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Viability of probiotic bacteria during refrigerated storage in commercial yogurt marketed in Mashhad, Iran

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Abstract

Probiotic yogurt is one of the most common and important probiotic products in the market. Researchers have revealed that the survival of probiotic organisms in yogurt during storage has often been low. This research aimed to investigate the viability of probiotic bacteria in yogurt (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) marketed in Mashhad during storage at refrigerator. Methods based on culture using two culture media (MRS/CL/CIP for *L.acidophilus* and TOS-MUP for *B.bifidum*) was used. Results showed in 40% of samples the number of viable *lactobacillus acidophilus* was lower than the standard limit (6 log cfu/ml) and about *B.bifidum* only 13% of samples had viable bacteria lower than the limit. Comparison between the number of two type of viable bacteria showed the average of viable *L.acidophilus* and *B.bifidum* in all samples of yogurt was 6.37 log CFU / ml and 6.06 log CFU/ml, respectively. These amounts were significantly different ($p<0.05$). It is concluded that the counts of probiotic bacteria in half of the tested yogurt was higher than the international standard limit.

Key words : probiotic yogurt, *Lactobacillus acidophilus, Bifidobacterium bifidum.*

INTRODUCTION

Yogurt has long been recognized as a product with many desirable effects for consumers, and it is also important that most consumers consider yogurt to be 'healthy'. The conventional yogurt starter bacteria, *L. bulgaricus* and *Streptococcus thermophilus*, lack the ability to survive passage through the intestinal tract and consequently do not play a role in the human gut [1]. Consumption of probiotic bacteria via food products is an ideal way to re-establish the intestinal microflora balance. Many microorganisms have been used or considered for use as probiotics. A probiotic preparation may contain one or several different strains of microorganisms. Because viable and biologically active microorganisms are usually required at the target site in the host, it is essential that the probiotic be able to withstand the host□s natural barriers against ingested bacteria [2]. The most commonly used probiotics are strains of lactic acid bacteria (e.g., *Lactobacillus*, *Bifidobacterium* and *Streptococcus*). The beneficial effects of *Lactobacillus* and *Bifidobacterium* have been discussed for decades. Bacteria in these two genera resist gastric acid, bile salts, and pancreatic enzymes, adhere to intestinal mucosa and readily colonize the intestinal tract [3].

To provide health benefits, the suggested concentration for probiotic bacteria is 10^6 CFU/g of a product [4, 5, 6]. However, studies have shown the low viability of probiotics in market preparations [7, 8 ,9,10]. The need to monitor survival of *L.acidophilus* and *bifidobacteria* in fermented products has often been neglected, with the result that a number of products reach the market containing a few viable bacteria [7,9,11]. To assess viability and survival of probiotic bacteria, it is important to have a working method for selective enumeration of these probiotic bacteria. Several media for selective enumeration of *L.acidophilus* and *Bifidobacterium* spp. have previously been proposed. However, most of these methods are based on pure cultures of these organisms.In this study viability of probiotic bacteria in commercial yogurts marketed in Mashhad was assessed.

MATERIALS AND METHODS

Sample collection:

Thirty kinds of probiotic yogurts were purchased from main market stores. The products were kept under refrigerated conditions during transportation and directly stored in the laboratory at 4°C.

Probiotic count:

Viable cell counts of probiotics (CFU/mL) were determined by plate count methodology. One gram yogurt sample was diluted with 9 mL of peptone water (Oxoid, W. Heidelberg, Australia) and mixed uniformly with a vortex mixer. Subsequent serial dilutions were prepared and viable numbers enumerated using pour plate technique. MRS - clindamycin - ciprofloxacin agar (MRS-CL/CIP Agar) (Sigma-Aldrich Inc.) was used for selective enumeration of *L. acidophilus* by incubating the plates anaerobically at 37ºC for 72h ± 3h [12]. Typically colonies on agar media are usually 2-5 micrometers, convex, entire, opaque, and without pigment.

Selective enumeration of *Bifidobacteria* was performed on TOS MUP agar (Sigma-Aldrich Inc.). Superior suppression of lactic acid bacteria found in milk products due to Lithium-Mupirocin occurred. The anaerobic incubation at 37ºC for 72h ± 3h was performed [13]. A confirmation of selected colonies is possible by microscope. *Bifidobacteria* show irregular formed rods when magnified 100 times using phase-contrast method [14].

Statistical analysis:

All data were analyzed using the one way ANOVA procedure of the SPSS, version 16. Duncan's multiple range test was used to compare the probiotic count between the probiotic samples marketed in Mashhad.

RESULT

Results showed 26.7% of yogurt samples contains *L.acidophilus* higher than 6 log CFU.ml^{-1} and 40% of samples had *L.acidophilus* lower than standard limit. The count of

Table 1: Viable counts (log cfu.ml^{-1}) of probiotic bacteria during the storage period

Probiotic yogurt	Viable count (log cfu.ml^{-1}) *L.acidophilus*	Viable count (log cfu.ml^{-1}) *B.bifidum*
Sample 1	6.07	6.24
Sample 2	5.85	6.4
Sample 3	6.04	6.26
Sample 4	5.93	6.72
Sample 5	5.72	6.59
Sample 6	6.04	6.65
Sample 7	6.13	6.37
Sample 8	6.17	6.51
Sample 9	5.89	6.28
Sample 10	6.07	6.19
Sample 11	6.09	6.43
Sample 12	5.89	6.5
Sample 13	5.66	6.27
Sample 14	5.77	6.06
Sample 15	6.09	6.33
Sample 16	5.85	6.5
Sample 17	6.07	6.62
Sample 18	5.11	5.7
Sample 19	5.77	5.25
Sample 20	4.81	5.87
Sample 21	5.93	6.28

Sample 22	6.23	6.56
Sample 23	5.98	6.4
Sample 24	6.36	6.45
Sample 25	6.42	6.2
Sample 26	6.26	6.2
Sample 27	6.09	6.29
Sample 28	6.2	5.99
Sample 29	6.26	6.37
Sample 30	6.32	6.54

L.acidophilus in other samples was 6 log CFU.ml^{-1}. The number of viable *B.bifidum* in 83% of samples was higher than the standard limit and in 13% of yogurts was lower than 6 log CFU.ml^{-1}. There were no significant differences in the cell numbers of bacteria ($p>0.05$). Results indicate that only in 10% of the samples (samples 18,19 and 20) the number of both viable *L.acidophilus* and *B.bifidum* was lower than standard limit. The average counts for *B.bifidum* and *L.acidophilus* at 30 yogurt samples were 6.37 and 6.06 (log CFU.ml^{-1}), respectively. There were significant differences at 0.05 level.

DISCUSSION:

Despite the importance of being life for probiotic bacteria in food products, many studies have shown the low viability of probiotics in yoghurt [9, 15, 16]. The low viability in yogurt is mainly attributed to the lower pH in yoghurt and further reduction of pH in yogurt during post-acidification [17, 18]. Using ABT (*L. acidophilus*, *Bifidobacteria* and *S. thermophilus*) starter cultures that are devoid of *L. delbrueckii* subsp. *Bulgaricus* help improvement viability of probiotic bacteria [19, 20]. *L. delbrueckii* subsp. *bulgaricus* produces lactic acid during refrigerated storage. This process is known in the industry as "post acidification" and, if it occurs, it causes a loss in viability of the probiotic bacteria [21]. L. delbrueckii subsp. bulgaricus plays a critical role in lactic acid and flavor production in yogurt and when excluded from the starter culture, it profoundly affects the texture, acidity and the aroma of the final product. Therefore, in recent years yogurt producers are not able to remove it from yogurt cultures [22, 23]. The use of high level of inoculums will ensure a high cell count at the end of the inoculation and survival of the probiotic bacteria during storage until consumption [24]. According to Sarvari et al., (2014) throughout the storage. *L. delbrueckii* ssp. *bulgaricus* lost its viability considerably until the end of storage. The viability loss of bifidobacteria was gradual and steady during the storage, and the organisms exhibited good stability during the storage as compared to lactobacilli [25]. In a study of Vinderola et al., 2000 the cell counts of *S. thermophilus, L. acidophilus* and *B. bixdum* gradually decreased through the cold storage of carbonated and non-acidified fermented milk, although the counts were always higher than 10^6 viable cells g^{-1} [26]. Gueimonde et al., 2004 shows that counts of Lactobacillus spp. always remained higher than 10^5 CFU. ml^{-1}, whereas the population of *Bifidobacterium* spp. decreased below this level in two commercial probiotic-fermented dairy products [27]. Changes in viable counts of starter and probiotic bacteria in yogurt supplemented with various ingredients during 33 days of refrigerated storage were examined by Naji et al., (2014). Statistical analysis showed, during storage for 33 days, the counts of L. delbrueckii subsp. bulgaricus declined gradually in all yogurts, except those supplemented with TC and yeast extract. All the products showed a decline in viable counts of *Bifidobacteria* during refrigerated storage except the yogurt supplemented with milk powder and inoculated with the fivefold starter culture [28]. In the Naji's work, five-fold increase in inoculum caused significant ($p< 0.05$) increase in the survival of *Bifidobacteria* during the storage period. This is in contrast with Dave & Shah, (1997) findings who showed that increased inoculum did not improve the viability of *Bifidobacteria* in yogurt made with ABT starter culture [29]. The viability of probiotic bacteria in yogurt depends on the strains used, the interaction between species present, culture conditions, production of hydrogen peroxide due to bacterial metabolism, the final acidity of the product, and the concentrations of lactic and acetic acids. The viability also depends on the availability of nutrients, growth promoters and inhibitors, the concentration of sugars (osmotic pressure), dissolved oxygen and oxygen permeation through the package (especially for *Bifidobacterium* spp.), inoculation level, incubation temperature, fermentation time and storage temperature [30].

CONCLUSION

Assessing probiotic bacteria in foods is a good measure to ensure the health of probiotic products. From this research is concluded that the counts of probiotic bacteria in fermented dairy products marketed in Mashhad is not always above the therapeutic dose of 6.0 log CFU/g. The average number of

B.bifidum and *L.acidophilus* at 30 yogurt samples were 6.37 and 6.06 (log $CFU.ml^{-1}$), respectively. In order to produce yogurt that has good taste and appropriate pH, it should discard the increase in the number of bacteria. The increase in the number of bacteria alone has an inappropriate effect on the taste and flavor. Therefore, to have a high probiotic population, it should focus on other factors that help in the survival of probiotic bacteria in the product.

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