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Evaluation of Probiotic Yoghurt Produced by *Lactobacillus paracasei ssp. tolerans*

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ABSTRACT: The objectives of this paper were production of probiotic yoghurt using *L. paracasei ssp. tolerans*, and evaluation of pH changes, viable counts, and organoleptic properties of the produced yoghurt. It was observed that the pH decrease during the fermentation period was faster in the milk inoculated with *L. paracasei ssp. tolerans* plus starter culture. There was a gradual decrease in pH in both of the fermented milks during the first 120 min, followed by a sharp drop between 180-210 min, and a steady reduction later until the desired pH was achieved. The viable count of the probiotic bacterium (*L. paracasei*) decreased almost linearly during the storage time, but remained higher than the standard limit for probiotic products. Incorporation of *L. paracasei ssp. tolerans* in yoghurt neither affected the viability of the starter cultures during 21 days of cold storage, nor the organoleptic properties of the yoghurt. There was no significant difference between organoleptic properties of the control and the probiotic yoghurts.

Keywords: *Lactobacillus paracasei ssp. Tolerans, Organoleptic Properties, Probiotic, Viability, Yoghurt.*

Introduction

Nowadays, manufacture of dairy products containing probiotics and prebiotics is an important issue of industrial and commercial significance (Champagne & Gardner, 2005). Probiotic fermented milk products such as yoghurt, kefir, buttermilk, and acidophilus milk are considered as important functional foods. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (WHO/FAO, 2001). They can be used in management, prevention and treatment of some important diseases (*e.g.,* intestinal- and immune-associated diseases). *Lactobacillus* and *Bifidobacterium* are the most important probiotic microorganisms used in yoghurt and other fermented milk products because of some potential characteristics such as their good tolerance toward harmful environmental factors (*e.g.,* low pH, hydrogen peroxide, and molecular oxygen) and beneficial effects on human health (Mortazavian & Sohrabvandi, 2006; Lee & Wong, 1998; Oliveira *et al*., 2009; Østlie *et al*., 2005). Viable lactobacilli and bifidobacteria are available commercially both in the form of milk-based products (*e.g.,* yoghurt, fermented milk drinks, and non-fermented acidophilus milk) and freeze-dried products (Korbekandi *et al*., 2011).

Technological aspects related to microbial systems and functional foods are composition and processing of raw materials, viability and productivity of the applied starter cultures, and technological and storage conditions of the final foods. Several parameters can control safety

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aspects, sensory properties, organoleptic characteristics, and stability of fermented milk products (Korbekandi *et al*., 2011; O'grady & Gibson, 2005; Shah, 2001). The viability of probiotic microorganisms in yoghurts is an important parameter. In addition to the viability of probiotics in yoghurts, their sensory attributes greatly influence consumer acceptance.

Lactobacillus paracasei is a probiotic bacterium showing compatibility with the yoghurt starters (Kristo, 2003), suitable viability and stability (Kristo *et al*., 2003; Maragkoudakis *et al*., 2006; Donkor *et al*., 2006), favorable organoleptic properties of produced probiotic yoghurt (Kristo *et al*., 2003; Maragkoudakis *et al*., 2006; Schwenninger & Meile, 2004), good tolerance to gastrointestinal conditions (Maragkoudakis *et al*., 2006), inhibition of the adhesion of *Escherichia coli* and *Salmonella typhimurium* to Caco-2 cells (Maragkoudakis *et al*., 2006), and induction of the secretion of pro- and anti-inflammatory cytokines by human peripheral blood mononuclear cells (Maragkoudakis *et al*., 2006). In spite of the latter health benefits, especially for the strain *L. paracasei ssp. tolerans*, there are very few studies about this strain and verification of some of the results seems to be necessary.

The objectives of this study were production of probiotic yoghurt using *L. paracasei ssp. tolerans*, and evaluation of its post-acidification during the storage period, viability of the starters and the probiotic bacteria during the storage period, and to evaluate organoleptic properties of the produced yoghurt.

Materials and Methods

- *Inoculum preparation*

To produce *L. paracasei ssp. tolerans* DSM 20258 inoculum, a single colony of the microorganism on MRS-Agar was transferred into MRS-broth and incubated at 37 °C for 24 hours. After adjusting the turbidity of the culture on 0.9, 100 mL pasteurized (95 °C, 15 min) reconstituted skimmed milk (14% total solid, 1.2% yeast extract) was inoculated with 6 mL of the culture. After incubation for 18 h at 37 °C, the resultant curdled milk was used to inoculate pasteurized reconstituted skimmed milk (14% total solid).

The lyophilized DVS commercial yoghurt starters (Chr. Hansen, YC-350, 50-unit pouches) consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* were suspended in pasteurized reconstituted skimmed milk (14% total solid, 42 °C). For production of yoghurt, 12 ml of the inoculum was added to 3 L reconstituted skimmed milk. Therefore, the resultant mixture contained 0.0002 U mL^{-1} of the DVS commercial yoghurt starter.

- *Production of control and probiotic yoghurts*

For production of the control yoghurt, the pasteurized reconstituted skimmed milk (14% total solid) was inoculated (0.4% v/v) with the commercial inoculum preparation. For the probiotic yoghurt, the milk was inoculated with *L. paracasei ssp. tolerans* (0.4% v/v), as well as the commercial inoculum preparation (0.4% v/v). Both inoculated milks were incubated at 42 °C until pH 4.6 was attained. The pH of yoghurt and bio-yoghurt were measured by using a digital pH-meter (TS Technology®, Tehran, Iran). To determine the microorganisms grown on both milks during fermentation, the generation number between the beginning and the end of process was measured.

- *Microbiological analysis*

Yoghurt and bio-yoghurt samples were decimally diluted in sterile NaCl solution (0.9%), and 1 mL aliquots were poured into various molten selective agar plates. M17 agar (Merck) was used for the enumeration

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of *S. thermophilus*. *L. delbrueckii* and *L. paracasei ssp. tolerans* and there were also inoculated on MRS-agar (pH= 5.2) and MRS-vancomycin-Agar, respectively. M17 was incubated aerobically at 37 °C where as MRS (pH= 5.2) and MRS-vancomycine were incubated anaerobically at 45 °C and 37 °C, respectively (Tharmaraj & Shah, 2003). Anaerobic jars (Merck) and Anaerocult® gas packs were used to produce anaerobic conditions, and Anaerotest® strips (Merck) were used to check this condition. Plates containing 30-300 colonies were counted and the results were reported as colony forming units per milliliter (CFU mL^{-1}) after calculations.

- *Generation number calculation*

The generation number of the bacteria during fermentation was determined by pour plate method and calculating the number of doublings between the beginning and the end of the fermentation, which was equal to (Log N/N_0) / Log 2, with N as the viable number of bacteria at the end of fermentation.

- *Sensory evaluation*

Thirty healthy and trained students in both sexes (58% female, 42% male, ages between 23-28 years) tasted the yoghurts in random order for the assessment of organoleptic properties, and Karl Ruher nine point scheme was used for scoring appearance, texture, flavor and total acceptability (Tamime & Robinson, 1999). The samples were tasted by the panel 1 day after the yoghurt production. The panellists were trained to have the same understanding of what was desirable. The samples were offered in booths, and their temperature was 23°C. The data were collected from each individual independently.

- *Statistical analysis*

Statistical analyses were carried out by non-parametric methods such as Mann-Whitney and Friedman and means such as *t*-test were applied using the statistical package SPSS for Windows version 13.0 (SPSS Inc, Illinois).

Results and Discussion

- *Changes of pH during fermentation*

The pH of the incubated milk was monitored during fermentation to study the pH changes, and to determine the proper time to remove the incubated milk from the incubator. There was a gradual decreases in the pH in both of the fermented milks during the first 120 minutes (Figure 1), followed by a sharp drop between 180- 210 minutes, and a steady reduction later until the desired pH was achieved. The pH in the probiotic yoghurt was reduced significantly ($P < 0.05$) faster than the control yoghurt and reached the desired pH, 30 minutes earlier. This might be due to the presence of more *Lactobacilli* and consequently more production of lactic acid which is consistent with the results of Vinderola *et al.* (Vinderola *et al*., 2002). The pH was reduced to below five that might be due to the inactivation of lactic acid bacteria after this point.

- *Effect of L. paracasei on growth of starters during fermentation period*

In order to study the effect of *L. paracasei ssp. tolerans* present in the probiotic yoghurt on the growth of yoghurt starters during fermentation period, generation number of starters during fermentation time was studied. The result indicated that the growth of both starters in the probiotic yoghurt was lower than their growth in the control yoghurt (Figure 2), but this difference was not significant ($P> 0.05$). This is consistent with the results of Donkor *et al.* (Donkor *et al*., 2006). This might be considered as an advantage for *L. paracasei* as a probiotic, because some other probiotics have shown inhibitory effects on starter cultures (Vinderola *et al*., 2002; Guler-Akin,

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2005; Avonts *et al*., 2004; Korbekandi et al., (In Press); Korbekandi *et al*., 2009). The growth of *L. paracasei ssp. tolerans*, itself was lower than the starters, but again this difference was not significant ($P > 0.05$).

- *Post-acidification during the storage period*

In order to study the pH changes during the storage period, pH of probiotic and control yoghurts were determined at 5 °C. The pH dropped significantly ($P < 0.05$) and linearly (Figure 3) on both control and probiotic yoghurts during 21 days of storage (5 °C) period. The pH of the probiotic yoghurt at this day was 0.1 unit lower than the pH of the control yoghurt. The pattern of pH decrease in both yoghurts were almost the same, and similar to the results of Donkor *et al.* (2006). They had also used *L. paracasei* as the probiotic strain.

- Post acidification of yoghurts during cold storage might be mainly addressed to lactic acid production, especially by *Lactobacillus bulgaricus* (Antunes *et al*., 2005). The range of pH (4.6-4.2) between days (1-21) was almost consistent with this range (4.4-4.1) in the study carried out by Maragkoudakis *et al.* (2006), which used *L. paracasei* as the probiotic bacterium in their yoghurt. The inoculation percentage in the current study was (0.4 v/v), where as this percentage in their work was (1 v/v), justifying their slightly lower range of pH. Kailasapathy *et al.* (2006) used *Bifidobacterium lactis* and *L. acidophilus* as the probiotic bacteria in yoghurt, but in contrast, these bacteria reduced acid production. On this basis, it would seem that the reduction of pH in yoghurts containing probiotic bacteria depends on the probiotic strain.

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- *Viability and stability of starters and L. paracasei*

In order to study the viability and stability of *L. bulgaricus* and *S. thermophilus* in probiotic and control yoghurts during the storage period, and to test the effect of *L. paracasei ssp. tolerans* on their viability, the viable counts of these two starters were determined during cold storage period in both probiotic and control yoghurts.

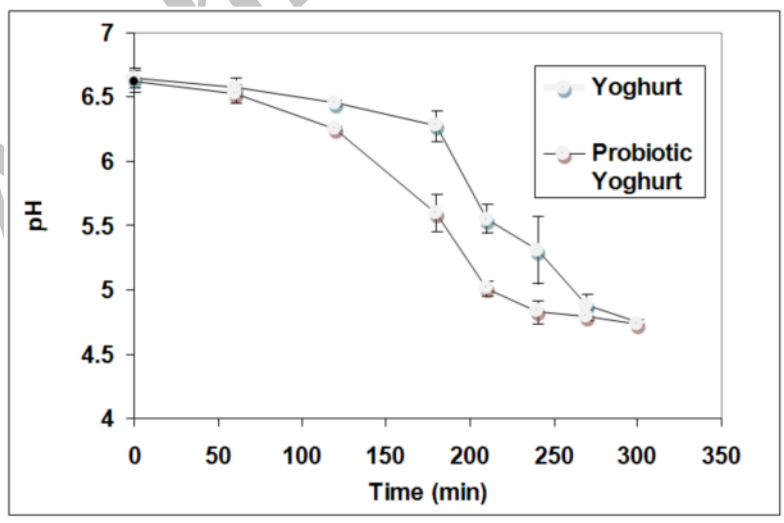

Fig. 1. Inoculated milk pH profile during fermentation period
- pH of probiotic (●) and control yoghurt (○) during fermentation period inside the incubator (42 °C) was recorded. Data points are means (triplicate), and error bars are data ranges.

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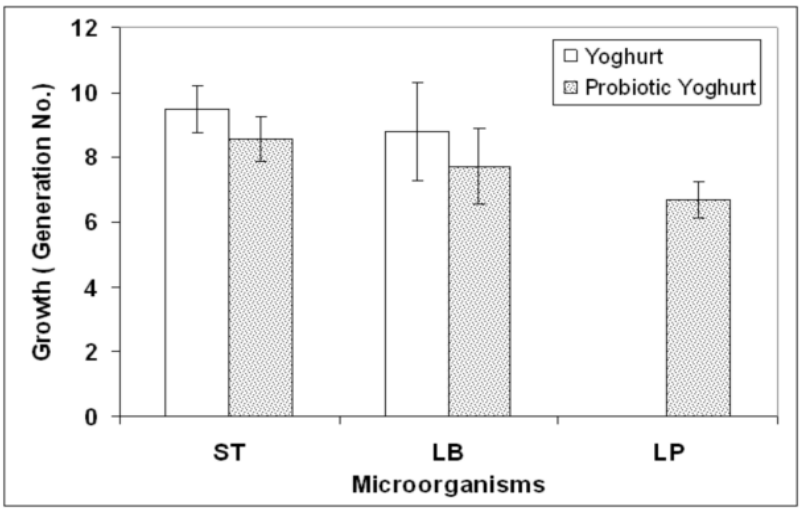

Fig. 2. Effect of *L. paracasei* on growth of starters during fermentation period
- Generation number (the number of doublings) of each bacterium between the beginning and the end of the fermentation (to reach pH 4.7) was calculated in the probiotic (dotted bars) and the control yoghurt (plain bars). Data points are means (triplicate), and error bars are data ranges. LB, ST and LP stand for *L. delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus* and *L. paracasei ssp. tolerans*, respectively.

Viable counts of *L. bulgaricus*, significantly ($P < 0.05$) decreased in both probiotic and control yoghurts, during 21 days of storage at 5 °C (Figure 4). The decrease in both yoghurts showed that *L. paracasei* was not responsible for this. Besides, the decrease of *L. bulgaricus* population during the storage period, in probiotic yoghurt (1 Log CFU mL^{-1}) was lower than the decrease in control yoghurt (1.4 Log CFU mL^{-1}). This is in agreement with the results of Donkor *et al.* (2006). They interpreted this, as an increase in necessary growth factors, such as peptides and amino acids, due to proteolytic effect of some probiotic bacteria including *L. paracasei ssp. tolerans*, which increases the survival of *L. bulgaricus*.

The viable counts of *S. thermophilus*, during 21 days of storage at 5 °C decreased (Figure 5) significantly ($P < 0.05$), which seems to be due to the increase in organic acids production, especially lactic acid and consistent with the results of Maragkoudakis *et al.* (2006).

Viability of *L. paracasei ssp. tolerans* decreased almost linearly and significantly ($P = 0.042$) during the storage time (21 days), but the viable count (1.77×10^7) was higher than the standard limit for probiotic products (10^6 CFU mL^{-1}) until the last day (Figure 6). Again, the decrease in viability might be interpreted as the increase in the production of lactic acid or other organic acids and this was in agreement with the results of Maragkoudakis *et al.* (2006), while it is inconsistent with the results of Donkor *et al.* (2006). This disagreement might be justified as the presence of two other probiotic bacteria (*L. acidophilus* and *B. lactis*) in the yoghurt, which produce free amino acids during the storage period and consequently increase the stability and viability of *L. paracasei*.

- *Sensory evaluation*

In order to study the acceptability of the produced probiotic yoghurt and compare it to the control, organoleptic properties of both yoghurts were assessed by trained students using nine point schemes for scoring. The average scores of appearance of probiotic yoghurt was higher, but the average scores for texture, flavor and total acceptability was lower (Table 1). There was no significant ($P > 0.05$) difference between

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organoleptic properties of the control and the probiotic yoghurts. This is in agreement with the results of Kristo *et al.* (2003) regarding *L. paracasei*. Schwenninger and Meile (2004) have also shown that *L. paracasei* not only did not influence the quality characteristics of dairy food, but exhibited inhibitory activities against spoiling yeasts.

Conclusion

Incorporation of *L. paracasei ssp. tolerans* in yoghurt neither affected the viability of the starter cultures during 21 days of cold storage, nor the organoleptic properties of the yoghurt. Viability of the probiotic bacterium decreased during the cold storage time, but the viable count until the day 21st was higher than the standard limit for probiotic products. This study verified the reports about compatibility of *L. paracasei* with the yoghurt starters, suitable viability and stability, and organoleptic acceptability of produced probiotic yoghurt.

Acknowledgements

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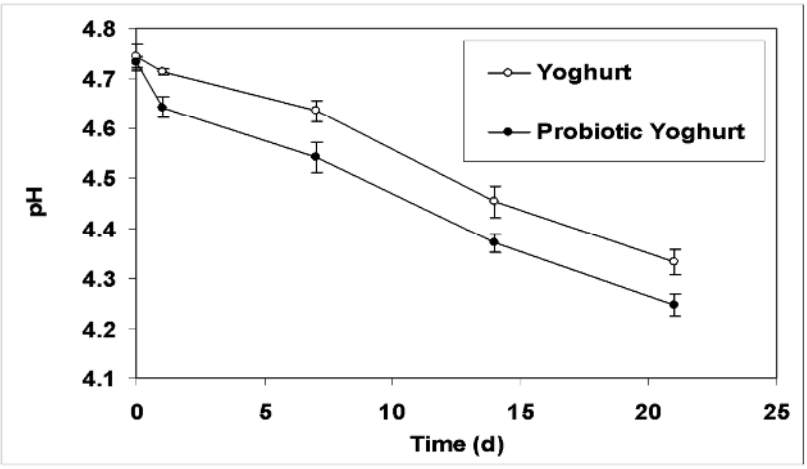

Fig. 3. Changes of pH during the storage period

- Probiotic **(●)** and control yoghurt **(**○**)** pH values were determined during the storage period at 5 °C. Data points are means (triplicate), and error bars are data ranges.

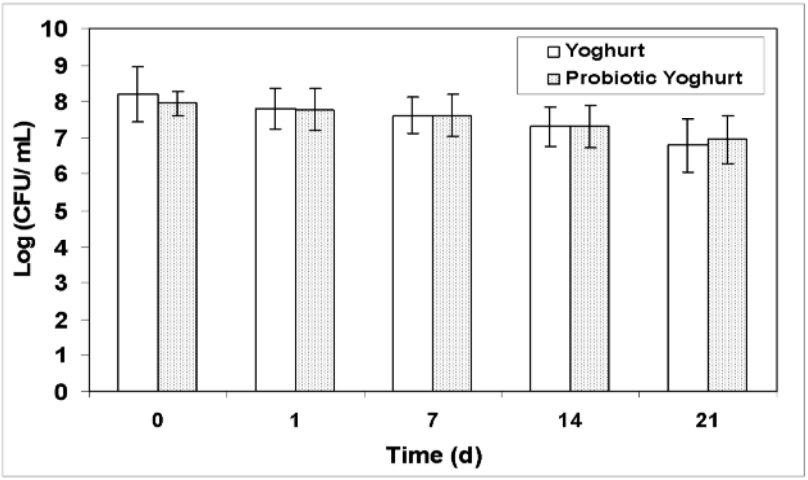

Fig. 4. Viability of *L. bulgaricus* during the storage period

- Viable count of *L. bulgaricus* during 21 days storage period at 5 °C was determined in probiotic (dotted bars) and control yoghurts (plain bars). Data points are means (triplicate), and error bars are data ranges.

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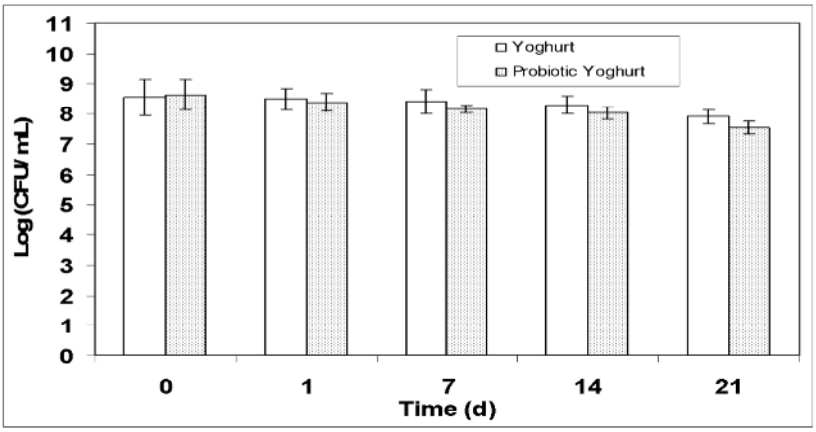

Fig. 5. Viability of *S. thermophilus* during the storage period
- Viable count of *S. thermophilus* in yoghurt (dotted bars) and probiotic-yoghurt (plain bars) during 21 days storage period at 5 °C was determined in probiotic (dotted bars) and control yoghurt (plain bars). Data points are means (triplicate), and error bars are data ranges.

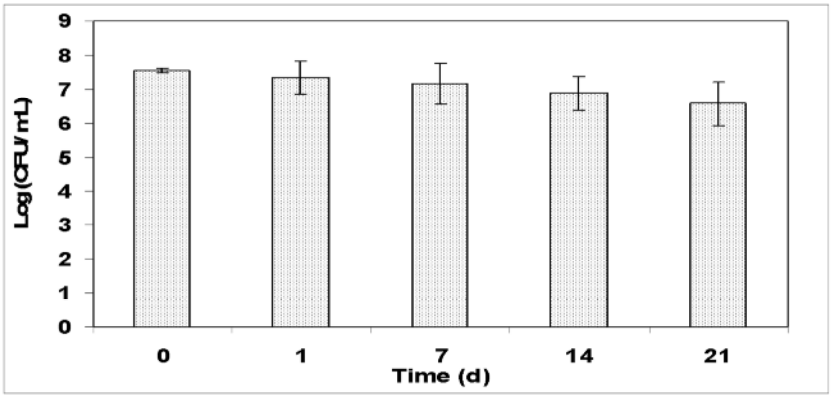

Fig. 6. Viability of *L. paracasei* during the storage period
- Viable count of *L. paracasei ssp. tolerans* during 21 days storage period at 5 °C was determined in probiotic yoghurt. Data points are means (triplicate), and error bars are data ranges.

Table 1. Organoleptic properties of control and probiotic yoghurt

Yoghurt	Properties			
	Appearance	Flavor	Texture	Total acceptability
Control	4.43±2.06*	4.30±2.26	4.67±2.2	4.63±2.32
Probiotic	4.6±2.415	4.13±2.03	4.50±2.46	4.6±2.15

* Mean ± standard deviation

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