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Effect of Potassium Sorbate And/or Probiotic Bacteria on Spoilage Bacteria During Cold Storage of Soft Cheese

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Abstract: In this study, the effect of potassium sorbate and /or probiotic bacteria on spoilage organisms during manufacturing and cold storage of soft cheese in attempt to improve the hygienic quality of soft cheese have been assessed. Ten batches of white soft cheese were prepared from cow's milk containing 5% Sodium Chloride and inoculated with *pseudomonas fluorescens*, the first batch was a control (containing no probiotic bacteria or potassium sorbate),the following four batches (2 – 5) containing different concentrations of potassium sorbate (0.02, 0.05, 0.1 and 0.2 %). The sixth batch contains *Bifidobacterium longum*, the rest four batches (7 – 10) containing both potassium Sorbate at concentrations of 0.02, 0.05, 0.1 and 0.2 and *B. longum*. The cheese batches were examined physically and bacteriologically at zero time, after 3, 9, 12, 18, 21, 27 and 30 days, The reduction percent of *Pseudomonas fluorescens* count at the end of storage period (30 days) were 93.6, 94.6, 97.78 and 99.5 for cheese containing potassium sorbate at concentrations of 0.02, 0.05, 0.1 and 0.2%, respectively. While the reduction percent in case of cheese containing *B. longum* only was 96.25%. But in case of combined addition of both Potassium Sorbate and *B. longum* the reduction percent were 96.39, 97.6, 99.40 and 99.47% for 0.02, 0.05, 0.1 and 0.2% added Potassium Sorbate and *B. longum*, respectively. In the same time, in the control batch, the reduction percent of *Pseudomonas fluorescens* count at the end of storage period (30 days) was 87.50%. A significant ($p < 0.05$) inhibition of growth of *Pseudomonas fluorescens* in batches (9,10) containing both potassium sorbate 0.1%, 0.2% and *B. longum* was observed all over the experiment. Sorbate above 0.1% although highly effective, cause unobjectionable sweet flavor, a condition that can acts as an effective check against excessive use of the preservatives. In conclusion the combined addition of potassium sorbate at concentration of 0.1% and *B. longum* had a great inhibitory effect up on existing microorganism and also improved the organoleptic quality of cheese.

Key words: Potassium sorbate · *B. longum* · *Pseudomonas fluorescens* · Soft cheese

INTRODUCTION

Milk is an excellent medium for growth of a variety of bacteria [1]. Spoilage bacteria may originate on the farm from the environment or milking equipment or in processing plants from equipment, employee, or the air. Lactic acid bacteria (LAB) are usually the predominant microbes in raw milk and proliferate if milk is not cooled adequately. Refrigeration suppresses growth of LAB and within one day psychro-philic bacteria (*Pseudomonas, Enterobacter, Alcali-genes* and some spore-formers) grow and can eventually produce rancid odors through the action of lipases and bitter peptides from protease action [2,3]. Pasteurization kills the psychrophiles and mesophilic bacteria (LAB). However, post-pasteurization contamination of milk, particularly with *Pseudomonas* and some Gram-positive psychrophiles does occur [4, 5]. Spoilage problems in cheese can sometimes be traced to low quality milk, but may also result from unhygienic conditions in the processing plant. Soft cheeses with a high pH of 5.0–6.5 and a moisture content of 50–80% may be spoiled by *Pseudomonas*, *Alcali-genes* and *Flavobacterium*. The growth of these organisms in cottage cheese can cause sliminess, bitter tastes, off-flavors and color defects, which lead to spoilage of the cheese due to proteolytic and lipolytic activities of the psychrotrophs in general and pseudomonas species in particular [3].

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Food preservatives are added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods and to prolong shelf life and quality of foods [6,7]. The most commonly used preservatives in many types of foods are benzoic acid, sorbic acids, nitrate and nitrite [8].

Potassium sorbate is the potassium salt of sorbic acid and is much more soluble in water than the acid. Potassium sorbate will produce sorbic acid once it is dissolved in water and is the most widely used food preservative in the world. It is effective up to pH 6.5, but effectiveness increases as the pH decreases. Potassium sorbate is effective against yeasts, molds and selected bacteria and is widely used at 0.025 to 0.10 % levels in cheeses, yogurt and sour cream. Maximum level allowable by law is 0.1%. It is important to know that the addition of potassium sorbate to a food product will raise the pH by approximately 0.1 to 0.5 pH units depending on the amount, pH and type of product. Additional adjustment of the pH might be needed to keep the pH at a safe level. Potassium sorbate may be individually used or in combination with other organic acids [9].

The incorporation of probiotic bacteria as adjuncts in different fermented milk products is currently an important topic with industrial and commercial consequences. The food application of probiotics has reinforced the acclaimed healthy properties and given rise to an increased consumption of these products in Europe and USA [10]. A number of dairy products are marketed as containing probiotic bacteria. Fermented milk and cheeses have been described as the most suitable carriers for the administration of such bacteria [11].

Among industrially used probiotics, *Bifidobacterium longum* strains which are often incorporated in different health products. Fermented dairy products containing *B. longum* have obtained "Food for specific health use" (FOSHU) approval in Japan, which are recognized to regulate intestinal microflora and allow a healthier gastrointestinal condition [12]. *B. logum* provides countless essential nutrients, boosts immune function, protects against "unfriendly" bacteria and supports a large network of cellular functions. In addition, several important antibiotic substances are produced as a result of *Bifidobacterium* metabolism. These substances inhibit growth of other bacteria [13]. Therefore, a combination of sorbate and *B. longum* might be expected to exert greater inhibitory action on spoilage organisms than would either alone.

The main objective of this study was to determine the effect of potassium sorbate and / or *B. longum* on inhibition of *pseudomonas fluorescence* in soft cheese during cold storage.

MATERIALS AND METHODS

Sources and Maintenance of Cultures: The culture of *Pseudomonas fluorescens* 1 used in this study was available from the stock culture collection of the *Microbiology* laboratory in the Faculty of Agriculture Ein-Shams University, Egypt. *Pseudomonas fluorescens* 1 was maintained by subculturing (1% inocula) in tryptic soy broth (Difco Laboratories) and incubating for 18 h at room temperature (23 to 25°C). The culture of *B. longum* BB 536 (morinaga lyophilized bacteria) was obtained from Mitsubishi international corporation, USA. Before the assay *B. longum* strain was cultured twice in de Man, Rogosa and Sharpe (MRS) broth (Difco Laboratories, Detroit, MI) and incubated for 18 h at 37°C under anaerobic condition. All cultures were stored at 2 to 5°C. Both cultures were sub cultured 3 times immediately before use in the experiments.

Enumeration of Bacteria: Microorganisms were enumerated by pour plate technique after preparation of serial dilutions [14]. Loopfuls from each dilutions for *Pseudomonas fluorescens* and *B. longum* BB 536 were streaked on previously prepared *Pseudomonas aeromonas* agar plate and MRS plate respectively and then the plates were incubated at 25°C for 3 days for *Pseudomonas* and 37°C for 24 hrs (under anaerobic condition) for *B longum*, the colony forming unite / ml for each organism was calculated.

Inoculation of Cultures and Preparation of White Soft Cheese: Ten batches of raw cow's milk (one liter each) were used in this study. All batches were pasteurized at 64°C for 30 minutes, cooled to 37°C, salted (5%) and calcium chloride (2 ppm) was added. The culture of *Pseudomonas fluorescens* strain was added to each batch of the pasteurized milk to provide concentration of $1x10^5$ cells /ml. The 1st batch was control containing *Pseudomonas fluorescens* only. To the 2nd, 3rd, 4th and 5th batches, potassium sorbate was added in concentration of 0.2, 0.5, 1 and 2 g/liter, respectively. While 6th batch was inoculated with culture of *B. longum* BB 536 only to provide concentration of $1x10^8$ cells/ml, while the 7th, 8th, 9th and 10th batches were inoculated with both potassium sorbate at concentration of 0.2, 0.5, 1 and 2 g/liter and *B. longum* (1x10^8 cells/ml).

All batches were coagulated with rennet at 37°C until the curds were formed, then each curd portion was ladled into sterile stainless steel molds and pressed to escape the whey to from a firm curd. Each cheese curd with its whey was kept in a sanitized plastic container at 7°C and examined physically for change in flavor, odor and color and bacteriologically for *Pseudomonas fluorescens* counts at zero time then after 3, 9, 12, 18, 21, 27 and 30 days.

Statistical Analysis: Statistical analyses were conducted by using the GLM procedure (PROC GLM) of SAS [15]. Separation of the means (p <0.05) was accomplished by Duncan's multiple range test. All experiments and analyses were replicated 3 times.

RESULTS

Results given in Table 1 and Figure 1 show the reduction percentages of *Pseudomonas fluorescens* count between the first (0) and the last (30) day of cold storage of cheese and revealed that the reduction percentages were 93.6, 94.6, 97.78 and 99.5 in 0.02, 0.05, 0.1 and 0.2 % potassium sorbate treated cheese, respectively. While the reduction percentage of *Pseudomonas fluorescens* counts in *B.longum* treated cheese samples was 96.25 but the reduction percentage of *Pseudomonas fluorescens* count were 96.39, 97.06 99.40 and 99.47 in 0.02, 0.05, 0.1 and 0.2% potassium sorbate treated cheese with *B. longum* , respectively. Concerning to the control batch, the reduction percent of *Pseudomonas fluorescens* count at the end of storage period (30 days) was 87.50.

Results in Table 2 summarize the effect of potassium sorbate and/or *B. longum* on *Pseudomonas fluorescens* counts at different stages of cold storage of cheese and showed that, no significant difference between the mean counts of *Pseudomonas fluorescens* in control cheese throughout the experiment. In the first stage of cold storage (day 0-9) the mean counts of *Pseudomonas fluorescens* in potassium sorbate treated cheese (0.02, 0.05, 0.1 and 0.2%) were 4.03 x 10^5±8.89 x 10^3, 3.87 x 10^5±8.82 x 10^3, 2.80 x 10^5±5.77 x 10^3 and 2.10 x 10^5±5.77 x 10^3 while, the mean counts of *Pseudomonas fluorescens* in *B. longum* treated cheese was 4.17 x 10^5±8.82 x 10^3, concerning the mean counts of *Pseudomonas fluorescens* in potassium sorbate treated cheese (0.02, 0.05, 0.1% and 0.2%) with *B. longum,* the mean counts were 3.77 x 10^5±1.20 x 10^4, 3.60 x 10^5±1.15 x 10^4, 2.53 x 10^5±8.82 x 10^3 and 1.60 x 10^5±5.77 x 10^3, respectively.

Table 1: Reduction percentage of *pseudomonas flourescence* count between the first and the last day of cold storage of cheese

Treatment		Day 0	Day 30	Reduction %
1.	Control	8.0 x 10^5	1.0 x 10^{5*}	87.50
2.	Potassium sorbate 0.02%	3.9 x 10^5	2.5 x 10^{4*}	93.60
3.	Potassium sorbate 0.05%	3.7 x 10^5	2.0 x 10^{4*}	94.60
4.	Potassium sorbate 0.10%	2.7 x 10^5	6.0 x 10^3	97.78
5.	Potassium sorbate 0.20%	2.0 x 10^5	1.0 x 10^3	99.50
6.	*B. longum*	4.0 x 10^5	1.5 x 10^4	96.25
7.	Potassium sorbate 0.02%+ *B. longum*	3.6 x 10^5	1.3 x 10^4	96.39
8.	Potassium sorbate 0.05%+ *B. longum*	3.4 x 10^5	1.0 x 10^4	97.06
9.	Potassium sorbate 0.10%+ *B. longum*	2.4 x 10^5	1.5 x 10^3	99.40
10.	Potassium sorbate 0.20%+ *B. longum*	1.5 x 10^5	8.0 x 10^2	99.47

* The last day of storage was 27th day

Table 2: Effect of potassium sorbate and/or *B. longum* on *pseudomonas flourescence* count at different stages of cold storage of cheese

Treatment		Day 0 - 9	Day 10 - 21	Day 22 - 30
1.	Control	8.67 x 10^5±4.41 x 10^4 a	7.80 x 10^5±9.17 x 10^4 a	5.00 x 10^4±5.00 x 10^4 a
2.	Potassium sorbate 0.02%	4.03 x 10^5±8.89 x 10^3 bc	3.17 x 10^5±5.90 x 10^4 b	1.25 x 10^4±1.25 x 10^4 a
3.	Potassium sorbate 0.05%	3.87 x 10^5±8.82 x 10^3 bcd	2.70 x 10^5±6.11 x 10^4 b	1.00 x 10^4±1.00 x 10^4 a
4.	Potassium sorbate 0.10%	2.80 x 10^5±5.77 x 10^3 e	1.87 x 10^5±5.90 x 10^4 bcd	8.00 x 10^3±2.00 x 10^3 a
5.	Potassium sorbate 0.20%	2.10 x 10^5±5.77 x 10^3 g	1.00 x 10^5±2.65 x 10^4 cd	3.00 x 10^3±2.00 x 10^3 a
6.	*B. longum*	4.17 x 10^5±8.82 x 10^3 b	3.33 x 10^5±5.24 x 10^4 ab	2.10 x 10^4±6.00 x 10^3 a
7.	Potassium sorbate 0.02%+ *B. longum*	3.77 x 10^5±1.20 x 10^4 cd	2.90 x 10^5±5.67 x 10^4 b	1.65 x 10^4±3.50 x 10^3 a
8.	Potassium sorbate 0.05%+ *B. longum*	3.60 x 10^5±1.15 x 10^4 d	2.37 x 10^5±6.01 x 10^4 bc	1.25 x 10^4±2.50 x 10^3 a
9.	Potassium sorbate 0.10%+ *B. longum*	2.53 x 10^5±8.82 x 10^3 f	2.4 7x 10^4±1.48 x 10^4 e	2.25 x 10^3±5.30 x 10^2 b
10.	Potassium sorbate 0.20%+ *B. longum*	1.60 x 10^5±5.77 x 10^3 h	1.40 x 10^4±2.03 x 10^3 e	9.00 x 10^2±7.00 x 10 c

Values are means ± standard errors

Means in the same column without a common letter differ significantly (P <0.05)

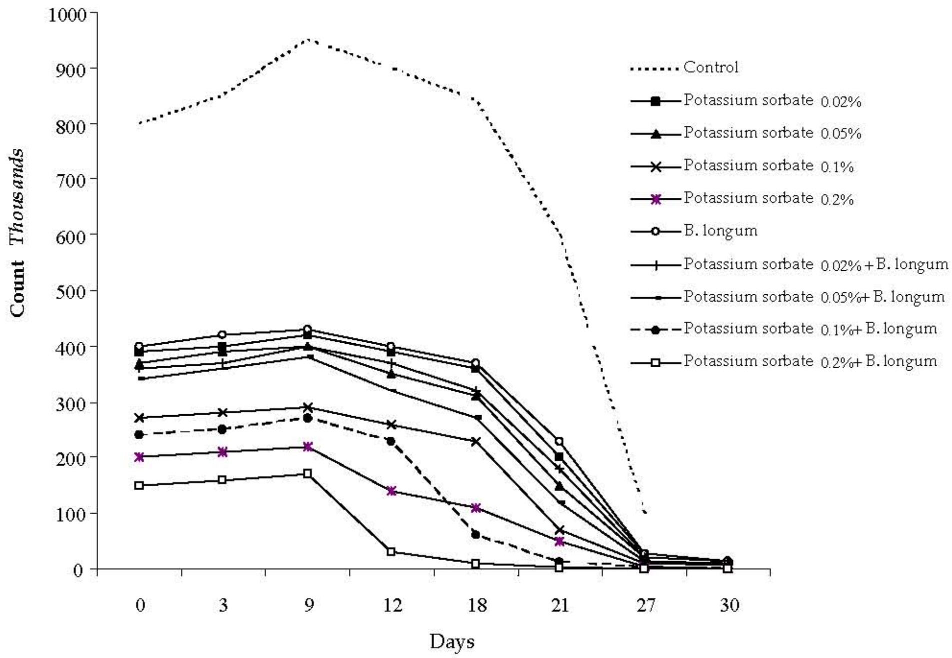

Fig. 1: Effect of potassium sorbate and/or B. longum on *Pseudomonas flourescence* count throughout the experiment

In the second stage of cold storage of cheese (day 10-21) the mean counts of *Pseudomonas fluorescens* in potassium sorbate treated cheese (0.02, 0.05, 0.1% and 0.2%) were $3.17 \times 10^5 \pm 5.90 \times 10^4$, $2.70 \times 10^5 \pm 6.11 \times 10^4$, $1.87 \times 10^5 \pm 5.90 \times 10^4$ and $1.00 \times 10^5 \pm 2.65 \times 10^4$, respectively, while, the mean counts of *Pseudomonas fluorescens* in *B. longum* treated cheese was $3.33 \times 10^5 \pm 5.24 \times 10^4$. The the mean counts of *Pseudomonas fluorescens* in potassium sorbate treated cheese (0.02, 0.05, 0.1 and 0.2%) with *B. longum* were $2.90 \times 10^5 \pm 5.67 \times 10^4$, $2.37 \times 10^5 \pm 6.01 \times 10^4$, $2.47 \times 10^4 \pm 1.48 \times 10^4$ and $1.40 \times 10^4 \pm 2.03 \times 10^3$, respectively.

The third stage of cold storage of cheese (day 22-30) revealed that, no significant difference between the mean counts of *Pseudomonas fluorescens* in all potassium sorbate treated cheese, *B. longum* treated cheese and potassium sorbate treated cheese (0.02 and 0.05%) with *B. longum*. The mean counts of *Pseudomonas fluorescens* in potassium sorbate treated cheese (0.1 and 0.2%) with *B. longum* were $2.25 \times 10^3 \pm 5.30 \times 10^2$ and $9.00 \times 10^2 \pm 7.00 \times 10$.

DISCUSSION

Many previous researches pointed out the need for preservatives to maintain the quality of milk and dairy products. Good milk and dairy products preservative must be non toxic, easily removable, leave no detectable residue, inexpensive and easy to apply. Sorbate appear to meet all of these criteria [3, 6, 16].

Bifidobacterium spp. is considered pro-biotic bacteria that are increasingly incorporated into dairy foods in order to provide health benefits [17]. A number of researchers have reported the use of lactic acid bacteria to control the growth of spoilage and pathogenic bacteria in a variety of foods. A major benefit of some of these lactic acid bacteria is their ability to produce inhibitory compounds at refrigeration temperatures, while themselves not growing. It has been well documented that hydrogen peroxide produced by these lactobacilli at refrigeration temperatures is a main cause for growth inhibition of undesirable organisms in refrigerated foods [18, 19]. Using potassium sorbate accompanied with *Bifidobacterium* has the ability to prolong the microbiological shelf life by 3 days [20].

In this study, the effect of potassium sorbate and/or B. longum on spoilage bacteria (*Pseudomonas fluorescens*) during cold storage of soft cheese was assessed. It is evident from the present results that *Pseudomonas fluorescens* count in soft cheese samples decreased during cold storage of cheese until the end of the storage period (30 days at 7°C).The reduction rate of *Pseudomonas fluorescens* populations was prominent in soft cheese batch number 5 (containing potassium sorbate 0.2%), batch 9 (containing potassium sorbate 0.1% + *B. longum*) and batch 10 (containing potassium sorbate 0.2% + *B. longum*). These results were in agreement with those reported by Lengkey and Adriani

[21] who found that the *Bifidobacterium* has the ability as bio-preservative against the pathogenic bacteria and *Pseudomonas spp.* and Sonia *et al.* [22] who mentioned that 0.1% potassium sorbate caused partial inhibition of *Pseudomonas fluorescens*.

The potential for the beneficial effects of adding potassium sorbate and / or *B. longum* on spoilage bacteria during cold storage of soft cheese was demonstrated in Table 2, which indicate that, no significant inhibition of growth of *Pseudomonas fluorescens* in soft cheese during cold storage at 7° C in control cheese throughout the experiment, this is close agreement with the reports of Neugebauer and Gilliland, [23].

In the first stage of storage (day 0-9) a significant ($P < 0.05$) inhibition of growth of *Pseudomonas fluorescens* in soft cheese during cold storage at 7°C in batches (4&5) containing potassium sorbate 0.1, 0.2% and batches (9&10) containing both potassium sorbate 0.1, 0.2% and *B. longum*. All cheese batches showed normal odor, color and consistency.

In the second stage of cold storage of cheese (day 10-21) all cheese batches still had normal odor, color and consistency and the results showed a significant inhibition of growth of *Pseudomonas fluorescens* in batches 9&10 containing both potassium sorbate 0.1, 0.2% and *B. longum*. While in the last stage of cold storage of cheese (day 22-30) the control cheese batch and cheese batches containing potassium sorbate 0.02 and 0.05% deteriorated at 27^{th} day of storage (spoiled odor, color and slim formation). A significant ($P < 0.05$) inhibition of growth of *Pseudomonas fluorescens* in batches (9&10) containing both potassium sorbate 0.1, 0.2% and *B. longum* was observed in this stage.

The cheese batch number 6 which contain *B. longum* only show a significant inhibition of growth of *Pseudomonas fluorescens* in soft cheese during cold storage at 7°C ($P < 0.05$) between the last stage of storage and the first stage of storage, but the reduction percent of *Pseudomonas fluorescens* count was less than the reduction percent of all other treatments (96.25%). *B. longum* combined with 0.1 and 0.2% potassium sorbate were much inhibitorier than *B. longum* alone. Sorbate alone had minimal inhibitory effects on growth of *Pseudomonas fluorescens* than the combined treatment of *B. longum* with 0.1 and 0.2% potassium sorbate, these results were in accordance with those reported by Gilliland and Ewell [24]. Thus the use of sorbate in conjunction with *B. longum* should increase significantly the inhibition of *Pseudomonas fluorescens* in soft cheese.

Although most treatments eventually showed evidence of spoilage by the end of the storage period, it was still evident that in the treatments containing potassium sorbate 0.1 and 0.2% with *B. longum* it is usually took longer for the soft cheese to exhibit spoilage defects (33 days). Sorbate above 0.1% although highly effective, cause unobjectionable sweet flavor, a condition that can acts as an effective check against excessive use of the preservatives [9].

In conclusion, potassium sorbate at concentration of 0.1% used in mixture with *B. longum* was very effective at controlling growth of spoilage organisms in refrigerated soft cheese.

REFERENCES

1. Burkepile, D.E., J.D. Parker, C.B. Woodson, H.J. Mills, J. Kubanek, P.A. Sobecky and M.E. Hay, 2006. Chemically mediated competition between microbes and animals: microbes as consumers in food webs. Ecol., 87: 2821-2831.
2. Boor, K. and H. Fromm, 2006. Managing microbial spoilage in the dairy industry, pp: 171-193. In: Blackburn C. deW (eds.), *Food Spoilage Microorganisms*. CRC Press LLC, Boca Raton FL.
3. Dogan, B. and K.J. Boor, 2003. Genetic diversity and spoilage potentials among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. Appl. Environ. Microbiol., 69: 130-138.
4. Mayr, R., K. Gutser, M. Busse and H. Seiler, 2004. Gram positive non-sporeforming recontaminants afrequent spoilage organisms of German retail ESL (extended shelf life) milk. Milchwissenschaft, 59: 262-266.
5. Stevenson, R.G., M.T. Rowe, G.B. Wisdom and D. Kilpatrick, 2003. Growth kinetics and hydrolytic enzyme production of Pseudomonas spp isolated from pasteurized milk. J. Dairy Res., 70: 293-296.
6. Saad, B., M.F. Bari, M.I. Saleh, K. Ahmad and M.K.M. Talib, 2005. Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatography. J. Chromatography A., 1073: 393-397.
7. Mota, F.J.M., Ferreira Implovo, S.C. Cunha, M. Beatriz and P.P. Oliveira, 2003. Optimisation of extraction procedures for analysis of benzoic and sorbic acids in foodstuffs. Food Chemistry, 82: 469-473.

8. Mihyar, G.F., A.K. Yousif and M.I. Yamani, 1999. Determination of benzoic and sorbic acids in labaneh by high-performance liquid chromatography. J. Food Composition and Analysis, 12: 53-61.
9. Igoe, R.S. and Y.H. Hui, 1996. Dictionary of Food Ingredients - 3rd ed. Chapman & Hall, New York.
10. Kristo, E.C., G. Biliaderis and N. Tzanetakis, 2003. Modelling of rheological, microbiological and acidification properties of a fermented milk product containing a probiotic strain of *Lactobacillus paracasei*. International Dairy J., pp: 517-528.
11. Saarela, M., G. Mogensen, R. Fonden, J. Matto and T. Mattila-Sandholm, 2000. Probiotic bacteria: safety, functional and technological properties. J. Biotechnol., 84: 197-215.
12. Denis, R., 2005. Technological aspects related to the use of bifidobacteria in dairy products. A review. Lait, 85: 39-56.
13. Fuller, R., 1997. Modification of the intestinal microflora using probiotics and prebiotics. Scand J. Gastroenterol Suppl., 222: 28-31.
14. APHA (American public health association), 1992. Standard Methods for Examination of Dairy Products, 16th Ed, American Public Health Association, Washington, DC.
15. SAS Institute, 1999. User's Guide: Statistics. Version 8 Edition. SAS Inst. Inc., Cary, NC.
16. FDA u.s. Food and Drug Administration, 2004. Food ingredient and colors. International food information council (IFIC).
17. Stanton, C., G. Gardiner, P.B. Lynch, J.K. Collins, G. Fitzgerald and R.P. Salminen, 1998. Probiotic cheese. International Dairy J., 8: 491-496.
19. Gilliland, S.E. and H.R. Ewell, 1983. Influence of combinations of *Lactobacillus lactis* and potassium sorbate on growth of psychrotrophs in raw milk. J. Dairy Sci., 66: 974-980.
18. Brashears, M.M., S.R. Reilly and S.E. Gilliland, 1998. Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157:H7 on refrigerated raw chicken meat. J. Food Prot., 61: 166-170.
20. Al-Dagal, M., M. Bazaraa and A. Wael, 1999. Extension of Shelf Life of Whole and Peeled Shrimp with Organic Acid Salts and Bifidobacteria. J. Food Protection, 62: 51-56.
21. Lengkey, H.A.W. and L. Adriani, 2009. Effect of milk fermented with *Lactobacillus acidophilus* and Bifidobacterium spp., on lactic acid and acetic acid content and on *staphylococcus aureus* and *pseudomonas aeruginosa*. Biotechnology in animal Husbandry, 25: 719-724.
22. Sonia, P., B. Irineu, M. Leonor Nunes and M. Fernando Bernardo, 2004. Desalted Cod Spoilage Flora Inhibition by Citric Acid and Potassium Sorbate Combination. Journal of Aquatic Food Product Technol., 13: 25-37.
23. Neugebauer, K.A. and S.E. Gilliland, 2005. Antagonistic action of *Lactobacillus delbrueckii* ssp. *lactis* RM2-5 toward spoilage organisms in cottage cheese. J. Dairy Sci., 88: 1335-1341.
24. Gilliland, S.E. and H.R. Ewell, 1983. Influence of combination of lactobacillus lactis an potassium sorbate on growth of Psychrotrophs in raw milk. J. Dairy Sci., 66: 974-980.