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Effect of Lactulose and Inulin on Physicochemical and Microbial Properties of Synbiotic Yogurt

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ABSTRACT

Over past years, a dairy product called probiotic yogurt has been manufactured known as a functional food. In order to increase activity and improve growth and survival of probiotics and to improve technological properties of probiotic yogurt, prebiotics are added to its formulation. The aim of this study was to evaluate the effect of prebiotics (lactulose and inulin individually, and as mixture) on physicochemical properties and survival of Lactobacillus casei in synbiotic yogurt. The samples were examined for pH value, acidity, syneresis and probiotic bactria counts on 1,7,14 and 21 days of cold storage and then were compared to the control sample (probiotic yogurt without prebiotics).The results showed that the highest and the lowest pH value measured in the samples containing lactulose and lactulose-inulin, respectively. The control sample and the sample containing lactulose-inulin mixture showed the highest and the lowest acidity, respectively. Control sample and the sample containing lactulose-inulin showed the highest and the lowest syneresis on 1 d, respectively. At the end of storage period, yogurt containing lactulose-inulin showed the lowest syneresis, having a significant difference ($p<0.05$) as compared with other samples. Viability of probiotic bacteria showed an ascending trend by the end of second week, but it declined from 14 d to 21 d. The highest and the lowest probiotic bacteria counts observed in the sample containing lactulose-inulin and the control sample, respectively.

Key words: *Lactulose, Inulin, Lactobacillus casei, Synbiotic Yogurt*

INTRODUCTION

In recent years, there has been an increasing trend for consuming foods with functional properties additional to their nutritional value [1]. Yogurt is one of the best known food products that containing probiotics and increasingly yogurts are being supplemented with prebiotics by manufacturers [2]. Ever since yogurt was proposed as a health-promoting food by Metchnikoff, the oldest and still most widely used way to increase the numbers of advantageous bacteria in the intestinal tract has been the direct consumption of live bacteria [3]. Probiotics are living, health-promoting microorganisms that are incorporated into different kinds of foods [4]. Yogurt that contains probiotic bacteria such as, Lactobacillus and bifidobacteria is becoming popular due to the health-promoting properties of the probiotics [5]. Probiotics have been used therapeutically to modulate immunity [6], production of antibacterial substances, e.g., organic acids, bacteriocins, hydrogen proxide [7], reducing the level of blood cholesterol and allergic reactions [8], preventing from all types of cancer specially colon cancer [9], improving intestinal microflora balance [10], alleviation from lactose intolerance [1], and preventing from growth and activity of pathogenic bacteria [11]. Prebiotics are substances that are used by specific bacteria as carbon or energy sources, thus they may be added to the medium to increase growth and survival of bacteria [12]. Inulin, a non-digestable carbohydrate containing naturally-occurring fructooligosaccharides, possesses some properties of dietary fibres. Besides its health

benefits, inulin is also considered to have prebiotic properties such as the ability to stimulate probiotic bacteria without adversely affecting flavor [13].

Lactulose is a disaccharide consisting of galactose and fructose resulting from milk heating process or alkaline isomerization of lactose [14]. It stimulates growth and activity of Bifidobacterium species so it has been known as bifidus factor [15]. Some studies showed that in most cases lactulose increases bifidobacteria counts, while fructooligosaccarides stimulate lactobacilli growth. Prebiotics have positive effect on both probiotics and starter bacteria [16]. Reduced viability of probiotics during storage is one of the problems of probiotic products, especially acidic ones such as yogurt. There are some techniques to improve the viability of probiotic bacteria, among them the most important is the use of prebiotics. The aim of this study was to determine the contribution of prebiotics to improving qualitative properties and survival of probiotics in synbiotic yogurt.

MATERIALS AND METHODS

Materials

Raw milk containing about 2.5% fat was purchased from a dairy farm, Kamalshahr, Karaj. Yogurt starter YC-X11 containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* and probiotic mono-strain culture of *Lactobacillus casei LC-01*, (freez-dried and DVS), were purchased from CHR. Hansen, Denmark. Prebiotics including lactulose and inulin were purchased from Buffalo, Us; Flocca, Swiss; and Mellaleosa, US, respectively.

Primary culture preparation

To prepare the primary culture, 2L of raw milk was heated at 80-85^{0}C for 15-20 min. Then the heated milk was transferred to two 1-L erlene – meyerflasks, then yogurt starter YC-X11 (50 unit) was added to one of the erlene-meyer flasks and *Lactobacillus casei LC-01* (25g) was added to other flask. They were incubated at 4^{0}C for 12h. At the end they were refrigerated [17].

Synbiotic yogurt production

To produce synbiotic yogurt, 250-mL pasteurized milk (2.5% fat) containing dried skimmed milk (1.5%) was inoculated simultaneously with 120 µl of yogurt starter and 140µl of probiotic bacterium. In the next stage, prebiotics (1.5%) were separately added. Then the samples were incubated at 40^{0}C. When pH value of the samples reached 4.5 – 4.7, they were refrigerated. It should be noted that control samples also were inoculated with the starters and probiotic bacterium at the above–mentioned ratios, but it contained no prebiotic compound [17].

Physicochemical analysis

pH value of samples was measured using pH-meter (Swiss, Metrohm 632) at 25^{0}C. Titratable acidity was determined by AOAC method [18]. Syneresis was measured according to Gonzalez–Martinez et al. method. To measure syneresis, first, 25g of yogurt was weighed in centrifuge tubes. Then the tubes were centrifuged in 350 G at 10^{0}C for 30 min. The headspace liquid separated from the sample was removed and the tubes were re-weighed. Syneresis rate was expressed as lost water per 100g of yogurt [19].

Microbial analysis

MRS vancomycin agar was used for the selective enumeration of probiotic bacteria in the presence of yogurt bacteria. The plates were incubated anaerobically at 37°C for at least 72 h [20].

RESULTS AND DISCUSSION

Variation curve of pH value of synbiotic yogurt samples during refrigeration is presented in Figure 1. L, I, LI and C indicated the samples containing lactulose, inulin, the mixture of lactulose-inulin and control sample, respectively. Variation trend of pH value of samples is descending as it reduced significantly during 3w storage. The sample containing lactulose-inulin mixture showed the lowest pH value at the end of storage, while the slope of this curve is more compared to the other samples. The sample containing lactulose showed the highest pH value having significant difference ($p<0.05$) from the control sample. Figure 1 shows the pH variation curve of probiotic yogurt samples during refrigeration.

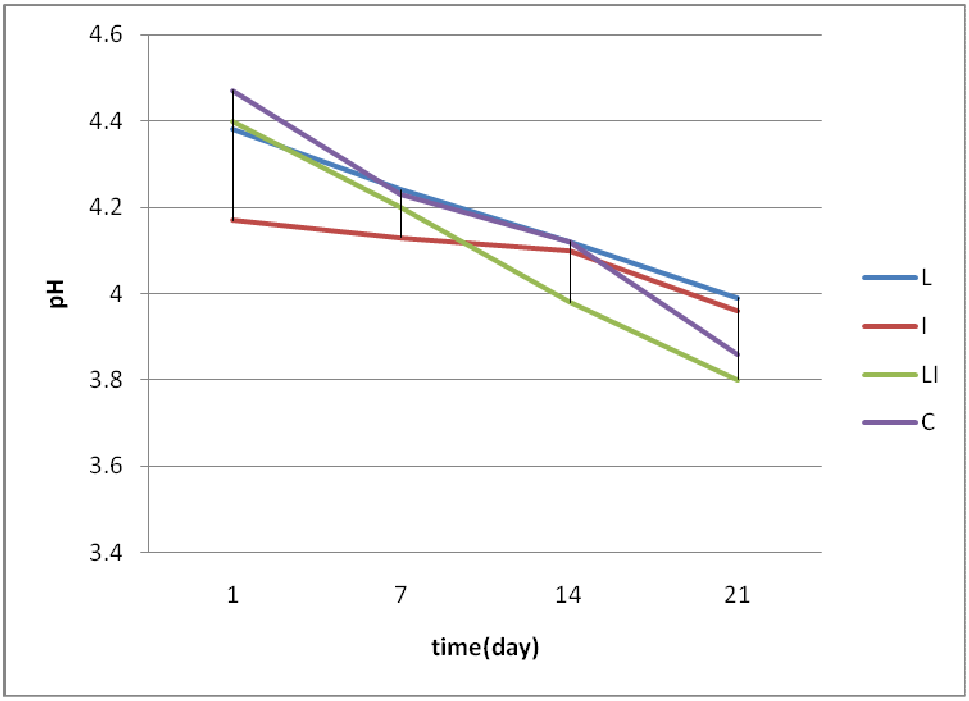

Figure 1: Variation curve of pH value of synbiotic yogurt samples during refrigeration

Figure 2 shows the variation curve of acidity of the samples during refrigeration. In contrast to pH curve, the variation trend of acidity was ascending as it increased significantly ($p<0.05$) during 3 w storage.

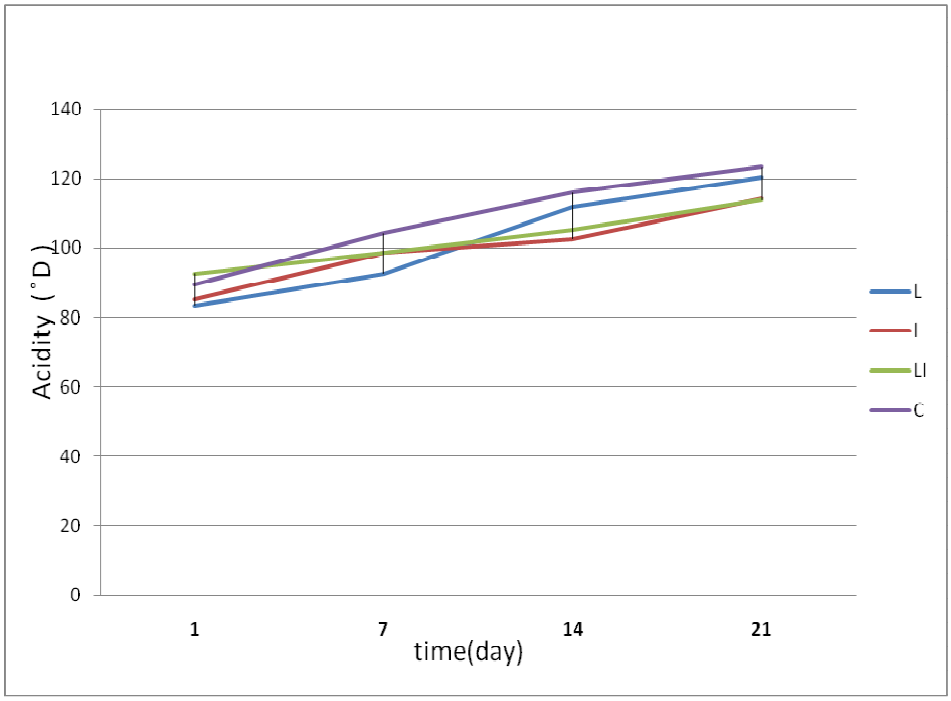

Figure 2: Variation curve of acidity of synbiotic yogurt samples during refrigeration

The control sample showed higher acidity compared to the other yogurt samples. Also, variation trend of acidity in the sample containing lactulose-inulin was moderate, i.e its sourness was less than the other samples. This sample had the lowest acidity on 21 d which had significant difference ($p<0.05$) from the control sample.

The results from syneresis of synbiotic yogurt samples over storage are presented in Table 1.

Table 1: Syneresis of the samples (%) during cold storage (values are means ± SD)

Yogurt samples	1th day	7th day	14th day	21th day
L	26.35 ± 0.012[a]	27.05 ± 0.089[a]	29.21 ± 0.180[a]	34.64 ± 0.084[a]
I	24.53 ± 0.076[c]	26.94 ± 0.089[b]	28.73 ± 0.180[b]	34.61 ± 0.084[a]
LI	24.79 ± 0.076[b]	27.04 ± 0.089[a]	28.67 ± 0.180[b]	33.45 ± 0.084[b]
C	24.15 ± 0.076[d]	25.05 ± 0.089[c]	29.43 ± 0.180[a]	34.56 ± 0.084[a]

** Values in the same row shown with similar letters are not significantly different.*

As Table 1 shows, the control sample and the sample containing lactulose-inulin showed the lowest and the highest synersis on 1 d, respectively.This situation continued by the end of 1st week. Control sample represented the highest syneresis at the end of second weed, showing a significant difference ($p<0.05$) with the samples containing inulin and inuline-lactulose mixture. At the end of storage period, the sample containing inulin-lactulose had a significant difference ($p<0.05$) from the other samples. Syneresis of this sample was the lowest.

Table 2 indicates probiotic bacteria count in synbiotic yogurt over refrigeratuion. Probiotic bacteria count increased significantly ($p<0.05$) from 1 d to the end of 2th week. In contrast, probiotic bacteria of control sample decreased as three logarithmic cycles at 3rd week and 21d, while this decrease was two logarithmic cycles for the other samples. The sample containing lactulose-inulin mixture had the highest bacteria count at the end of refrigeration. The sample containing inulin showed no significant difference from the sample containing lactulose. The obtained results suggest that as the storage period increases to 21 d, pH value of probiotic yogurt samples decreases significantly ($p<0.05$).

Table 2: Probiotic bacteria counts (log cfu/mL) of synbiotic yogurt samples during cold storage (values Means± SD)

Yogurt samples	1th day	7th day	14th day	21th day
L	8.18 ± 0.001[b]	8.33 ± 0.005[b]	8.40 ± 0.004[a]	6.18 ± 0.009[b]
I	8.27 ± 0.013[a]	8.36 ± 0.005[a]	8.44 ± 0.004[a]	6.20 ± 0.009[b]
LI	8.23 ± 0.013[a]	8.33 ± 0.005[b]	8.45 ± 0.004[a]	6.27 ± 0.009[a]
C	8.27 ± 0.013[a]	8.37 ± 0.005[a]	8.27 ± 0.004[c]	5.78 ± 0.009[c]

** Values in the same row shown with similar letters are not significantly different.*

In this study, probiotic bacteria were added prior to fermentation. Among the yogurt samples, the sample containing lactulose-inulin mixture had the lowest pH value. The investigations have shown that lactulose has no effect on acidity development and lowering pH value, while lactulose–inulin mixture reduces pH value significantly because inulin stimulates growth and activity of yogurt starter cultures and probiotic bacteria [21]. Significantly reduced pH value of condensed milk using permeate in the presence of lactulose-inulin has been reported by some researchers. Investigations showed that addition of inulin to probiotic yogurt could promote lactic acid production [22]. Reduced fermentation time and increased acid production using inulin in yogurt have been confirmed by some researchers [23].

Some researchers used commercially available cultures and evaluated acidification in the yogurt samples over storage for 2 w in 6°C. The results suggested increased titratable acidity at 3.22 % in the samples [24]. The results showed that the longer the storage duration, the more acidity of yogurt. Syneresis in gel means separation of aqueous phase from continuous phase namely gel network. The most important factors affecting yogurt syneresis include fat percentage, starter bacteria characteristics, solids not fat, exopolysaccharides production, addition of fibers and stabilizers, temperature of fermentation and pH value of the product [17]. In general, the longer the storage duration of control or probiotic yogurt, the more syneresis percentage, while as the results shows, synbiotic yogurt shows less syneresis. Reduced syneresis percentage of probiotic yogurt in the presence of prebiotic compounds has been confirmed in another study [25]. The sample containing lactulose-inulin mixture had lower pH value compared to the other samples. It can be the result of reduction in syneresis. But yogurt gel with a low pH value is susceptible to a mechanical tension, facilitating its reduced syneresis of synbiotic yogurt is increased firmness and water complex capacity index, making prebiotics enable to bind water firmly to reduce syneresis [26].

Viability of probiotics in food products is the most important consideration in fermented milk products. The most important factors affecting viability of probiotics include the used microbial species, milk total solids, the nutrients, soluble oxygen (especially for Bifiodobacterium), inoculum level and temperature, and fermentation time [27].

As indicated in Table 2, the sample containing lactulose-inuline showed the highest viability of probiotic bacteria on 21 d, having a significant difference ($p<0.05$) from the control sample which showed the lowest probiotic bacteria count at the end of storage period. Despite low pH of the sample containing lactulose-inulin mixture, this sample had the highest live bacteria on 21 d.

CONCLUSION

Lactulose-inulin mixture could be improved viability of probiotics in synbiotic yogurt. The sample containing lactulose-inulin had the lowest syneresis and acidity.

REFERENCES

[1] Hekmat S., Reid G. *Nutrition Research*, **2006**, 26, 163-166.
[2] Laparra J.S., Tako E., Glahn R.P., Miller D.D. *Food Chemistry*, **2008**, 109, 122–128.
[3] Saarela M., Lahteenma L., Crittenden R., Salminen S., Mattila-Sandholm T. *International Journal of Food Microbiology*, **2002**, 78, 99-117.
[4] Chukeatirote E. *Songklanakarin Journal Science Technology*, **2003**, 25(2), 275-282.
[5] Farnsworth J.P., Hendricks J.L., Guoa M.R. *Small Ruminant Research*, **2006**, 65,113–121.
[6] Khan S.H., Ansari F.A. *Pakistan Journal Pharmacology Science*, **2007**, 20(1), 71-76.
[7] Grajek W., Olejnik A., Sip A. *Acta Biochemica Polonica*, **2005**, 52(3), 665-671.
[8] Kaur I.P., Chopra K., Saini A. *European Journal of Pharmaceutical Sciences*, **2002**, 15, 1–9.
[9] Suvarna V.C., Boby V.U. *Current Science*, **2005**, 88(11), 1744-1748.
[10] Rasdhari M., Parekh T., Dave N., Patel V., Subhash R. *Pakistan Journal of Biological Sciences*, **2008**, 11(17), 2101-2108.
[11] Liptakova1 D., Valik1 L., Laukova A., Strompfova V. Czech *Journal of Food Science*, **2007**, 25, 272–282.
[12] Stanton C., Desmond C., Fitzgerald G.F., Collins K., Ross R.P. *International Dairy Journal*, **2005**, 12, 183-190.
[13] Akın M.B., Akın M.S., Kirmac Z. *Food Chemistry*, **2007**, 104, 93–99.
[14] Thammarutwasik P., Hongpattarakera T., Chantachum S., Kijroongrojana K., Itharat A., Reanmongkol W., Tewtrakul S., Buncha O. *Songklanakarin Journal of Science and Technology*, **2009**, 31(4), 1-8.
[15] Matijevic B., Bozanic R., Tratnik L. *Mljekarstvo*, **2009**, 59(1), 20-27.
[16] Kosin B., Rakshit S.K. *Food Technology*, **2006**, 44(3), 371-379.
[17] Aghajani A.R., Pourahmad R., Mahdavi Adeli H.R. *Journal of Food Biosciences and Technology*, **2012**, 2, 13-822.
[18] AOAC. Official methods of analysis of the AOAC, 15th ed. (Ed.S.Williams). Arlington, USA: Association of Official Analytical Chemists, **2002**.
[19] Gonzalez– Martinez C., Becerra M., Chafer M., Albors A., Carot J.M., Chiralt A. *Trends Food Science and Technology*, **2002**, 13, 334-340.
[20] Tharmaraj N., Shah N.P. *Journal of Dairy Science*, **2003**, 86, 2288-2296.
[21] Tabatabaie F., Mortazavi A. *World Applied Sciences Journal*, **2008**, 3(1), 88-90.
[22] Donkor O.N., Nilmini S.L.I., Stolic P., Vasilgevic T., Shah N.P. *International Dairy Journal*, **2007**, 17, 92-151.
[23] Oliveira R.P.S., Perego P., Converti A., Oliveira M.N. *Journal of Food Science*, **2009**, 14, 1-7.
[24] Kneifel W., Jaros D., Erhard F. *International Journal of Food Microbiology*, **1993**, 18, 179-189.
[25] Paseephol T. (2008) Characterisation of prebiotic compounds from plant sources & food industry wastes. Inulin from Jerusalem artichoke and lactulose from milk concentration permeate, **2008**, 1-21.
[26] Mortazavian A., Razavi S.H., Sohrabvandi S. *Iranian Journal of Biotechnology*, **2007**, 5(1), 1-18.
[27] Lourens-Hattingh A., Viljoen B.C. *International Dairy Journal*, **2001**, 11(1-2), 1-17.