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Effects of Different Contents of Yogurt Starter/Probiotic Bacteria, Storage Time and Different Concentration of Cysteine on the Microflora Characteristics of Bio-Yogurt

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Abstract: The objectives of this study, were to investigate the effects of different content of yogurt starter/probiotic bacteria, storage time and different concentration of cysteine on the microflora characteristics of bio-yogurt. The number of *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* were found to be higher in the samples with higher levels of starter. The numbers of *L. acidophilus* and *B. bifidum* were found to be higher in the samples with higher levels of added probiotic bacteria. The counts of *S. thermophilus* increased slowly during storage up to day 7 and decreased later *L. delbrueckii* subsp. *Bulgaricus*, *B. bifidum* and *L. acidophilus* counts, decreased during the storage period. Increasing of cysteine improved the viability of *B. bifidum* and *L. delbrueckii* subsp. *Bulgaricus* and it had no important effect on the viability of *L. acidophilus* and made the environment unfavourable for the growth of *S. thermophilus*.

Key words: Bio yogurt, cysteine, probiotic, *S. thermophilus*, *L. acidophilus*, *B. bifidum*

INTRODUCTION

Products of milk, especially cheeses and yogurt are very popular in Iran. Yogurt is produced by adding 2 starter cultures, *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus* to milk (Tamime and Marshall, 1997). Irrespective of what type of milk isused, the technology of yoghurt-making is standard and includes the following processing stages: Standardization of the fats content and fortification of the Solids-Not-Fat level (SNF), the latter process, is achieved by the addition of dairy powders and/or concentration of the milk using vacuum-evaporation or membrane concentration, such as Ultrafiltration (UF) or Reverse Osmosis (RO), homogenization, followed by heat-treatment, partial cooling and fermentation of the milk base by a thermophilic starter culture and at the desired pH value, the fermentate is cooled and finally stored at <5°C (Alichanidis and Polychroniadou, 1995; Kurmann, 1986; Tamime and Robinson, 1999). During the fermentation, hydrolysis of the milk proteins occurs, the pH drops, the viscosity increases and bacterial metabolites are produced that contribute to the taste and possibly to the health promoting properties of yogurt. Several health benefits have been reported for traditional yogurt (Boudraa *et al*., 1990; Marteau *et al*., 1990; Bakalinsky *et al*., 1996; Rachid *et al*., 2002) and this healthy image is enhanced by supplementation with probiotic bacteria. Probiotic bacteria are defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host (FAO, 2001). Fermented foods that have potential probiotic properties are produced world wide from a variety of food substrates (Farnworth, 2005). Probiotics have been used for the treatment of various types of diarrhoea (Sarker *et al*., 2005; Szymanski *et al*., 2006), urogenital infections (Reid *et al*., 2003) and gastrointestinal diseases such as Crohn's disease and pouchitis (Kuehbacher *et al*., 2006) although, there is still no consensus about their effectiveness (Lin, 2003; Reid and Hammond, 2005; Senok *et al*., 2005). Lactic acid bacteria including lactobacilli and bifidobacteria are the most common bacterial species considered as potential probiotics (Sanders, 1997). Yogurt produced from cows' milk is consumed in both developing and industrialized countries. Probiotic bacteria generally do not grow rapidly in cows'milk. Thus, in yoghurt manufacture, they do not attain as high numbers as the starter cultures (Champagne *et al*., 2005). Probiotic bacteria grow slowly in milk, due to lack of proteolytic activity. Dave and Shah (1997a, 1998) and Klaver *et al*. (1993) have shown that milk supplemented with peptides and amino acids, such as cysteine, improved the survival of bifidobacteria. Cysteine is a sulphur-containing amino acid that is incorporated into agar media for the growth of bifidobacteria To produce therapeutic benefits, a sufficient number of

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viable microorganisms must be present throughout the entire shelf life of the product. In this regard, minimum levels for probiotic bacteria in fermented milks ranging from 105-106 CFU mL^{-1} (Samona and Robinson, 1994) have been suggested. Schuller-Malyoth *et al.* (1968) considered that a good probiotic culture should contain between 106 and 108 viable cells per millilitre. However, these organisms often show poor viability in market preparations (Dave and Shah, 1997a; Klaver *et al.*, 1993; Ravula and Shah, 1998). Several factors have been involved in affecting the viability of probiotic cultures in fermented milks such as pH, acidity, the presence of other microorganisms, temperature, oxygen content and others (Shah, 2000).

The aim of this study, was to determine the effects of different storage time, different ratios of yogurt starter and probiotic and addition of different concentration of cysteine on the viable bacteria counts in bio-yogurt produced from cow's milk.

MATERIALS AND METHODS

Materials: Fresh raw milk obtained from Animal Husbandry of Urmia University, Iran, in December 2007, was used in manufacturing of yogurt. The milks were inoculated with mixed yogurt culture consisting of Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *Bulgaricus* and the probiotic strains of *Lactobacillus acidophilus* PTCC 1643 and *Bifidobacterium bifidum* PTCC 1644.... were obtained from the Persian Type Culture Collection.

Production of yogurt and bio-yogurt: Different trials were performed for the manufacture of yogurt and bio-yogurt. Diffrent mixtures of yogurt starter/probiotic strains, three storage time (1, 8, 15 days) and different concentration of cystein(0.25, 0.5, 0.75%) were used in manufacturing of samples.

Whole cow's milk was fortified with 2% skim milk powder., then was heat-treated at 90°C for 10 min, cooled to 45°C.

The samples were inoculated with yogurt culture (at rates of 1, 2, 3% w/v) and *Lactobacillus acidophilus* PTCC 1643 and *Bifidobacterium bifidum* PTCC 1644 at rates of (1, 2, 3% v/v). A solution of L-cysteine HCl (5%) (Sigma) was added to batches at rates of 0.25, 0.5 and 0.75% w/v and dispensed into plastic cups (100 mL). Batches were incubated at 42°C until reaching pH 4.6. Then they were immediately transferred to a cold store (4±1°C) and stored for 1, 8, 15 days.

Analytical methods: Bio-yogurt samples (10 g) were decimally diluted in 100 mL sterile peptone water (0.1%) and 1 mL aliquot dilutions were poured onto plates of the various selective and differential agars in triplicate. M17 agar was used for the enumeration of *S. thermophilus L. delbrueckii* subsp. *Bulgaricus*, *L. acidophilus* and *B. bifidum* were incubated by using MRS, MRS with sorbitol agar and MRS-NNLP, respectively. All plates were incubated at 37°C for 72 h. M17 was incubated aerobically, whereas all other media plates were incubated anaerobically. Anaerobic conditions were created using Anaerocult A sachets (Merck). Plates containing 20-200 colonies were counted and the results expressed as cfu g^{-1} of sample. Sensory evaluation was carried out with a trained panel of 10 judges. Attributes evaluated were flavour of each sample. Evaluations were made using a 150 mm line scale anchored by the appropriate references.

Experimental design and statistical analysis: A D-Optimal response surface design was used to explore the effect of four studied factors on microbiological properties of bio-yoghurt. Following quadratic polynomial was fitted to experimental data applying regression analysis methods.

$$\hat{y}=\beta_0+\sum_{i=1}^{n}\beta_i x_i+\sum_{i=1}^{n}\beta_{ii} x_i^2+\sum_{i=1}^{n}\sum_{j>1}^{n}\beta_{ij} x_i x_j$$

Where:

$\hat{y}$ = Predicted response
β_0 = A constant
β_i = Linear coefficient
β_{ii} = Quadratic coefficient
β_{ij} = Interaction coefficient

Statistica version 6.0 (Statsoft, Tulsa, Okla., U.S.A.) was used to statistical analysis of data and creation of plots.

RESULTS AND DISCUSSION

Bacterial counts

Starter bacterial count: As shown on Fig. 1, the number of *S. thermophilus* were found to be higher in the samples with higher levels of starter. The counts of *S. thermophilus* increased slowly during storage up to day 7 and decreased later by about 1 log cycle (Fig. 1). Similar results were reported by Birollo *et al.* (2000) and Dave and Shah (1997a).

Addition of cysteine made the environment unfavourable for the growth of *S. thermophilus* (Fig. 2).

L. delbrueckii subsp. *Bulgaricus* counts in the samples with higher levels of starter were slightly higher than in others. Increasing in probiotic content led to a

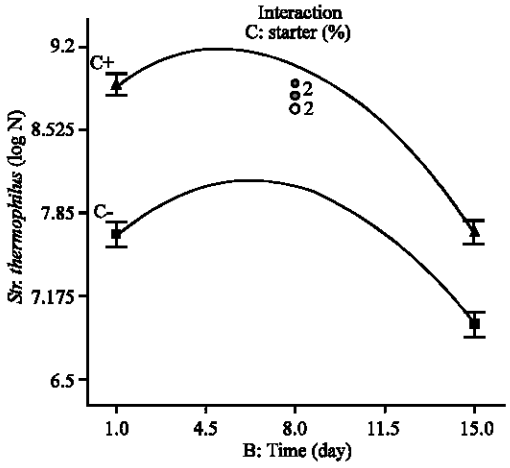

Fig. 1: The effect of storage time starter contents on *S. thermophilus* count

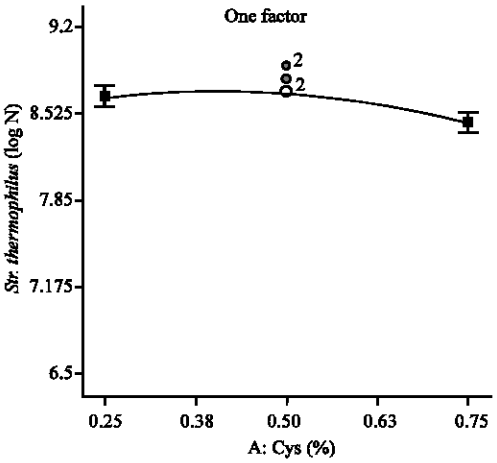

Fig. 2: The effect of cystein concentration on *S. thermophilus* count

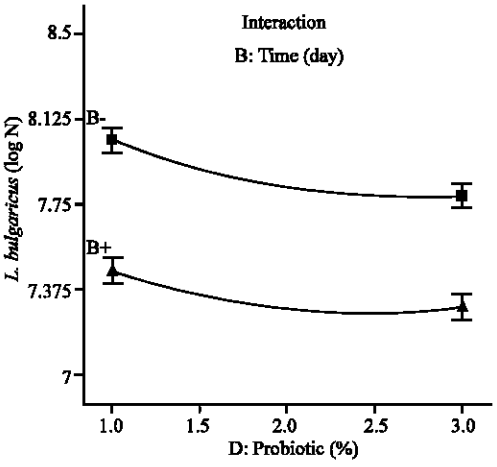

Fig. 3: The effect of probiotic content and storage time on *L. delbrueckii* subsp. *Bulgaricus* count

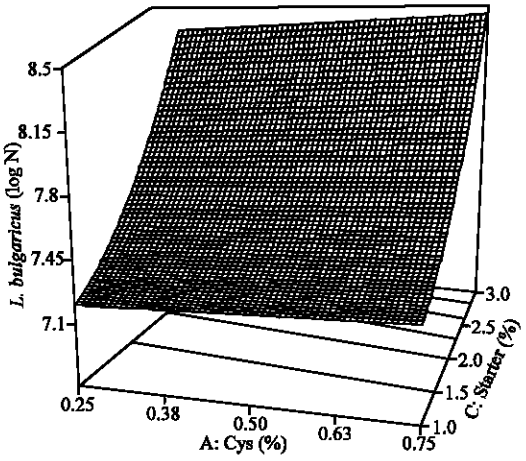

Fig. 4: The effect of starter and cystein percents on *L. delbrueckii* subsp. *Bulgaricus* count

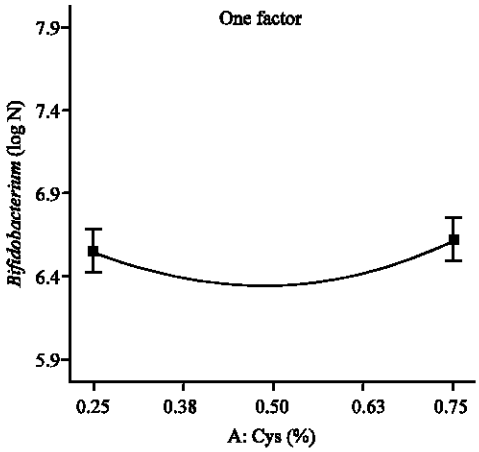

Fig. 5: The effect of cystein concentration on *B. bifidum* count

significant decrease in *L. delbrueckii* subsp. *Bulgaricus* counts at the both first and last days of storage (1 and 15). This could be attributed to the mechanism of nutritional competition and restrictions in the growth of *L. delbrueckii* subsp. *Bulgaricus*, due to the presence of probiotic bacteria (Fig. 3). *L. delbrueckii* subsp. *Bulgaricus* counts in the samples with higher concentration of cysteine were higher than other sampels (Fig. 4).

Probiotic bacterial count: Addition of cysteine improved the viability of *B. bifidum* (Fig. 5). Bifidobacteria are reported to be weakly proteolytic, thus requiring growth factors. Cysteine is a redox-potential reducing agent and is an essential amino acid required for the growth of bifidobacteria (Ravula and Shah, 1998). Thus, improved

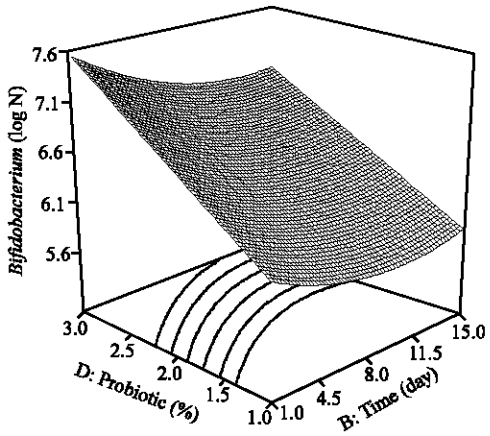

Fig. 6: The effect of storage time and probiotic content on *B. bifidum* count

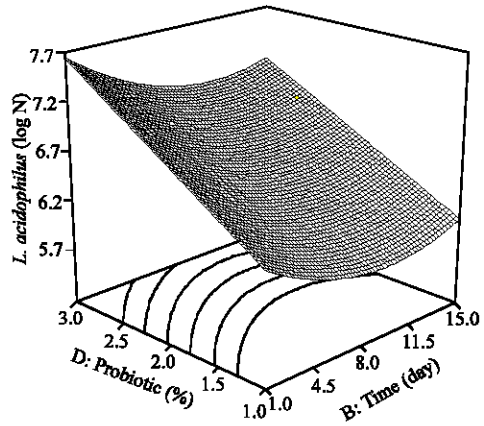

Fig. 7: The effect of storage time and probiotic content on *L. acidophilus* count

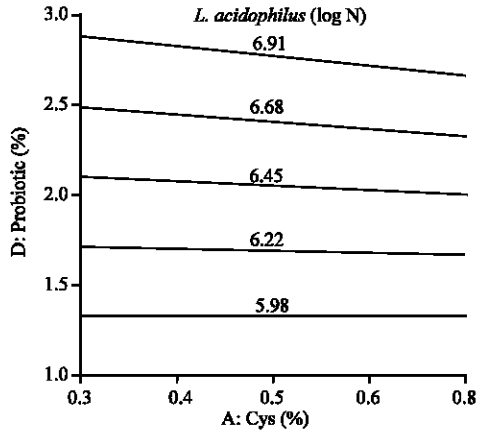

Fig. 8: The effect of cystein and probiotic contents on *L. acidophilus* count

survival of probiotic bacteria, in particular bifidobacteria. *B. bifidum* counts, decreased during the storage period (Fig. 6). This could be attributed to antagonistic relationships between yogurt bacteria and probiotic strains.

The counts of *L. acidophilus* decreased during storage (Fig. 7). The most important factors affecting the viability of *L. acidophilus* are acidity and hydrogen peroxide (Dave and Shah, 1997b). Acidity of the samples increased during the storage period). Addition of cysteine had no important effect on the viability of *L. acidophilus* (Fig. 8).

CONCLUSION

The number of *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* were found to be higher in the samples with higher levels of starter. The number of *L. acidophilus* and *B. bifidum* were found to be higher in the samples with higher levels of probiotic bacteria. The counts of *S. thermophilus* increased slowly during storage up to days 7 and decreased later *L. delbrueckii* subsp. *Bulgaricus*, *B. bifidum* and *L. acidophilus* counts, decreased during the storage period. Increasing of cysteine improved the viability of *B. bifidum* and *L. delbrueckii* subsp. *Bulgaricus* and it had no important effect on the viability of *L. acidophilus* and made the environment unfavourable for the growth of *S. thermophilus*.

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