Indian Standards was adopted for estimating the fat, total solids and titratable acidity⁽⁹⁾ of yoghurt samples. The fat content of yoghurt samples was determined according to the procedure where 11.3 g of well mixed yoghurt sample was weighed accurately and added into the milk butyrometer. 10 ml of Gerber's sulphuric acid and one millilitre of iso-amyl alcohol were added. The butyrometer was closed and well mixed. The butyrometer was placed in a water bath at $65 \pm 2^\circ\text{C}$ for tempering. It was then centrifuged at 1200 rpm for five minutes. The percentage of fat was read by adjusting the fat column within the scale of the butyrometer.

Total solid of the yoghurt sample were determine according to BIS standards were, five gram of yoghurt sample was weighed into the prepared dish with its lid and it was left on boiling water bath for 30 minutes. Then the dish with its lid was transferred to the oven maintained at 100°C and it was allowed to dry for at least three hours. After three hours the dish was immediately

transferred to desiccator for cooling and recorded the weight. Procedure was repeated until loss of weight between successive weighing did not exceed 0.5 mg. The percentage of total solids was calculated by using the following formula (Percentage of Total Solids = $100 \times (M_2 - M_1) / M$; M_1 = mass in g of empty dish, M_2 = mass in g of the sample with dish after drying, and M = mass in g of the sample taken for the test).

The titratable acidity of yoghurt samples was also determined according to Bureau of Indian Standards. Nine gram of test yogurt sample was weighed into conical flasks. Few drops of the phenolphthalein solution were added into the conical flask, and it was mixed by slight swirling. The contents in the conical flask were titrated against 0.1 N sodium hydroxide solution from the burette until a faint pink colour developed for about five seconds. Titratable acidity expressed as percentage of lactic acid according to the formula (Titratable acidity = $9VN/W$; N = Normality of sodium hydroxide solution used for titration, V = Volume of 0.1 N sodium hydroxide required for titration, W = Mass in g of sample of yoghurt).

The pH of yoghurt samples was estimated using Cyber Scan 2500 digital pH meter (Eutech). Syneresis and water holding capacity was determined as per the procedure described by Brodziak et al.⁽¹⁰⁾. Five ml of yoghurt was centrifuged at 5000 rpm for 20 minutes at 4° C and separated whey was measured after one minute. Amount of whey separation was expressed in volume of separated whey per 100 ml of yoghurt as syneresis. To determine the water holding capacity 20g of yoghurt (W) was centrifuged for 10 min at 4000 rpm at 4°C. The whey expelled (Y) was removed and weighed. Water Holding Capacity (g/ kg) was measured using the given formula $(W-Y) \times 1000$. The viscosity of the yoghurt sample was determined using a Brookfield Viscometer (D 445 /446).

2.3 Microbiological analysis of yoghurt

Bifidobacterium selective agar (Hi-media Laboratories Mumbai 400086) was used for enumerating *Bifidobacterium bifidum* in the product.

2.4 Sensory evaluation of yoghurt

The fresh yoghurt samples were evaluated for their sensory characteristics viz., colour & appearance, flavours, body & texture and overall acceptability on a 5-point hedonic scale as per the method recommended by Rafiq et al.⁽¹¹⁾. An expert panel consisting of seven members belonging to Department of Dairy Science, CVAS, Mannuthy evaluated the samples.

2.5 Statistical analysis

The data obtained from various studies were subjected to statistical analysis by using SPSS 21 version. To obtain the mean value and standard deviation of all the parameters, six replications of yoghurt samples were performed for all control and treatment samples. Repeated measures analysis of variance was done for comparing chemical and microbiological parameters among different samples. Duncan's Multiple Range Test (DMRT) was carried out for pair wise comparison, if F values are found to be significant in ANOVA. With regard to the sensory evaluation data, acceptability of different samples was compared by Friedman's test.

3 Result and Discussion

3.1 Standardization of yoghurt mix and preparation of yoghurt

On analysis of quality parameters such as fat and total solids, the mean fat content of yoghurt prepared was observed to be 0.3 per cent. The total solid content of control plain yoghurt (C_1), control probiotic yoghurt (C_2), synbiotic yoghurt incorporated with one per cent oats (T_1) and synbiotic yoghurt with two per cent oats (T_2) were 14.91 ± 0.493 , 14.88 ± 0.460 , 17.21 ± 0.669 and 18.26 ± 0.560 percent respectively. Significantly higher total solids content was observed for samples containing two per cent oats when compared with control. Milk fat has a significant impact in the texture, taste, and colour development of yoghurt products. As a result, unless different stabilisers are utilised, the reduction in fat will diminish the total solids content (in low-fat and non-fat yoghurt), resulting in a weak body, poor texture, and increased whey separation⁽¹²⁾.

3.2 Analysis of physico-chemical properties of yoghurt

Properties such as acidity, pH, syneresis, water holding capacity and viscosity of newly developed product were assessed and compared with control. There was a statistically significant ($p < 0.05$) increase in the titratable acidity of yoghurt samples during storage. No significant difference existed between the treatments. The titratable acidity of the control and treatment samples in

this investigation varies from 0.79 to 1.1 percentage of lactic acid. It is evident from table 2 that the titratable acidity increases as storage duration increases. According to Diep et al. (13) acidity of yoghurt shall be between 0.7 to 1.2 percentage of lactic acid. Result of the current study agrees with the legal standard. The finding can also be related with Prittam et al. (14) who observed variations in the titratable acidity (TA) profile of probiotic and synbiotic yoghurt samples during refrigeration over a period of 28 days. There was a sharp increase in the TA levels in all the synbiotic yoghurt samples. The ascending trend of TA corresponds to the sharp decline in the pH values of the yogurt samples during storage. Nur et al. (15) reported that the acidity of yoghurt tends to increase continuously with storage length. In their study it was found that the acidity determines the quality and taste of yoghurt. The present finding is in agreement with all of the above reports. The increase in titratable acidity observed during storage may be attributed to the growth of microbes and production of lactic acid (Table 2).

Table 2. Titratable acidity of yoghurt samples during storage

Sam- ple	1 st day(Mean±S.E) (% of lactic acid)	3 rd day(Mean±S.E) (% of lactic acid)	5 th day(Mean±S.E) (% of lactic acid)	7 th day(Mean±S.E) (% of lactic acid)	Overall(Mean±S.E) (% of lactic acid)
C1	0.81±0.006	0.91±0.186	0.99±0.017	1.10±0.006	0.80 ^{ns} ±0.008
C2	0.80±0.020	0.90±0.200	0.98±0.018	1.08±0.011	0.91 ^{ns} ±0.009
T1	0.82±0.016	0.91±0.025	1.02±0.013	1.08±0.009	0.99 ^{ns} ±0.008
T2	0.79±0.018	0.91±0.019	0.98±0.015	1.05±0.015	1.08 ^{ns} ±0.008
Over- all	0.80 ^D ±0.008	0.91 ^C ±0.011	0.98 ^B ±0.008	1.08 ^A ±0.006	

ns- Non significant

Means having same letters as superscripts (A-D) doesn't differ significantly at 0.05 level.

The mean pH values decrease progressively, along with the days of storage and a significantly lower pH observed on 7th day of storage. According to Australian Food Standards Code (Standard H8) yoghurt is prepared with *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* or other suitable lactic acid bacteria and required to have a pH < 4.5. Results of the present study can be compared with the standard. Similar observations were made by Kamel et al. (16). In their study fat replacers did not negatively influence the activity of yoghurt starter bacteria. The pH values of the yoghurt samples gradually decreased during storage and the decrease in pH was statistically significant (P < 0.01) (Table 3).

Table 3. pH of yoghurt samples

Sample	1 st day (Mean±S.E)	3 rd day (Mean±S.E)	5 th day (Mean±S.E)	7 th day (Mean±S.E)	Overall (Mean±S.E)
C1	4.39±0.011	4.31±0.004	4.31±0.004	4.15±0.012	4.27 ^B ±0.009
C2	4.40±0.009	4.29±0.017	4.23±0.012	4.14±0.018	4.26 ^B ±0.009
T1	4.43±0.010	4.29±0.017	4.23±0.012	4.14±0.018	4.31 ^A ±0.009
T2	4.44±0.007	4.36±0.010	4.27±0.010	4.18±0.020	4.31 ^A ±0.009
Overall	4.42 ^A ±0.005	4.33 ^B ±0.006	4.25 ^C ±0.005	4.16 ^D ±0.008	

Means having same letters as superscripts (A-D) doesn't differ significantly at 0.05 level.

The syneresis percentage of different treatments and controls were estimated as a part of the study. With respect to this parameter, treatment T2 with 2 percent oats showed lowest syneresis percentage (2.80±0.02, 2.87±0.01, 2.99±0.02 and 3.08±0.01 percent respectively on first, third, fifth and seventh days of storage). Significant increase in syneresis rate was observed (p < 0.01) during storage (Table 4). Diep et al. (13) stated that an increase in total solids could increase the density of yoghurt matrices and result in decreased syneresis. According to Valérie et al. (17) the value of syneresis in fermented milk increases with the storage time and he observed an initial value of syneresis for fresh yoghurt 4.7 per cent which after storage of 28 days increased to 8.3 per cent. The present findings are in agreement with the above report. Syneresis increased with the advancement of storage period in all the yoghurt samples.

Sample T2 with two percent oats showed significantly higher (p < 0.01) water holding capacity (WHC) than other treatment and control. The mean WHC values decreased progressively during the storage period (Table 5). The value differed significantly (p < 0.01) between days of storage among different treatments. Ranok et al. (18) observed that increase in fat and total solids content increased the water holding capacity yoghurt products. When skim milk was replaced by whey protein concentrates, yoghurt showed an improved water-holding capacity. In the current study sample T1 and T2 containing oats showed higher WHC than plain yoghurt it can be concluded that increase in total solids content might have resulted in improved water holding capacity of the product.

Table 4. Syneresis of yoghurt sample during storage

Sam-ple	1 st day(Mean±S.E) (%)	3 rd day(Mean±S.E) (%)	5 th day(Mean±S.E) (%)	7 th day(Mean±S.E) (%)	Overall(Mean±S.E) (%)
C1	3.33±0.02	3.41±0.03	3.63±0.08	3.74±0.08	3.53 ^A ±0.036
C2	3.03±0.03	3.15±0.03	3.30±0.03	3.45±0.03	3.23 ^B ±0.036
T1	2.86±0.02	2.98±0.02	3.08±0.02	3.22±0.01	3.04 ^C ±0.036
T2	2.80±0.02	2.87±0.01	2.99±0.02	3.08±0.01	2.94 ^C ±0.036
Over-all	3.01 ^D ±0.013	3.10 ^C ±0.015	3.22 ^B ±0.026	3.37 ^A ±0.024	

Means having same letters as superscripts (A-D) doesn't differ significantly at 5% level.

Table 5. Water holding capacity of yoghurt samples during storage

Sam-ple	1 st day(Mean±S.E) (g/ kg)	3 rd day(Mean±S.E) (g/ kg)	5 th day(Mean±S.E) (g/ kg)	7 th day(Mean±S.E) (g/ kg)	Overall(Mean±S.E) (g/ kg)
C1	499.58±0.80	483.20±2.58	470.58±4.35	455.91±2.16	477.33 ^D ±2.94
C2	517.33±3.70	507.08±4.16	500.58±9.29	484.33±8.57	502.33 ^C ±3.01
T1	539.91±2.31	526.33±4.94	511.66±3.76	500.50±4.25	519.60 ^B ±3.23
T2	547.00±9.66	538.33±5.03	528.23±6.25	515.16±5.31	532.18 ^A ±3.95
Over-all	525.95 ^A ±2.66	513.75 ^B ±2.14	502.76 ^C ±3.15	488.97 ^D ±2.79	

Means having same letters as superscripts (A-D) doesn't differ significantly at 5% level.

When viscosity of different treatments was analysed, it was observed that there was a significant difference in viscosity between treatments and control. Significantly high viscosity (3120.00±20.65 Cp at 20oC) was observed for samples containing two per cent oats when compared to control (Table 6). Najgebauer-Lejko et al.⁽¹⁹⁾ opined that measuring viscosity of yoghurt is challenging because it is non-Newtonian, ie. viscosity changes as shear stress changes. Since both of the treatment yoghurts showed increase in their apparent viscosity it can be assumed that oat flour caused a significant increase in viscosity.

Table 6. Viscosity of yoghurt samples

Sample	Viscosity (Mean±S.E) (cP at 20°C)
C1	706.07 ^D ±22.31
C2	873.33 ^C ±19.09
T1	2253.33 ^B ±36.76
T2	3120.00 ^A ±20.65
F value	2013.52

** - Significant at 0.01 level

3.3 Microbiological analysis of yoghurt

The mean *Bifidobacterium bifidum* count of synbiotic yoghurt with one per cent oats (T₁) was 18.24±0.02, 18.02±0.02, 17.83±0.02 and 17.58±0.03log₁₀ cfu/g respectively for the first, third, fifth and seventh day of storage. The corresponding values for synbiotic yoghurt with two per cent oats (T₂) were 18.98±0.03, 18.46±0.04, 17.96±0.05 and 17.62±0.05 log₁₀ cfu/g respectively.

Among different treatments the mean Bifidobacterium count differed significantly (p< 0.01) and reduced according to duration of storage. Treatment T2 with two percent oats gave highest count of Bifidobacterium when compared to samples with other level of oats and control. The probiotic count within the product on 7th day of storage was 17.62±0.05 log₁₀ cfu/g which indicated that minimum therapeutic requirement of probiotic organism was maintained even after 7 days of storage (Table 7). According to Richard et al.⁽²⁰⁾ probiotic bacteria grow slowly in milk due to their lack of proteolytic activity, thus requiring the incorporation of essential growth factors such as peptides for enhancing their growth. Co-culturing with proteolytic yogurt bacteria ie. Lactobacillus delbrueckii subsp. bulgaricus (LB) and Streptococcus thermophilus (ST) increases the growth and viability of probiotics.

Table 7. *Bifidobacterium bifidum* count in yoghurt samples during storage

Sample	1 st day (Mean±S.E) (log ₁₀ cfu/g)	3 rd day (Mean±S.E) (log ₁₀ cfu/g)	5 th day (Mean±S.E) (log ₁₀ cfu/g)	7 th day (Mean±S.E) (log ₁₀ cfu/g)	Overall (Mean±S.E) (log ₁₀ cfu/g)
C2	18.10±0.03	17.95±0.04	17.32±0.05	17.06±0.05	17.06 ^C ±0.03
T1	18.24±0.02	18.02±0.02	17.83±0.02	17.58±0.03	17.91 ^B ±0.03
T2	18.98±0.03	18.46±0.04	17.96±0.05	17.62±0.05	18.25 ^A ±0.03
Over-all	18.25 ^A ±0.01	18.04 ^B ±0.02	17.84 ^C ±0.02	17.60 ^D ±0.02	

Means having same letters as superscripts (A-D) doesn't differ significantly at 5% level.

3.4 Sensory evaluation of yoghurt

The mean overall acceptability scores noted during sensory evaluation were 18.30±0.894 and 17.83±0.872 for control yoghurt samples (C1) and control probiotic yoghurt samples (C2) respectively. The corresponding values for yoghurt samples added with one per cent oats (T1) and yoghurt added with two per cent oats (T2) were 18.17±0.667, 18.22±0.843 respectively (Table 8). When the sensory scores among different yoghurt samples were analysed by Friedman test, no significant difference could be observed among the samples. A study conducted by Kaur and Riar⁽²¹⁾ the consumer panels did not detect significant difference in the appearance, mouth feel, flavor, or overall quality among yoghurts prepared with different concentrations of oats when compared to the control yoghurt. In the present study also no significant difference in the sensory scores observed between control and treatment groups of yoghurt.

Table 8. Sensory scores of yoghurt samples

Sample	Appearance and colour (Mean ± S.E)	Body and texture (Mean ± S.E)	Flavours (Mean ± S.E)	Overall scores (Mean ± S.E)
C1	4.50±0.224	4.50±0.224	4.50±0.224	18.30±0.894
C2	4.17±0.167	4.00±0.365	4.33±0.211	17.83±0.872
T1	4.17±0.167	4.13±0.307	4.33±0.211	18.17±0.667
T2	4.50±0.224	4.48±0.307	4.50±0.224	18.22±0.843
Chi-square value	4.800	4.76	1.00	3.980
p-value	0.187ns	0.261ns	0.801ns	0.272ns

Probiotics such as the *Bifidobacterium* genera, that are helpful to the host's health when given in sufficient amounts on a regular basis. Prebiotics are now defined as non-digestible dietary ingredient(s), that have a favourable effect on the health of the host by selectively boosting the development and activity of probiotic bacteria. Furthermore, there may be synergistic effects from mixing probiotics and prebiotics, i.e., symbiotic benefits. This study claims that there isn't enough research on the creation of functional dairy products, particularly those containing probiotics, *Bifidobacterium*, prebiotics, oats and yoghurt.

4 Conclusion

In the current study an attempt was made to develop synbiotic yoghurt using skim milk and probiotic strain of *Bifidobacterium bifidum* (NCDC 255), incorporating oats as prebiotic at two different levels. Chemical and microbiological quality parameters of fresh yoghurt samples were evaluated. Rheological properties and sensory attributes of the product were also assessed. Furthermore shelf-life study was conducted. Result of the study revealed that different concentrations of oat flour had diverse effects on the quality of yoghurt. Synbiotic yoghurt (T2) with 2 per cent oat flour was the best suited with correct proportion of ingredients. The product showed seven days shelf life. Minimum therapeutic requirement of probiotic organism was maintained within the product even after seven days of storage, in order to transfer the probiotic effect. Probiotics, such as those from the *Bifidobacterium* family, are beneficial to the health of the host when provided in appropriate doses on a regular basis. Furthermore, combining probiotics and prebiotics may have synergistic effects, resulting in symbiotic advantages. According to the study, when provided in appropriate proportions on a regular basis, the developed functional dairy product containing probiotics (*Bifidobacterium*), prebiotics (oats) and yoghurt, are capable to provide health benefits to the host.

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