EVALUATION OF VARIOUS PROPERTIES OF SYMBIOTIC YOGHURT OF BUFFALO MILK

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ABSTRACT

In present study the set-type yoghurt samples were prepared from buffalo milk, *Lactobacillus acidophilus* and *Bifidobacterium bifidium* as probiotics and lactoluse, oligofructose and inulin as prebiotics. Changes in pH value, syneresis and probiotic counts along with sensorial properties were tested in all of yoghurt samples after preparation up to 21 days at 4C. According to the results of pH test, post acidification with a range of 4.53–3.93 was observed. Using prebiotics simultaneously led to the significant lower syneresis rate during the storage. Probiotic bacteria used in this study were found to have survived above 7 log cfu/mL throughout the study period. The present study proved that prebiotics improved physicochemical properties and enhanced probiotic bacteria survival in the experimental yoghurts. On the other hand, control and probiotic yoghurts were preferred by the sensory panel over symbiotic yoghurt.

PRACTICAL APPLICATIONS

There is a high demand for consumption of buffalo milk and its derived products worldwide. Buffalo yoghurt is a good carrier for developing symbiotic yoghurt. Probiotics just in adequate amounts can have beneficial effects for the host and therefore their viability should be monitored throughout storage time of symbiotic yoghurt, as they must survive in the gut environment. Sensory properties of yoghurt are important factors for its popularity as well. Therefore symbiotic yoghurt was made using Lactobacillus acidophilus and Bifidobacterium bifidium along with prebiotics including inulin, lactulose and oligofructose to settle the most beneficial probiotic–prebiotic combination, that have at least the minimum requirement of probiotics as well as good sensory properties for development of a marketable symbiotic yoghurt of buffalo milk.

INTRODUCTION

In the last decade, milk production has increased worldwide. Production of buffalo milk is in the second place after bovine milk production in ranking. Nevertheless, buffalo milk is preferred over by consumers, since it is either drunk or transformed into dairy products such as yoghurt as an increasing popular product of buffalo milk (Han *et al.* 2012). Recently high demand of buffalo milk and its derived products is making buffaloes part of landscapes unimaginable which is due to high sensory quality of buffalo dairy products as well as high adaptability of the animals. In addition, buffalo milk is more nutritive than cow milk based on a comparison of gross compositions showing the higher amounts of fat, protein, lactose, minerals and vitamins (Han *et al.*, 2012).

Yoghurt is the most popular fermented dairy product and has the most acceptability worldwide. This dairy product is regularly produced through lactic fermentation of milk by two starter bacteria, namely, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* resulting in unique texture of yoghurt due to complex interactions among casein, lactic acid and bacterial polysaccharides produced by starter cultures as well as in unique aroma mostly due to the

TABLE 1. LIST OF COMBINATIONS AND TREATMENTS IN THE PRESENT STUDY

	Treatment	Description
1	**Control**	Samples without probiotics and prebiotics
2	**LA**	Samples with *Lactobacillus acidophilus*
3	**BB**	Samples with *Bifidobacterium bifidium*
4	**MIX**	Samples with both probiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidium)*
5	**O-LA**	Samples with *Lactobacillus acidophilus* and oligofructose
6	**L-LA**	Samples with *Lactobacillus acidophilus* and lactulose
7	**I-LA**	Samples with *Lactobacillus acidophilus* and inulin
8	**O-BB**	Samples with *Bifidobacterium bifidium* and oligofructose
9	**L-BB**	Samples with *Bifidobacterium bifidium* and lactulose
10	**I-BB**	Samples with *Bifidobacterium bifidium* and inulin
11	**O-MIX**	Samples with both probiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidium)* and oligofructose
12	**L-MIX**	Samples with both probiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidium)* and lactulose
13	**I-MIX**	Samples with both probiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidium)* and inulin

production of acetaldehyde by these starter bacteria (Aghajani *et al.* 2012; Ranathunga and Rmusk 2013).

Probiotics in adequate amounts are beneficially healthy for the host. These microorganisms including *Lactobacillus* and *Bifidobacterium* spp. are bacterial members of the normal human intestinal flora, which apply several beneficial effects on human health and well-being (Aghajani *et al.* 2012; Marhamatizadeh *et al.* 2012). The expediency of probiotic bacteria added to foods depends on the dose levels. Their viability should be monitored throughout storage time, as they must survive in the gut environment. In order to improve these characteristics of probiotic bacteria, fermented food should be fulfilled with prebiotics (Majchrzak *et al.* 2010).

Prebiotics are indigestible nutrients having health promoting benefits for the host through promoting growth or activity of one or more probiotic bacteria as well as inhibition of harmful bacteria in the colon and improving bioavailability of minerals such as magnesium, calcium and iron. Lactulose, inulin and oligofructose are among the most important prebiotics used in food products specially fermented dairy products including yoghurt (Gustaw *et al.* 2011). Lactulose is composed of galactose and fructose and is produced from lactose through heat processing of milk or alkaline isomerization (Shaghaghi *et al.* 2013). Inulin and oligofructose are indigestible and fermentable fructans which are associated with dietary fibers. They promote Ca^{++} absorption resulting in bone density improvement, cholesterol and triglycerides level reduction, facile digestion of diets containing high protein, prevention of constipation by providing roughage, having sense of being sated without carrying any extra calories, control of blood glucose – which is very important in diabetic people and decrease of colon cancer incidence (Oliveira *et al.* 2012; Srisuvor *et al.* 2013).

Symbiotic products are known as products containing both probiotics and prebiotics. Yoghurt plays an important role due to its own nutritional properties and proposing an appropriate environment as a carrier for the growth and survival of probiotics (Marhamatizadeh *et al.* 2012). It is important for subsequent products to yield adequate content at the end of shelf-life, because probiotics are live microorganisms and consumers expect their beneficial effects to be observed after ingestion of the products. Probiotic dairy products should contain at least 10^7 cfu/mL of viable probiotic bacteria at the time of consumption (Vinderola *et al.* 2000; Ali *et al.* 2013).

The aim of this study was to investigate the influence of selected prebiotics: lactulose, inulin and oligofructose on the growth and viability of probiotic bacteria as well as on the changes in pH value, syneresis and sensorial properties in probiotic and symbiotic yoghurt of buffalo milk up to 21 days during the refrigerated storage and settle the most beneficial probiotic–prebiotic combination, which fulfills the therapeutic requirement of presence 10^7 cfu/mL (g) for all storage period of the experimental yoghurts.

MATERIALS AND METHODS

Preparation of Milk, Prebiotics and Probiotics

Raw buffalo milk containing about 7% fat and 5% protein (Ekomilk Ultra pro, Stara Zagora, Bulgaria) was purchased from a dairy farm, Urmia, Iran. Microbial strains consisted of combined culture of yoghurt YC-x11 containing *L. delbrueckii* subsp. *bulgaricus* and *S. thermophiles* and The probiotic monostrain cultures of *L. acidophilus* (LA-05) and *B. bifidium* (BB-12), both freeze-dried, were purchased from CHR Hansen, Denmark. L-cysteine and prebiotics including lactulose, inulin and oligofructose were all purchased from Sigma (Sigma chemical Co. St. Louis, MO). All culture media were purchased from Merck (Darmstadt, Germany).

TABLE 2. CHANGES IN PH OF MILK SAMPLES DURING STORAGE (ACCORDING TO MEAN ± STANDARD DEVIATION)

Samples (h)	Control	BB	LA	MIX
2	6.36 ± 0.05^{Aa}	6.13 ± 0.05^{Ab}	6.16 ± 0.05^{Ab}	6.16 ± 0.05^{Ab}
5	6.36 ± 0.05^{Aa}	5.93 ± 0.05^{Bb}	5.9 ± 0.1^{Bb}	5.93 ± 0.05^{Bb}
18	6.03 ± 0.05^{Ba}	5.03 ± 0.05^{Cb}	5.06 ± 0.05^{Cb}	4.56 ± 0.05^{Cc}

Different superscript uppercase letters and superscript lowercase letters donate significant differences in columns and rows, respectively.

Primary Culture Preparation

In order to produce buffalo milk containing the probiotic bacteria (*L. acidophilus* and *B. bifidium*), four sterilized 250 mL clear-glass containers (TGI company), each containing 200 mL of pasteurized buffalo milk, were considered including *L. acidophilus*, *B. bifidium*, a mixture of both probiotic bacteria (0.33% [w/v] of each probiotic bacteria) and a control without any addition of probiotic bacteria. Then all four containers were incubated at 37C and pH changes of samples were measured every 1 h. Samples were transferred to the refrigerator (4C) as soon as the pH reached to 5.4. Bacterial count was operated for each container at 0, 2, 5 and 18 h after inoculation (Aghajani *et al.* 2012).

Symbiotic Yoghurt Production

To produce symbiotic yoghurt, 13 sterile containers (250 mL) containing pasteurized buffalo milk (7% fat) were inoculated with starter cultures according to the manufacturer's protocol. In the next step, 0.33% (w/v) of probiotic bacteria was inoculated ($\sim 10^7$ cfu/mL). Afterward, 1.5% (w/v) of prebiotics was separately added to make combinations as are provided in Table 1. All containers were incubated at 44C until the pH dropped to 4.6. Containers were finally refrigerated at 4C in order to stop fermentation (Boeni and Pourahmad 2012).

Experimental Factors

Measurement of pH Changes. The pH of the experimental yoghurts were determined by a pH meter (Switzerland, Metrohm Herisau E520) at 25C.

Syneresis Measurement. To measure syneresis, 25 g of yoghurt samples were at first weighed in centrifuge tubes, and then the tubes were centrifuged in 350× g at 10C for 30 min. The separated liquid from the samples that was collected at the top of tubes were removed and the tubes were re-weighed. Syneresis rate was expressed as lost water per 100 g of yoghurt (Boeni and Pourahmad 2012).

Bacterial Test. Microbial test consisted of sample culture in MRS-Agar supplemented with filter sterilized 0.05% (w/v) L-cysteine hydrochloride for *B. bifidium* (Ding and Shah 2009) and in MRS-Bile Agar for *L. acidophilus* (Ashraf and Shah 2011). In order to provide this, proper dilution of samples were made in sterile peptone water solution and the plates were incubated at 37C following the culture preparation. Colony counts were measured following 72 h incubation period on following days of storage: 1, 3, 7, 14 and 21.

Sensory Evaluation. The samples were evaluated using a nine-point Hedonic scale (1: extremely dislike to 9: extremely like) by 12 semi-trained panelists from scientific staff and PhD students of food hygiene and quality control. They were selected based on their performance in initial evaluation trials. The panel members were trained about characteristics of the product and its possible defects. Sensory descriptors of the samples were aroma, taste, texture and overall acceptability (Clark *et al.* 2009).

Statistical Analysis. The results were presented as mean ± SD. Statistical analysis of the data was performed using the analysis of variance (ANOVA) by the SPSS software, version 18.00. Means with a significant difference ($P < 0.05$) were compared by Duncan's post hoc test.

RESULTS AND DISCUSSION

The pH values of milk samples during storage are shown in Table 2. The pH values of all milk samples ranged from 4.56

TABLE 3. CHANGES IN MICROBIAL COUNT (CFU/ML) OF MILK SAMPLES DURING STORAGE (ACCORDING TO MEAN ± STANDARD DEVIATION)

Samples (h)	LA	BB	MIX-LA	MIX-BB
0	7.51 ± 0.23^{Aa}	7.44 ± 0.1^{Aa}	7.58 ± 0.07^{Aa}	7.52 ± 0.02^{Aa}
2	7.69 ± 0.09^{ABa}	7.65 ± 0.11^{Ba}	7.76 ± 0.09^{Aa}	7.72 ± 0.1^{Ba}
5	7.72 ± 0.04^{ABa}	7.71 ± 0.09^{Ba}	7.79 ± 0.04^{Aa}	7.75 ± 0.02^{Ba}
18	7.92 ± 0.08^{Ba}	7.82 ± 0.06^{Ba}	8.25 ± 0.21^{Bb}	8.24 ± 0.02^{Cb}

Different superscript uppercase letters and superscript lowercase letters donate significant differences in columns and rows, respectively.
MIX-LA: enumeration of *Lactobacillus acidophilus* in milk sample with both probiotics, MIX-BB: enumeration of *Bifidobacterium bifidium* in milk sample with both probiotics.

TABLE 4. CHANGES IN PH OF YOGHURT SAMPLES DURING STORAGE (ACCORDING TO MEAN ± STANDARD DEVIATION)

Days Samples	1	3	7	14	21
Control	4.53 ± 0.05^{Aa}	4.43 ± 0.05^{Ab}	4.4 ± 0.0^{Ab}	4.26 ± 0.05^{Ac}	4.13 ± 0.05^{Ad}
LA	4.43 ± 0.05^{ABa}	4.33 ± 0.05^{ABb}	4.4 ± 0.0^{Aab}	4.23 ± 0.05^{Ac}	4.03 ± 0.05^{BCd}
BB	4.46 ± 0.05^{ABa}	4.36 ± 0.05^{ABb}	4.33 ± 0.05^{ABbc}	4.26 ± 0.05^{Ac}	4.1 ± 0.0^{ABd}
MIX	4.46 ± 0.05^{ABa}	4.36 ± 0.05^{ABb}	4.26 ± 0.05^{BCc}	4.13 ± 0.05^{BCd}	4 ± 0.0^{CDe}
O-LA	4.43 ± 0.05^{ABa}	4.33 ± 0.05^{ABb}	4.26 ± 0.05^{BCbc}	4.2 ± 0.0^{ABcd}	4.13 ± 0.05^{Ad}
L-LA	4.4 ± 0.0^{Ba}	4.33 ± 0.05^{ABab}	4.33 ± 0.05^{ABab}	4.26 ± 0.05^{Ab}	4.1 ± 0.0^{ABc}
I-LA	4.43 ± 0.05^{ABa}	4.33 ± 0.05^{ABb}	4.3 ± 0.0^{BCbc}	4.23 ± 0.05^{Ac}	4.1 ± 0.0^{ABd}
O-BB	4.46 ± 0.05^{ABa}	4.33 ± 0.05^{ABb}	4.3 ± 0.0^{BCbc}	4.23 ± 0.05^{Ac}	4.03 ± 0.05^{BCd}
L-BB	4.46 ± 0.05^{ABa}	4.36 ± 0.05^{ABb}	4.33 ± 0.05^{ABbc}	4.26 ± 0.05^{Ac}	4.1 ± 0.0^{ABd}
I-BB	4.53 ± 0.05^{Aa}	4.43 ± 0.05^{Ab}	4.4 ± 0.0^{Ab}	4.26 ± 0.05^{Ac}	4.1 ± 0.0^{ABd}
O-MIX	4.4 ± 0.0^{Ba}	4.26 ± 0.05^{Bb}	4.23 ± 0.05^{Cb}	4.06 ± 0.05^{Cc}	3.93 ± 0.05^{Dd}
L-MIX	4.46 ± 0.05^{ABa}	4.4 ± 0.0^{Aab}	4.33 ± 0.05^{ABb}	4.2 ± 0.0^{ABc}	3.96 ± 0.05^{CDd}
I-MIX	4.46 ± 0.05^{ABa}	4.36 ± 0.0^{ABb}	4.26 ± 0.05^{BCc}	4.2 ± 0.05^{ABc}	4.03 ± 0.05^{BCd}

Different superscript uppercase letters and superscript lowercase letters donate significant differences in columns and rows, respectively.

to 6.4 during the storage, while average pH values of samples with the 0.33% (w/v) probiotic was lower than the control samples. Milk samples with 0.33% (w/v) mix probiotic exhibited the lowest pH values throughout the storage period. At the moment of inoculation, pH of all samples was equal (6.4). *Lactobacillus acidophilus* sample needed less incubation period for reaching desirable pH value than *B. bifidium* and MIX samples.

The bacterial count of milk samples during storage are shown in Table 3. According to the data, all the bacterial counts were more than 10^7 cfu/mL where MIX samples had the most bacterial count for both probiotics. This data also revealed that there were no significant differences among samples before 18 h after inoculation ($P > 0.05$) but at this time bacterial counts for both probiotics in MIX samples were higher than those in other samples and the count of *L. acidophilus* in MIX sample was the highest that can be due to the associative behavior of *B. bifidum* and *L. acidophilus* on their survival.

Bifidobacterium bifidum, is a slow-growing bacteria as a single species in milk due to its weak proteolysis activity and lack of nonprotein nitrogen (NPN) in milk that make milk into an unsuitable environment for growth of *B. bifidium*. Coexistence with *L. acidophilus* can give rise to its enhanced growth (Marhamatizadeh *et al.* 2012).

In probiotic yoghurt lactic acid and acetic acid are produced by *L. acidophilus* and *Bifidobacteria*, respectively. In natural yoghurt only lactic acid is produced (Ranathunga and Rathnayaka 2013). The pH values of yoghurt samples were during storage range from 4.53 to 3.93, where control samples had the highest pH value and O-MIX samples had the lowest (Table 4). Also, pH values decreased almost constantly during storage time for all treatments. The results showed that the addition of prebiotics did not lead to a significant decrease in pH value while using probiotic bacteria simultaneously led to a significant decrease in pH values after 3 weeks of storage. These results are similar to the findings of other researchers (Hekmat *et al.* 2009; Ramchandran

TABLE 5. CHANGES IN SYNERESIS RATE (%) OF YOGHURT SAMPLES DURING STORAGE (ACCORDING TO MEAN ± STANDARD DEVIATION)

Days Samples	1	3	7	14	21
Control	10.2 ± 0.54^{Aa}	11.32 ± 0.36^{Ab}	12.74 ± 0.44^{Ac}	13 ± 038^{Ac}	14.82 ± 0.55^{Ad}
LA	8.85 ± 0.59^{Ba}	8.84 ± 0.34^{Ba}	9.63 ± 0.43^{Ba}	10.72 ± 0.52^{BCb}	12.95 ± 0.56^{BCc}
BB	8.94 ± 0.31^{Ba}	9.2 ± 0.29^{Ba}	9.62 ± 0.58^{Ba}	11.06 ± 0.67^{Bb}	13.07 ± 0.33^{Bc}
MIX	8.85 ± 0.67^{Ba}	9.11 ± 0.4^{Bab}	10.01 ± 0.4^{Bbc}	10.93 ± 0.57^{BCc}	12.63 ± 0.42^{BCd}
O-LA	8.28 ± 0.32^{Ba}	8.59 ± 0.38^{Ba}	9.34 ± 0.34^{Bb}	10.55 ± 0.26^{BCc}	12.73 ± 0.36^{BCd}
L-LA	8.3 ± 0.4^{Ba}	8.71 ± 0.4^{Bab}	9.24 ± 0.38^{Bb}	10.39 ± 0.63^{BCc}	12.7 ± 0.43^{BCd}
I-LA	8.32 ± 0.34^{Ba}	9 ± 0.15^{Bb}	9.35 ± 0.25^{Bb}	10.48 ± 0.36^{BCc}	12.28 ± 0.34^{BCDd}
O-BB	8.87 ± 0.24^{Ba}	9.11 ± 0.27^{Bab}	9.56 ± 0.3^{Bb}	10.54 ± 0.22^{BCc}	12.13 ± 0.23^{CDd}
L-BB	8.89 ± 0.25^{Ba}	9.06 ± 0.17^{Bab}	9.5 ± 0.19^{Bb}	10.49 ± 0.20^{BCc}	11.77 ± 0.53^{Dd}
I-BB	8.87 ± 0.18^{Ba}	8.8 ± 0.22^{Ba}	9.44 ± 0.22^{Ba}	10.73 ± 0.14^{BCb}	12.21 ± 0.72^{BCDc}
O-MIX	8.55 ± 0.3^{Ba}	8.71 ± 0.18^{Ba}	9.56 ± 0.19^{Bb}	10.16 ± 0.27^{Cc}	11.62 ± 0.31^{Dd}
L-MIX	8.61 ± 0.33^{Ba}	8.71 ± 0.45^{Ba}	9.58 ± 0.17^{Bb}	10.39 ± 0.26^{BCc}	11.63 ± 0.58^{Dd}
I-MIX	8.38 ± 0.12^{Ba}	8.82 ± 0.18^{Ba}	9.68 ± 0.4^{Bb}	10.62 ± 0.29^{BCc}	11.62 ± 0.32^{Dd}

Different superscript uppercase letters and superscript lowercase letters donate significant differences in columns and rows, respectively.

TABLE 6. CHANGES IN *LACTOBACILLUS ACIDOPHILUS* COUNT (CFU/ML) OF YOGHURT SAMPLES DURING STORAGE (ACCORDING TO MEAN ± STANDARD DEVIATION)

Days Samples	1	3	7	14	21
LA	7.15 ± 0.07^{Aa}	7.27 ± 0.01^{Aa}	8.26 ± 0.05^{Ab}	7.64 ± 0.08^{Ac}	7.43 ± 0.1^{Ad}
O-LA	7.62 ± 0.08^{BCa}	8.23 ± 0.09^{BCc}	8.43 ± 0.1^{ABCd}	8.33 ± 0.07^{Bbcd}	8.1 ± 0.07^{BCDb}
L-LA	7.33 ± 0.08^{ADa}	8.06 ± 0.13^{Bbc}	8.35 ± 0.1^{ABd}	8.23 ± 0.18^{Bcd}	7.95 ± 0.15^{Bb}
I-LA	7.54 ± 0.11^{BDa}	8.19 ± 0.13^{BCbc}	8.39 ± 0.11^{ABc}	8.31 ± 0.12^{Bc}	8.06 ± 0.08^{BCb}
MIX	7.34 ± 0.08^{ADa}	8.14 ± 0.11^{BCc}	8.34 ± 0.11^{ABd}	7.95 ± 0.07^{Cc}	7.64 ± 0.14^{Ab}
O-MIX	7.84 ± 0.18^{Ca}	8.38 ± 0.14^{Cb}	8.55 ± 0.11^{BCc}	8.41 ± 0.2^{Bbc}	8.23 ± 0.1^{BCb}
L-MIX	7.45 ± 0.12^{BDa}	8.35 ± 0.17^{Cbc}	8.58 ± 0.12^{BCc}	8.32 ± 0.14^{Bb}	8.13 ± 0.08^{BCDb}
I-MIX	7.58 ± 0.25^{BDa}	8.37 ± 0.17^{Cb}	8.64 ± 0.22^{Cb}	8.39 ± 0.25^{Bb}	8.31 ± 0.19^{Db}

Different superscript uppercase letters and superscript lowercase letters donate significant differences in columns and rows, respectively.

and Shah 2010; Shaghaghi *et al*. 2013). Paseephol reported that the addition of prebiotic regardless of the type used did not affect the initial pH of yoghurt and showed that the low level of post-acidification in these yoghurts is attributed to the type of probiotic and yoghurt starters used (Paseephol 2008). Probiotic bacteria are slow acid producers (Marshal and Tamime 1997). The yoghurt starter cultures including *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are active even at refrigerated temperature and yet can produce small amounts of lactic acid by fermentation of lactose which cause to noticeable pH decrease (Ali *et al*. 2013). Various investigations indicated that the activity of starter bacteria of yoghurt resulted in significant decrease in pH during refrigeration (Ozer *et al*. 2007; Aghajani *et al*. 2012). It has been also reported that prebiotics stimulate the growth and the activity of probiotic bacteria while stimulating acid production by starters, resulting in a reduced pH value of the product over the time (Tabatabaie and Mortazavi 2008; Aghajani *et al*. 2012).

Syneresis rate of yoghurt samples during storage are presented in Table 5. It increased constantly during the storage for all treatments and all treated samples showed significantly lower syneresis rate than control samples at whole of the storage time. The results obtained using prebiotics simultaneously led to a significant lower syneresis rate during the storage and samples containing lactulose had the lowest syneresis rate at the end of storage. These used prebiotics are soluble fibers which are recognized as water-structuring agents (Kip *et al*. 2006). Prebiotic compounds prevent the syneresis by increasing the water-binding capacity (Shaghaghi *et al*. 2013). These results are confirmed by findings of other researchers who reported the addition of prebiotics to set yoghurt and cause the significant decrease in syneresis (Boeni and Pourahmad 2012).

The most important factor for the food products containing probiotic especially the yoghurt that have an acidic environment is survival of probiotic. Some important factors affecting the survivability of probiotics in fermented dairy products are culture conditions, the used specific strain, final acidity, inoculation level, fermentation time and the nutrients (Boeni and Pourahmad 2012; Han *et al*. 2012).

The results of *L. acidophilus* and *B. bifidium* counts of yoghurt samples during storage are shown in Tables 6 and 7, respectively. *Bifidobacterium bifidium* and *L. acidophilus* counts in all samples decreased followed by a first increase during the storage and both probiotic bacteria had a maximum level of count on seventh day of storage time. The viability of *L. acidophilus* and *B. bifidium* in yoghurt increased in samples with both probiotics showing a synergistic effect between these strains due to mutual interactions and by addition of prebiotics especially inulin, confirming the symbiotic effects already mentioned by other researchers (Šimunek and Evačić 2009; Oliveira *et al*. 2012). Various reports on more survivability of probiotic bacteria in the

TABLE 7. CHANGES IN *BIFIDOBACTERIUM BIFIDIUM* COUNT (CFU/ML) OF YOGHURT SAMPLES DURING STORAGE (ACCORDING TO MEAN ± STANDARD DEVIATION)

Days Samples	1	3	7	14	21
BB	7.24 ± 0.19^{Aa}	7.81 ± 0.16^{Ab}	8.24 ± 0.19^{Ac}	7.57 ± 0.17^{Ab}	7.25 ± 0.12^{Aa}
O-BB	7.65 ± 0.21^{Ba}	8.36 ± 0.19^{Bb}	8.63 ± 0.22^{BCb}	8.39 ± 0.14^{BCb}	7.96 ± 0.16^{BCDb}
L-BB	7.44 ± 0.19^{ABa}	7.96 ± 0.13^{ACbc}	8.35 ± 0.14^{ABd}	8.17 ± 0.14^{Bcd}	7.77 ± 0.08^{BEb}
I-BB	7.6 ± 0.13^{Ba}	8.22 ± 0.19^{BCb}	8.56 ± 0.18^{ABCd}	8.25 ± 0.10^{BCbc}	7.85 ± 0.2^{BCa}
MIX	7.44 ± 0.19^{ABa}	7.9 ± 0.15^{ACb}	8.41 ± 0.18^{ABCc}	7.78 ± 0.13^{Ab}	7.47 ± 0.15^{AEba}
O-MIX	7.72 ± 0.24^{Ba}	8.55 ± 0.27^{Bbc}	8.77 ± 0.18^{Cd}	8.46 ± 0.19^{Cbc}	8.26 ± 0.23^{Db}
L-MIX	7.7 ± 0.13^{Ba}	8.34 ± 0.17^{Bbc}	8.62 ± 0.22^{BCc}	8.34 ± 0.02^{BCbc}	8.06 ± 0.16^{BCDb}
I-MIX	7.74 ± 0.15^{Ba}	8.44 ± 0.17^{Bbc}	8.7 ± 0.22^{BCc}	8.46 ± 0.17^{Cbc}	8.14 ± 0.19^{CDb}

Different superscript uppercase letters and superscript lowercase letters donate significant differences in columns and rows, respectively.

TABLE 8. SENSORY ATTRIBUTES OF YOGHURT SAMPLES DURING STORAGE (ACCORDING TO MEAN ± STANDARD DEVIATION)

Sensory attributes	Days Samples	1	3	7	14	21
Taste	Control	8.3 ± 0.48^{ABa}	8.1 ± 0.56^{ABa}	7.5 ± 0.52^{Bb}	7.2 ± 0.63^{ABCbc}	6.9 ± 0.56^{ABc}
	LA	8.5 ± 0.52^{Aa}	8.3 ± 0.67^{Aab}	8.1 ± 0.56^{Aab}	7.7 ± 0.67^{ABb}	6.9 ± 0.73^{ABc}
	BB	8.7 ± 0.48^{Aa}	8.5 ± 0.52^{Aab}	8.3 ± 0.67^{Aab}	7.9 ± 0.87^{Abc}	7.3 ± 0.82^{Ac}
	MIX	7.6 ± 0.69^{CDa}	7.7 ± 0.67^{BCa}	7.5 ± 0.52^{Ba}	7.2 ± 0.63^{ABCa}	6.3 ± 0.67^{BCb}
	O-LA	6.6 ± 0.96^{EFa}	6.7 ± 0.67^{DEa}	6.7 ± 0.67^{DEFa}	6.4 ± 0.84^{DEFa}	5.3 ± 0.94^{DEFb}
	L-LA	7.1 ± 0.56^{DEa}	6.8 ± 0.63^{Dab}	6.5 ± 0.52^{EFb}	6.3 ± 0.67^{EFbc}	5.8 ± 0.63^{CDEc}
	I-LA	7.1 ± 0.73^{DEa}	6.9 ± 0.73^{Da}	6.8 ± 0.78^{CDEa}	6.7 ± 0.82^{CDEa}	6 ± 0.66^{CDb}
	O-BB	6.9 ± 0.56^{EFa}	6.7 ± 0.48^{DEa}	6.5 ± 0.70^{EFa}	5.5 ± 0.84^{Gb}	5.3 ± 0.67^{DEFb}
	L-BB	6.8 ± 0.63^{EFa}	6.6 ± 0.51^{DEab}	6.5 ± 0.52^{EFabc}	6.1 ± 0.73^{EFGbc}	5.9 ± 0.73^{CDEc}
	I-BB	7.8 ± 0.63^{BCa}	7.6 ± 0.51^{BCa}	7.3 ± 0.48^{BCab}	6.8 ± 0.63^{CDEbc}	6.5 ± 0.84^{BCc}
	O-MIX	6.4 ± 0.84^{Fa}	6.2 ± 0.42^{Ea}	6.1 ± 0.56^{Fa}	5.9 ± 0.73^{FGa}	4.8 ± 0.91^{Fb}
	L-MIX	7.6 ± 0.69^{CDa}	7.5 ± 0.52^{Ca}	7.2 ± 0.63^{BCDa}	6.3 ± 0.67^{EFb}	5.2 ± 0.78^{EFc}
	I-MIX	7.8 ± 0.63^{BCa}	7.6 ± 0.69^{BCab}	7.5 ± 0.7^{Bab}	7.1 ± 0.73^{CDb}	6.4 ± 0.69^{BCc}
Aroma	Control	8.7 ± 0.48^{Aa}	8.6 ± 0.51^{Aa}	8.2 ± 0.42^{Aa}	7.5 ± 0.52^{ABb}	6.8 ± 0.78^{ABc}
	LA	8.5 ± 0.52^{ABa}	8.4 ± 0.51^{ABa}	8.2 ± 0.63^{Aa}	7.5 ± 0.7^{ABb}	7 ± 0.66^{ABb}
	BB	7.9 ± 0.73^{BCa}	7.9 ± 0.73^{BCa}	7.9 ± 0.73^{ABa}	7.7 ± 0.67^{Aab}	7.1 ± 0.56^{Ab}
	MIX	7 ± 0.66^{Da}	7.2 ± 0.78^{DEFa}	7.1 ± 0.73^{CDa}	6.7 ± 0.82^{BCDab}	6.1 ± 0.99^{BCb}
	O-LA	6.8 ± 0.91^{Da}	6.7 ± 0.67^{EFGa}	6.6 ± 0.69^{DEa}	6.5 ± 0.84^{CDEa}	6.3 ± 0.94^{ABCa}
	L-LA	7.2 ± 0.91^{CDa}	7.2 ± 0.63^{DEFa}	6.9 ± 0.56^{CDEab}	6.6 ± 0.51^{CDEab}	6.3 ± 0.67^{ABCb}
	I-LA	6.9 ± 0.99^{Da}	6.6 ± 0.51^{FGab}	6.3 ± 0.67^{Eabc}	6.1 ± 0.73^{CDEbc}	5.7 ± 0.94^{Cc}
	O-BB	6.8 ± 0.63^{Da}	6.5 ± 0.84^{Gab}	6.4 ± 0.69^{Eab}	5.8 ± 1.03^{Eb}	5.8 ± 0.91^{Cb}
	L-BB	7.8 ± 0.63^{BCa}	7.3 ± 0.48^{CDEab}	6.9 ± 0.56^{CDEb}	6.8 ± 1.13^{BCb}	6.5 ± 1.17^{ABCb}
	I-BB	7.9 ± 0.73^{BCa}	7.9 ± 0.73^{BCa}	7.3 ± 0.67^{BCa}	6.5 ± 0.84^{CDEb}	6.3 ± 0.67^{ABCb}
	O-MIX	6.6 ± 0.96^{Da}	6.6 ± 0.69^{FGa}	6.4 ± 0.69^{Eab}	5.9 ± 0.87^{DEab}	5.8 ± 0.63^{Cb}
	L-MIX	7.9 ± 0.73^{BCa}	7.5 ± 0.7^{CDa}	7.2 ± 0.63^{CDab}	6.7 ± 0.82^{BCDbc}	6.3 ± 1.15^{ABCc}
	I-MIX	7.9 ± 0.73^{BCa}	7.8 ± 0.63^{BCDa}	7.5 ± 0.7^{BCa}	6.8 ± 0.91^{BCb}	6.5 ± 0.84^{ABCb}
Texture	Control	7.7 ± 0.94^{ABa}	7.3 ± 0.82^{Aab}	7.2 ± 1.03^{ABCab}	7.1 ± 0.737^{Aab}	6.8 ± 0.421^{ABb}
	LA	8.3 ± 0.82^{Ba}	8.4 ± 0.69^{Ba}	8 ± 0.81^{Bab}	7.7 ± 0.674^{Aab}	7.3 ± 0.674^{Bb}
	BB	8.4 ± 0.69^{Ba}	8.4 ± 0.51^{Ba}	7.7 ± 1.25^{ABCab}	7.4 ± 1.074^{Ab}	7.3 ± 0.948^{Bb}
	MIX	6.8 ± 1.03^{Ca}	7.5 ± 0.84^{ACa}	7 ± 0.66^{ACa}	7 ± 0.81^{Aa}	6.8 ± 0.63^{Aba}
	O-LA	8.2 ± 0.78^{ABa}	8.2 ± 0.91^{BCa}	7.9 ± 1.19^{BCab}	7.4 ± 1.42^{Aab}	6.9 ± 0.99^{ABb}
	L-LA	8.3 ± 0.67^{Ba}	7.8 ± 0.91^{ABCab}	7.6 ± 0.51^{ABCabc}	7.4 ± 0.69^{Abc}	6.9 ± 0.87^{ABc}
	I-LA	7.8 ± 0.78^{ABa}	7.9 ± 0.73^{ABCa}	7.6 ± 0.84^{ABCa}	7.4 ± 0.84^{Aa}	6.4 ± 0.96^{ABb}
	O-BB	8.4 ± 0.69^{Ba}	8 ± 0.81^{ABCab}	7.5 ± 1.17^{ABCab}	7.3 ± 1.15^{Abc}	6.5 ± 1.17^{ABc}
	L-BB	8 ± 0.81^{ABa}	7.8 ± 0.63^{ABCab}	7.3 ± 0.67^{ABCabc}	7.2 ± 0.78^{Abc}	6.9 ± 0.87^{ABc}
	I-BB	7.4 ± 1.07^{ACa}	7.3 ± 1.15^{Aa}	7 ± 0.81^{ACa}	7 ± 0.81^{Aa}	6.5 ± 1.17^{Aba}
	O-MIX	7.7 ± 0.94^{ABa}	7.7 ± 0.48^{ABCa}	7.1 ± 0.73^{ABCa}	7 ± 0.94^{Aa}	6 ± 1.15^{Ab}
	L-MIX	7.8 ± 0.63^{ABa}	7.6 ± 0.69^{ABCa}	6.9 ± 0.56^{Ab}	6.8 ± 0.63^{Abc}	6.2 ± 1.03^{Ac}
	I-MIX	7.7 ± 0.94^{ABa}	7.3 ± 1.05^{Ab}	7.2 ± 0.91^{ABCa}	6.9 ± 0.73^{Aab}	6.2 ± 1.03^{Ab}
Overall acceptability	Control	8.3 ± 0.5^{Aa}	8 ± 0.66^{ABab}	7.8 ± 0.42^{Abc}	7.4 ± 0.69^{Ac}	6.4 ± 0.69^{ABd}
	LA	8.7 ± 0.44^{Aa}	8.4 ± 0.69^{Aab}	7.9 ± 0.56^{Abc}	7.5 ± 0.52^{Ac}	6.9 ± 0.56^{Ad}
	BB	8.6 ± 0.5^{Aa}	8.3 ± 0.48^{Aab}	7.9 ± 0.56^{Ab}	7.3 ± 0.67^{Ac}	6.5 ± 0.52^{Ad}
	MIX	7 ± 0.70^{CDa}	6.9 ± 0.73^{CDEa}	6.6 ± 0.84^{BCDa}	6.3 ± 0.67^{BCab}	5.8 ± 0.78^{BCb}
	O-LA	7.2 ± 0.44^{BCDa}	7.2 ± 0.63^{CDa}	6.6 ± 0.84^{BCDab}	6.3 ± 0.67^{BCb}	5.3 ± 0.67^{CDc}
	L-LA	7.2 ± 0.66^{BCDa}	7.1 ± 0.56^{CDab}	6.6 ± 0.69^{BCDbc}	6.2 ± 0.63^{BCcd}	5.8 ± 0.63^{BCd}
	I-LA	7.2 ± 0.66^{BCDa}	7.2 ± 0.63^{CDa}	6.7 ± 0.67^{BCDab}	6.2 ± 0.63^{BCb}	5.5 ± 0.52^{Cc}
	O-BB	7.2 ± 0.83^{BCDa}	7.2 ± 0.63^{CDa}	6.9 ± 0.73^{Ba}	6.2 ± 0.63^{BCb}	5.7 ± 0.82^{Cb}
	L-BB	7.2 ± 0.66^{BCDa}	7.3 ± 0.67^{CDa}	6.9 ± 0.73^{Bab}	6.5 ± 0.7^{Bb}	5.7 ± 0.82^{Cc}
	I-BB	7.4 ± 0.72^{BCa}	7.1 ± 0.73^{CDa}	6.8 ± 0.78^{BCa}	6.1 ± 0.99^{BCb}	5.6 ± 0.51^{Cb}
	O-MIX	6.6 ± 0.7^{Da}	6.4 ± 0.51^{Eab}	6 ± 0.66^{Db}	5.3 ± 0.82^{Dc}	4.8 ± 0.91^{Dc}
	L-MIX	7 ± 0.7^{BCDa}	6.8 ± 0.42^{DEa}	6.1 ± 0.73^{CDb}	5.7 ± 0.67^{CDbc}	5.1 ± 0.73^{CDc}
	I-MIX	7.7 ± 0.66^{Ba}	7.5 ± 0.52^{BCab}	7 ± 0.66^{Bb}	6.2 ± 0.63^{BCc}	5.3 ± 0.82^{CDd}

Different superscript uppercase letters and superscript lowercase letters donate significant differences in columns and rows, respectively.

presence of prebiotics in yoghurt have been presented (Mohebbi and Ghoddusi 2010; Aghajani *et al*. 2012; Boeni and Pourahmad 2012; Shaghaghi *et al*. 2013).

The production of high acid level by yoghurt starter bacteria and lack of prebiotics as stimulating growth agents could be the reasons for significant reduction of probiotic bacteria count in control samples in this study (Aghajani *et al*. 2012). The basic low pH and further reduction of pH during post-acidification can cause the low viability of probiotics in yoghurt (Ali *et al*. 2013). Rybka and Kailasapathy stated that *L. bulgaricus* was the main factor responsible for *Bifidobacterium* sp. mortality and pH was significantly reduced when *L. bulgaricus* was excluded from yoghurt manufacturing, for its over acidification during manufacturing and storage (Rybka and Kailasapathy 1995). Besides, Dave and Shah reported that the presence of *L. bulgaricus* had a negative effect on the viability of *L. acidophilus* (Dave and Shah 1997). Many other studies have reported the low viability of probiotics in yoghurt (Gustaw *et al*. 2011; Ali *et al*. 2013).

The standard of probiotic products specifies that the minimum acceptable level of probiotics in dairy products should be 10^7 cfu/g to be able to provide beneficial effects, while daily consumption of these products must be 100 mL or 100 g at least (Boylston *et al*. 2004; Shaghaghi *et al*. 2013). In all the samples of present study, *B. bifidium* and *L. acidophilus* counts were higher than 10^7 cfu/g till the end of storage period. The major differences observed between the probiotics survival were related to species differences and there was little variance between the different commercial strains of the same *Bifidobacterium* or *L. acidophilus* (Ali *et al*. 2013).

The most important factor of yoghurt popularity is its sensory properties (Aghajani *et al*. 2012). Table 8 demonstrates the scores for sensory attributes of yoghurt samples during the storage. Evaluating the taste scores of samples showed that samples containing probiotics were higher in scores when they were prebiotic free. Higher scores were observed for the sample with *B. bifidium*, sample with *L. acidophilus* and control respectively and the lower scores were obtained for the sample with both probiotics and oligofructose. The results also showed that the taste scores of samples with both probiotics were lower than the samples with one probiotic and the samples containing oligofructose were lower in scores than the lactulose- and inulin-containing samples ($P < 0.05$), while the samples containing inulin had higher taste scores than others.

Comparing texture scores of samples revealed that the highest mean was recorded for the sample with *L. acidophilus* while the sample with *B. bifidium*, sample with both probiotics and oligofructose, sample with both probiotics and lactulose and sample with both probiotics and inulin were lower in score than other samples, respectively. As shown, texture scores of the samples with both probiotics were lower than those of the samples with one probiotic.

For aroma scores, higher scores were recorded for the sample with *B. bifidium*, sample with *L. acidophilus* and control, respectively.

Total acceptability scores ranged $4.8 < A < 6.9$ during the storage. The mean scores recorded for the sample with *L. acidophilus* and sample with *B. bifidium* were greater than those recorded for the other samples. Total acceptability data also showed a higher preference for natural yoghurt compared with symbiotic yoghurt. However, the most acceptable samples were those containing probiotics separately. It should be mentioned that the scores of all samples were higher than unacceptable limit.

Acetaldehyde, the main cause of yoghurt flavor, which is majorly made from conversion of threonine to acetaldehyde catalyzed by threonine aldolase *of L. delbrueckii* subsp. *bulgaricus*, is converted to ethanol by probiotics that produce alcohol dehydrogenase. Therefore, probiotic yoghurts do not have the typical yoghurt flavor. The typical yoghurt flavor is familiar and probiotic yoghurt flavor is unfamiliar to the consumers. Hence, this could be the reason for the above results (Ranathunga and Rmusk, 2013).

CONCLUSION

High quality symbiotic yoghurt was made using *L. acidophilus* and *B. bifidium* along with prebiotics including inulin, lactulose and oligofructose. The simultaneous use of *L. acidophilus* and *B. bifidum* and similarly the use of prebiotics in the symbiotic yoghurt containing these probiotics increased the growth and viability of probiotic bacteria. Although, more studies should be carried out to determine organic acid profile of these products due to the determination of associative behavior of the probiotics as well as prebiotics in terms of synergistic and symbiotic effects. During the cold storage, pH of symbiotic yoghurt decreased whereas the syneresis increased. The number of probiotic bacteria in the symbiotic yoghurt was above 7 log cfu/mL. It should be considered, besides their desired health properties, that probiotic bacteria should have several other requirements including their survival and activity in the product and stability during storage, for the development of marketable probiotic products. The results of present study indicated that the buffalo yoghurt may be a good carrier for developing the symbiotic yoghurt. However, natural and probiotic yoghurt were preferred by the sensory panel over symbiotic yoghurt for some sensory aspects. Hence, further research should be carried out to find ways that could improve or mask those sensory properties.

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