

Full Length Research Paper

# Survivability of probiotics in symbiotic low fat buffalo milk yogurt

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In present study, symbiotic low fat buffalo milk yogurt prototypes (plain and blueberry) were developed using a commercial starter containing probiotics. Samples were analyzed for physicochemical and microbiological properties, and the survivability of probiotics during 10 weeks of storage. Gross composition results were: total solids  $11.60 \pm 0.58$  and  $17.12 \pm 0.36\%$ , ash  $0.82 \pm 0.06$  and  $0.78 \pm 0.02\%$ , protein  $4.49 \pm 0.31$  and  $4.16 \pm 0.11\%$ , fat  $0.68 \pm 0.03$  and  $0.55 \pm 0.05\%$ , carbohydrates  $5.68 \pm 0.18$  and  $11.38 \pm 0.18\%$  for plain and blueberry flavored samples, respectively. Mineral contents (mg/g) were: Calcium  $1.97 \pm 0.20$  and  $1.72 \pm 0.06$ , magnesium  $1.63 \pm 0.02$  and  $1.69 \pm 0.01$ , zinc  $0.07 \pm 0.01$  and  $0.07 \pm 0.00$ , sodium  $0.87 \pm 0.15$  and  $0.94 \pm 0.12$  for the plain and blueberry flavored yogurt respectively. The values of pH, titratable acidity and viscosity ranged from 4.34 to 4.01 and 4.42 to 3.70, 0.96 to 1.13% and 0.94 to 1.30%, 1.40 to 1.67 and 2.15 to 1.56 Pa.s for the plain and blueberry flavored yogurts respectively. The initial population of *Bifidobacterium* spp. and *Lactobacillus casei* were above 10<sup>8</sup> CFU/g for both the plain and blueberry flavored yogurts. *Lactobacillus acidophilus* was viable only for the first two weeks; however, *Bifidobacterium* spp and *L. casei* remained viable (>10<sup>6</sup> CFU/g) during the storage. The results indicate that the low fat buffalo milk yogurt is a good vehicle for developing symbiotic yogurt.

**Key words:** Buffalo milk, symbiotic yogurt, refrigerated storage, probiotic survivability, physicochemical properties.

## INTRODUCTION

Buffalo milk represents an important animal product in South and East Asia. Buffalo milk differs from bovine milk in a number of ways. Compared with cow milk, buffalo milk has a higher content of fat ( $7.59 \pm 1.31\%$ ), crude protein ( $4.86 \pm 0.44\%$ ), lactose ( $4.74 \pm 0.20\%$ ) and total solids ( $18.44 \pm 1.56\%$ ) (Fundora et al., 2001; Lindmark-Månsson et al., 2003). These properties give the buffalo milk a rich flavour and taste, and make it highly suitable for the manufacture of a wide variety of milk products, such as cheese, butter, ice cream, and yogurt (Han et al., 2007). Buffalo milk products have become popular and

have their own niche in the US market, owing to an attractive nutrient profile (Amarjit and Toshihiko, 2003).

Yogurt products have been supplemented with probiotic bacteria such as *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacteria* to enhance their therapeutic value and to enhance their marketability as a functional food (Samona et al., 1996; Shah, 2007). Therefore, incorporation of probiotic bacteria in various dairy products has become an increasing trend (Özlem and Nursel, 2007; Kailasapathy et al., 2008). Today yogurt has moved from being a "health food" to being a mainstream "healthy food" that people of all ages enjoy. It has been suggested that minimum level for probiotic bacteria in yogurt is 10<sup>6</sup> viable cells per mL or g of product, in order to produce the therapeutic benefits (IDF, 1992; Dave and Shah, 1997; Bibiloni et al., 2001;

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Martin et al., 2007). Some researchers stipulate that the viable count of probiotic bacteria should be above  $10^7$  or  $10^8$  CFU/mL as satisfactory levels (Lourens-Hattingh and Viljeon, 2001) however, studies have shown that most probiotic foods have a low population of probiotics and that these organisms are not able to survive during the storage period of yogurts (Shah, 2007). Many factors may affect the viability of probiotic bacteria in yogurt: acidity, pH, hydrogen peroxide, oxygen content, concentration of organic acid, milk composition and temperature of storage, etc. during manufacture and storage of yogurt (Laroia and Martin, 1991; Dave and Shah, 1998; Kailasapathy et al., 2008). Therefore, it is important to ensure adequate and viable probiotic bacteria throughout the shelf-life of yogurts.

However, information about the physicochemical properties and the survivability of probiotics in low-fat buffalo milk yogurt during storage is limited compared to cow's yogurt. In the present study, two prototypes of low fat buffalo milk yogurt were developed. Physicochemical and microbiological properties and survivability of probiotics of two low fat buffalo milk yogurt products were evaluated during 10 week storage.

## MATERIALS AND METHODS

### Yogurt samples preparation

Blueberry and plain flavor low fat buffalo milk set-style yogurts were manufactured and yielded by Woodstock buffalo milk yogurt company (now Bufala di Vermont) (Woodstock, Vt., U.S.A.) with the inulin and pectin additives. The commercial starter culture was used including *L. bulgaricus*, *S. thermophilus*, *L. acidophilus*, *Bifidobacterium* and *L. casei*. For each flavor, three productions were carried out. Samples from the batch fermentations were taken to the laboratory and stored at 4°C for up to 10 weeks. Cell counts and pH measurements were performed weekly.

### Analysis of total solids, fat, protein, ash, carbohydrate, pH and titratable acidity

Total solids (TS) of the yogurt samples were measured by the forced-draft oven at 105°C until a steady weight was achieved, while ash content was measured gravimetrically (Wehr and Frank, 2004). Fat content was determined by the Babcock method according to procedures of Wehr and Frank (2004). Crude protein in yogurt was determined in the yogurt using the Kjeldahl method (Delwiche, 2002). Carbohydrate content was determined by the difference of TS minus other solid components. The pH values of the yogurt samples were measured using pH meter (model IQ 240, IQ Scientific Instruments, Inc, San Diego, Calif., U.S.A.). Lactic acid, reported as titratable acidity (TA), was estimated by titrating a 9 g sample, diluted with 18 mL water with 0.1 N sodium hydroxide using phenolphthalein as an indicator.

### Mineral analysis

For determination of mineral concentrations, yogurt samples (10 g) were dry-ashed in porcelain crucibles at 550°C for 6 h,

solubilized with 10 ml of 6 M HCl, quantitatively transferred into 25 ml volumetric flasks, and diluted to volume with double-deionized water. Calcium (Ca), zinc (Zn), sodium (Na) and magnesium (Mg) contents were determined by inductively coupled plasma atomic emission spectroscopy (ICPAES, Leeman Labs Plasma Spec Z.5, Lowell, Mass., U.S.A.) (Guler, 2007).

### Viscosity measurement

Viscosity measurements were carried out at room temperature ( $22 \pm 2^\circ\text{C}$ ) using Brookfield Programmable DV-II+ Viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, Mass., U.S.A.) equipped with a No. 4 spindle at 20 rpm.

### Shelf-life tests

The counts of probiotic bacteria (*L. acidophilus*, *Bifidobacterium* and *L. casei*) were evaluated each week over 10 week storage at 4°C. The values of pH, titratable acid, viscosity and yeast and mould counts were also determined each week.

*L. acidophilus* was counted with de Man, Rogosa and Sharpe (MRS)-IM agar with maltose using spread plate method, followed by 72 h incubation at 37°C under aerobic conditions. *Bifidobacterium* were enumerated using the pour plate method with MRS-IM agar and glucose containing 0.05% dichloroxacillin, 0.1% lithium chloride and 0.05% cysteine hydrochloride, followed by 72 h incubation at 37°C under anaerobic conditions. *L. casei* was determined with MRS-IM agar and glucose using the spread plate method, followed by six days incubation at 20°C under aerobic conditions (Farnsworth et al., 2006). The counts of probiotics, performed in triplicate, were calculated from the colonies on agar plates and thus expressed as colony forming units per gram (CFU/g).

### Statistical analysis

Statistical analyses were conducted using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) with Duncan's post-test were used. A probability level of  $P < 0.05$  was used throughout this study. Data were expressed as mean values  $\pm$  standard deviations. Each flavor had three trials and each trial of samples was performed in triplicate.

## RESULTS AND DISCUSSION

### Gross composition

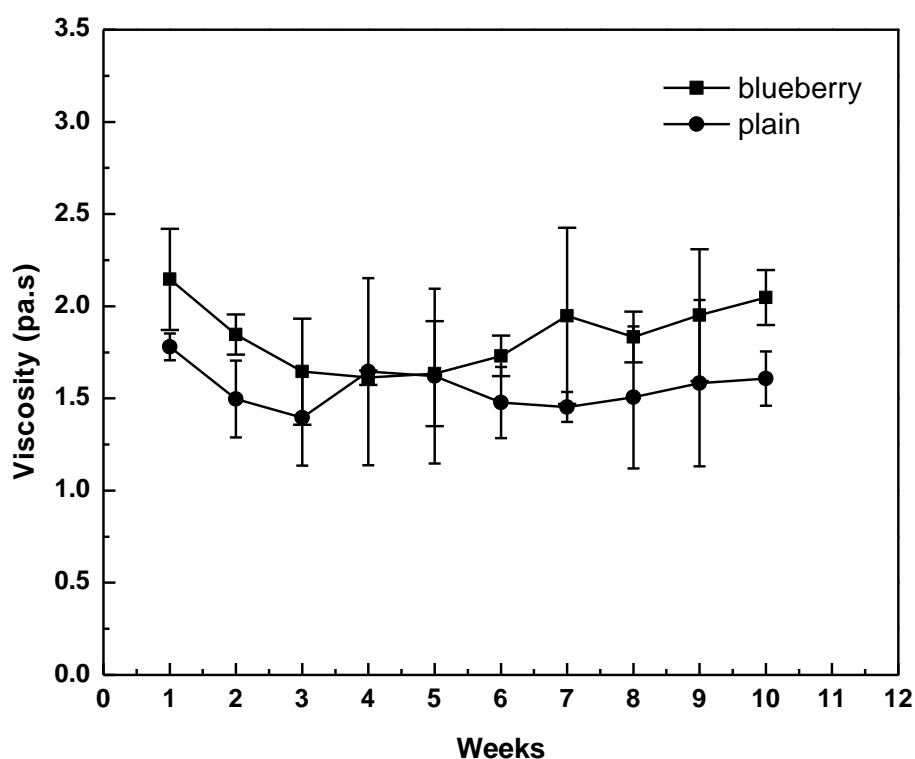
Gross composition and mineral contents of the buffalo milk yogurt are listed in Tables 1 and 2. Buffalo milk yogurt showed higher contents of protein, TS, carbohydrate and ash than those reported for cow milk (Fundora et al., 2001; Lindmark-Månsson et al., 2003), indicating higher nutrient density in buffalo milk yogurt. The average protein ( $4.49 \pm 0.31$  and  $4.16 \pm 0.11\%$ ) and ash ( $0.82 \pm 0.06$  and  $0.78 \pm 0.02\%$ ) for plain and blueberry flavor yogurt were within the normal range for buffalo milk composition (Han et al., 2007). The average fat were matched to the low fat yogurt (fat content below the 2%), that were  $0.68 \pm 0.03\%$  and  $0.55 \pm 0.05\%$  (w/w) for plain and blueberry yogurt, respectively. The TS of plain flavor buffalo milk yogurt was  $11.60 \pm 0.58\%$ , which

**Table 1.** Gross composition of low fat buffalo milk yogurt ( $X \pm SD$ ,  $n = 9$ )

Flavor	Protein (%)	Fat (%)	Total solid (%)	Ash (%)	Carbohydrate (%)
Plain	4.49 $\pm$ 0.31	0.68 $\pm$ 0.03	11.60 $\pm$ 0.58	0.82 $\pm$ 0.06	5.68 $\pm$ 0.18
Blueberry	4.16 $\pm$ 0.11	0.55 $\pm$ 0.05	17.12 $\pm$ 0.36	0.78 $\pm$ 0.02	11.38 $\pm$ 0.18

**Table 2.** Mineral contents (mg/g) of low fat buffalo milk yogurt ( $X \pm SD$ ,  $n=9$ )

Flavor	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Zn <sup>2+</sup>	Na <sup>+</sup>
Plain	1.97 $\pm$ 0.20	1.63 $\pm$ 0.02	0.07 $\pm$ 0.01	0.87 $\pm$ 0.15
Blueberry	1.72 $\pm$ 0.06	1.69 $\pm$ 0.01	0.07 $\pm$ 0.00	0.94 $\pm$ 0.12

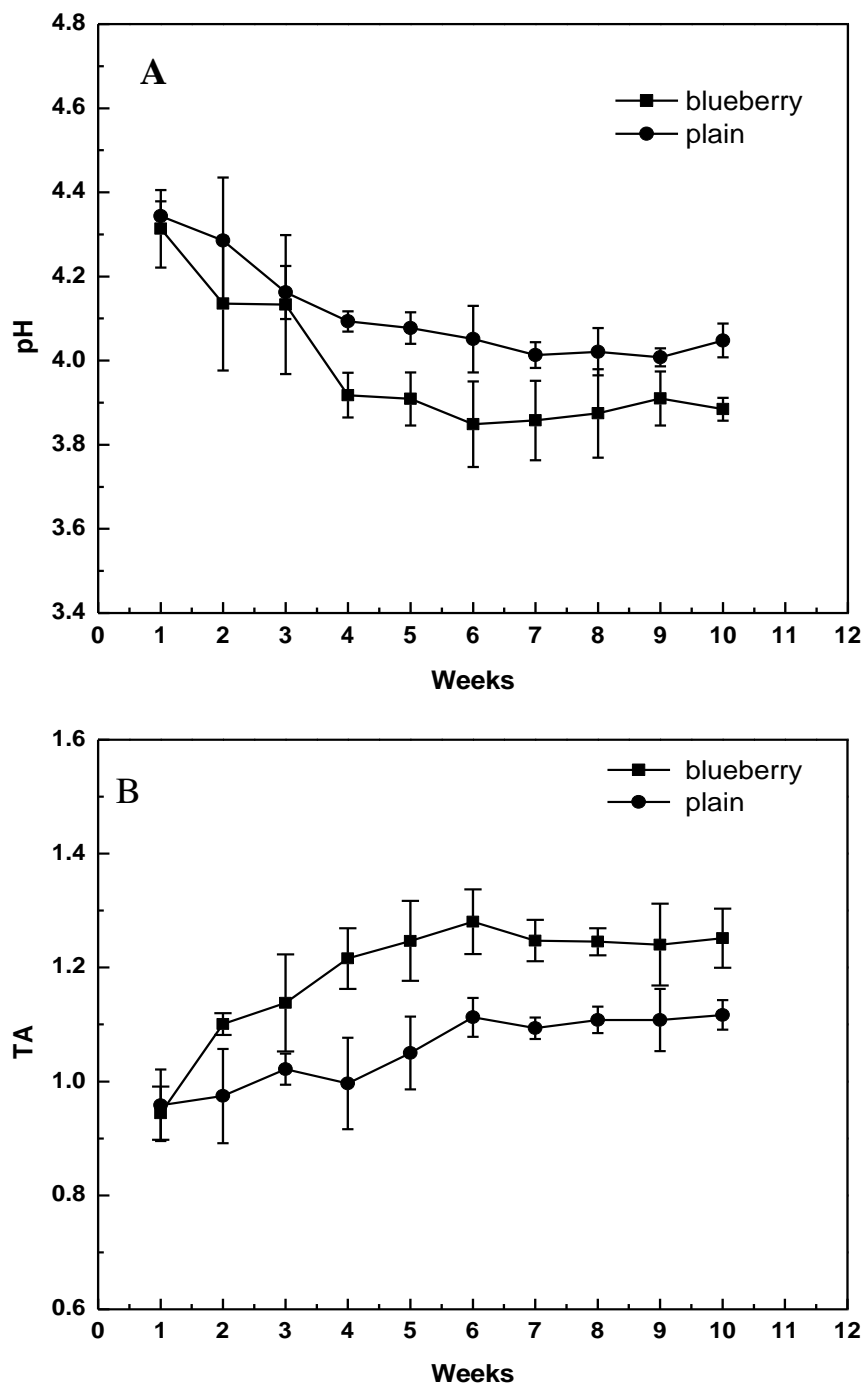
**Figure 1.** Changes in viscosity of blueberry flavoured (■) and plain (●) low fat buffalo milk yogurt during storage.

was lower than that of buffalo raw milk (18.44%), because most fat in buffalo milk was removed (the average fat content of buffalo milk was 7.59% in China) (Han et al., 2007). However the TS in blueberry yogurt (17.12%  $\pm$  0.36%) were higher than that in plain yogurt (11.60%  $\pm$  0.58%). The higher TS in blueberry yogurt may be because of the added blueberry solids. In terms of important minerals calcium was 1.97  $\pm$  0.20 and 1.72  $\pm$  0.06 mg/g for plain and blueberry yogurt, respectively. It was superior to cow milk (1.22 mg/g) (Yun, 2006; Park et al., 2007) and goat milk (1.34 mg/g) (Park et al., 2007).

Gross composition of buffalo milk yogurt varies depending on the type of raw materials used, type of yogurt manufactured and fortification methods, etc.

### Viscosity changes during storage

Figure 1 represents the changes in viscosity during 10 week storage. The yogurt had a viscosity between 1.78 and 1.40 Pa.s for plain flavor and 2.15 and 1.61 Pa.s for blueberry flavor. Analysis of variance showed that there



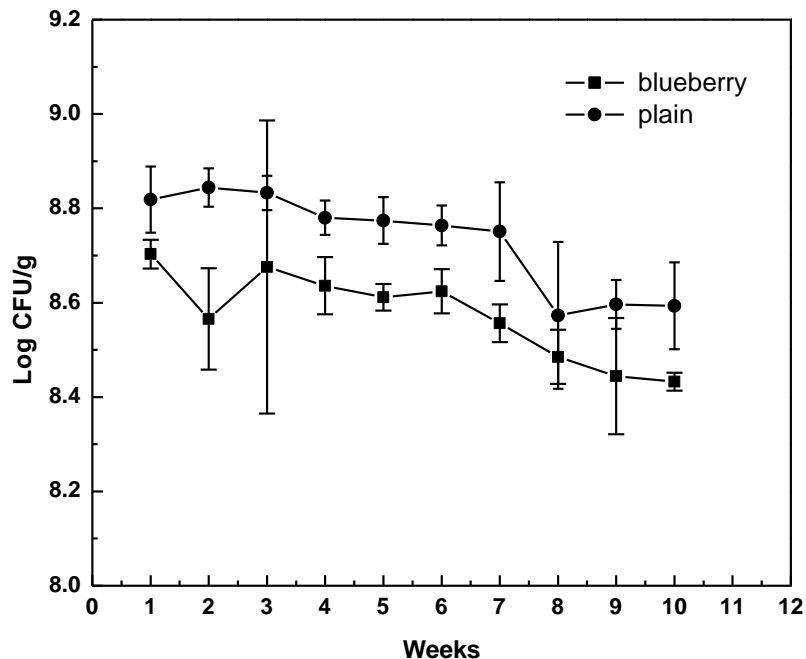
**Figure 2.** Changes in pH (A) and titratable acidity (B) of low fat buffalo milk yogurt during storage.

was no significant change in viscosity during storage for 10 weeks. However the viscosity of blueberry flavor yogurt was higher than that of the plain flavor because the blueberry flavor yogurt has higher levels of carbohydrate and total solids (Table 1). This may have improved the yogurt gel stability. Similar results have also been reported by Farnsworth et al. (2006) showing that

increasing total solids of the goat milk can improve yogurt viscosity.

#### Changes in pH and titratable acidity during storage

Figure 2 represents the changes in pH and titratable



**Figure 3.** Survivability of *Bifidobacterium* in low fat buffalo milk yogurt during storage.

acidity during storage. The initial (1st week) pH was 4.34 and 4.31, and the final (10th week) pH was 4.05 and 3.89 for plain and blueberry yogurt, respectively. Analysis of variance showed that there was a significant difference ( $P < 0.05$ ) between the pH at week 1 and week 10 across the two types of stored yogurts. The pH of the two types of yogurt significantly decreased from one to six week's storage and then stabilized with no further significant change up to 10 weeks of storage. Shah (2000) observed similar decreases in pH values during storage of commercial yogurts containing *L. acidophilus* and *B. bifidum*. The decline in pH was presumably due to continued fermentation by the lactic acid bacteria during storage (Shah, 2000; Kailasapathy, 2006).

The initial pH of the plain and blueberry yogurt was approximately the same (pH about 4.3), however, the blueberry yogurt showed the lower pH at the end of 10 week shelf-life (Figure 2). It is possible that addition of the blueberry into yogurt may have decreased pH during storage (Kailasapathy et al., 2008).

There was a similar change in trend in pH and titratable acidity during storage. The titratable acidity increased with storage time, and it peaked at six weeks storage, the average values were 1.11 and 1.28% for plain and blueberry flavor, respectively, but there were no significant differences in TA between 6 and 10 weeks.

### Survivability of probiotic bacteria during storage

Figures 3 to 5 show the survivability of *Bifidobacterium*,

*L. casei* and *L. acidophilus*, respectively for 10 weeks in refrigerated condition. Of the three probiotic bacteria, *Bifidobacteria* and *L. casei* survived very well throughout the storage. The population of *Bifidobacterium* ranged from  $6.58 \times 10^8$  to  $2.78 \times 10^8$  CFU/g and  $5.05 \times 10^8$  to  $2.52 \times 10^8$  CFU/g for plain and blueberry flavor yogurt respectively over 10 weeks storage. The population of *L. casei* ranged from  $3.49 \times 10^8$  to  $6.90 \times 10^7$  CFU/g and  $3.39 \times 10^8$  to  $1.42 \times 10^7$  CFU/g for plain and blueberry flavor yogurt respectively during the 10 week storage. Though, the population of *L. acidophilus* was  $1.44 \times 10^6$  and  $5.01 \times 10^6$  CFU/g for plain and blueberry flavor yogurt respectively at the beginning of storage (1<sup>st</sup> week). At the 3rd week, it was only  $1.00 \times 10^5$  and  $1.70 \times 10^5$  CFU/g for plain and blueberry yogurt respectively, and became too low to count by week sixth. Similar results were found by Dave and Shah (1997), they reported that the survival of *L. acidophilus* in yogurts after 35 days of storage was only approximately 0.1 to 5% compared to after five days of storage. Shah et al. (1995) found different brands of yogurt contained different quantities of viable cells of *L. acidophilus* and Olson and Aryana (2008) also reported that there is a wide range of survival of *L. acidophilus* in yogurt.

Analysis of variance for the probiotic counts showed that there was no significant change in *Bifidobacterium* and *L. casei* populations during storage, whereas, with *L. acidophilus*, there was significant change after the 3rd week (Figure 5). Among the 3 cultures, *L. acidophilus* differed significantly in survivability from the other 2 probiotics. The results of the present study indicated that

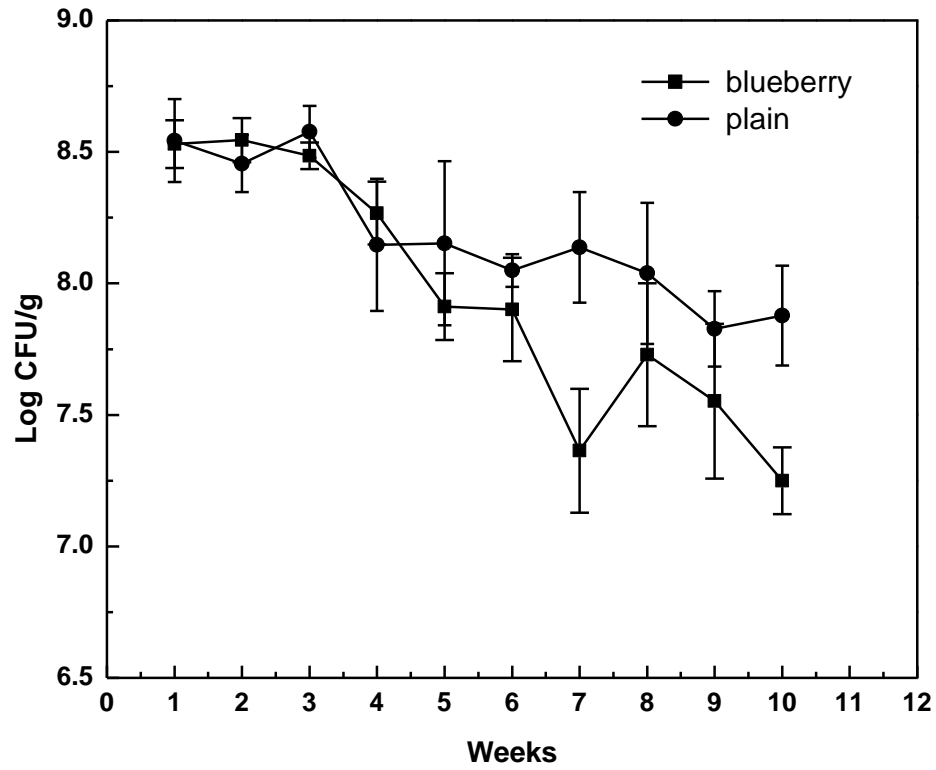


Figure 4. Survivability of *Lactobacillus casei* in low fat buffalo milk yogurt during storage.

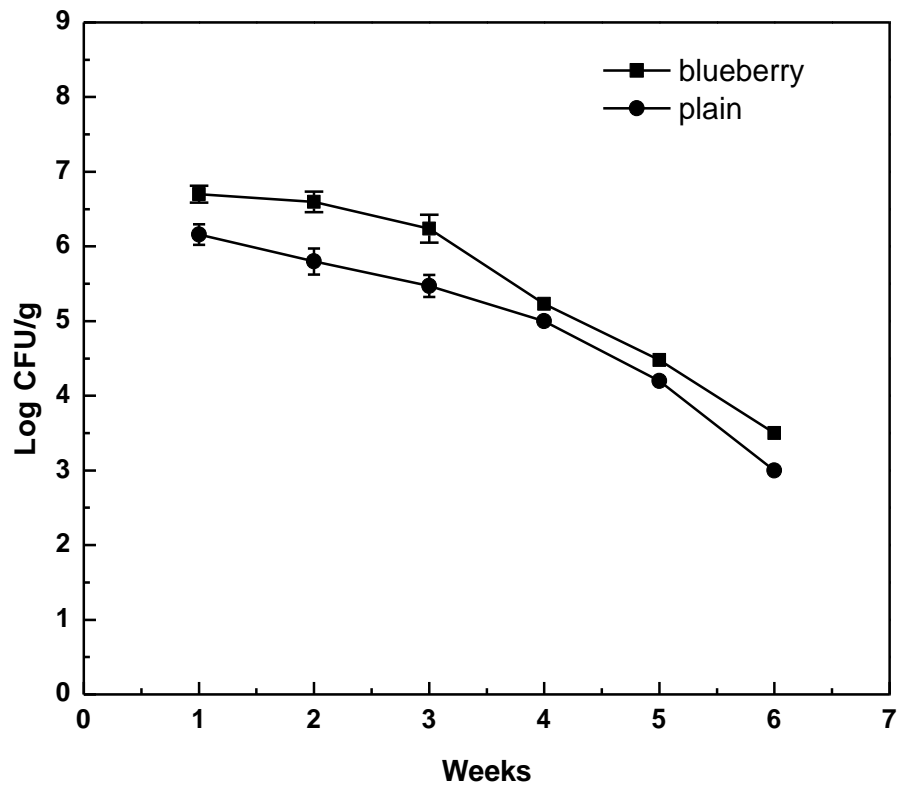


Figure 5. Survivability of *L. acidophilus* in low fat buffalo milk yogurt during storage.

**Table 3.** Counts of mold and yeast in low fat buffalo milk yogurt during storage

Week	Plain (CFU/ml)		Blueberry (CFU/ml)	
	Yeast	Mold	Yeast	Mold
1	None	None	None	None
2	None	None	None	None
3	None	None	None	None
4	None	None	None	None
5	None	None	None	None
6	None	None	None	None
7	7	None	None	None
8	13	1	1	1
9	19	3	3	10
10	2	8	8	1

the low fat buffalo milk yogurt may be a good carrier for developing probiotic yogurt containing *Bifidobacterium* and *L. casei*.

In addition, the survivability of *Bifidobacterium* and *L. casei* in the plain was higher than in the blueberry yogurt during storage. The survivability of *L. acidophilus* in the plain flavor, however, was lower than that in the blueberry yogurt. Similar results were also reported by Kailasapathy et al. (2008) for cow milk yogurts, indicating blueberry juice had a negative impact on the viability of some probiotics. However Apostolidis et al. (2006) indicated that blueberry yogurt has the highest phenolic content relevant to Type 2 diabetes compared with the plain, strawberry and peach flavor yogurt. Furthermore blueberry can add the good flavor to the yogurt.

### Counts of mould and yeast

The population of mold and yeast in yogurt has a substantial bearing on the safety and shelf-life of the product. The counts of mold and yeast in the low fat buffalo milk yogurt during refrigerated storage are shown in Table 3. After eight week's storage the yeast was detected and with prolonged storage time the mold was also found. After 10 week's storage the mold in plain yogurt reached 8 CFU/g. So the shelf-life of low fat buffalo milk yogurt should be less than eight weeks.

### Conclusion

The gross composition of the low fat buffalo milk yogurts indicates that they are a rich source of nutrients and meet the public need for nutrition. The survivability of probiotics indicate that *Bifidobacterium* and *L. casei* survived in good numbers ( $10^7$  to  $10^8$  CFU/g) for both plain and blueberry yogurt throughout the storage period in the refrigeration condition. However, *L. acidophilus* only survived at sufficient levels for about two weeks. Mold

and yeast can be found after eight week's storage indicating that the low fat buffalo milk yogurt was shelf-life stable for up to eight week refrigerated storage. Further study should be focused on how to improve survivability of *L. acidophilus* in the low fat buffalo milk yogurt.

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