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The Effect of Oligofructose, Lactulose and Inulin Mixture as Prebiotic on Physicochemical Properties of Synbiotic Yogurt

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ABSTRACT: Desirability and good background of yogurt have led the manufacturers to use this product mostly for producing milk-based probiotic products. The aim of this study was to evalutate the effect of a mixture of prebiotics including oligofructose, lactulose, and inulin on physicochemical properties (pH value, acidity, and syneresis) of synbiotic yogurt containing *lactobacillus casei*. Pasteurized milk was inoculated with certain amount of starter, prebiotic, and probiotic bacteria, then yogurt samples were prepared, and at 1, 7, 14 and 21 d, pH value, acidity, and syneresis were measured by pHmeter, titration method, and centrifuge, respectively at certain intervals. The results showed that the sample containing the mixture of lactulose, inulin and oligofructose (LIO) had the lowest pH value, highest acidity and syneresis. In contrast, the sample containing lactulose and inulin mixture (LI) had the lowest acidity and syneresis showing a significant difference from the control (probiotic yogurt without prebiotic). The variation trend of acidity and syneresis was rising and the sample containing lactulose and inulin (LI) mixture contained sample was selected as the best synbiotic yogurt sample.

Keywords: *Inulin, Lactobacillus casei, Lactulose, Oligofructose*.

Introduction

Yogurt is one of the most popular milk products produced through lactic fermentation by two starter bacteria, *lactobacillus delbrueckii subsp. Bulgaricus* and *streptococcus thermophilus*. This product is the best well-known carrier of probiotic organisms transferring them to the consumers (Zacarchenco & Massagur – Roing, 2006). Probiotics have been introduced as living effects and optimum concentration (Stanton *et al*., 2005). The most common probiotic bacteria belong to lactobacillus and bifidobacterium genera. *Lactobacillus casei* is a positive – gram, negative – catalase, mesophyl, microaerophil, and non-producing spore bacterium (Iyer & Hittinahalli, 2008). *Lactobacillus casei* is characterized by antioxidant property, activity against some bacteria such as *E.coli*, *staphylococcus aureus*, *salmonella thyphimorium*, high resistance to antibiotics vancomycin and ampicillin (Xanthopulos *et al*., 2000), growth and activity at all of suger – based media (tharmaraj & Shah, 2003), high stability in fermented milk products such as yogurt during storage (Khan & Ansari, 2007), boosting body immune system by producing cytokinins (moller & vrese, 2004) and preventing from metastasis of bladder cancer (Khurana & Kanawjia, 2007).

The activity of *L. casei* is higher than the other lactobacilli found in fermented milk products and it has been able to ferment a wide range of carbohydrates contained in the

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medium (Vahcic & Hruskar, 2000). *L. casei* is added to yogurt as a probiotic starter, improving the technological and nutritional properties of the final product (Matsuzaki, 2003). Prebiotics are indigestible food compounds having beneficial effects on the host through selective stimulating growth or activity of one or more bacteria in the intestine (Guarner, 2008). These compounds are considered as the second factor, following probiotics for controlling intestinal flora (Crittenden *et al*., 2005). Applying prebiotics in food products has resulted in increased bioavailability, stimulate the growth and the activity of probiotics (Stanton *et al*., 2005; Cumminges *et al*., 2004), improve the texture (Short, 2004; Tungland, 2003), mend the mouthfeel (Pereira & Gibson, 2002), generate the creamy texture (Tamime, 2005) and increase the amount of short chain fatty acids (Kai, 2007). Prebiotics also lead to reduce cholesterol level of blood serum (Lim *et al*., 2004), Prevent the reduction of bone density, increase magnesium, iron and especially calcium absorption resulting in increased mineral density of bones, decrease the activity of enzymes converting pre-carcinogenic to carcinogenic matters (Scholz–Ahrens *et al*., 2001), prevent from tumor formation in colon (Roller et al.,2004), reduce the incidence of diabetes (Tamime, 2005), increase laxative properties (Salminen *et al*., 2000) ,prevent from colon cancer incidence and boost body immune system(Crittenden *et al*., 2005).

Lactulose, inulin and oligofructose are among the most important prebiotics used in food products especially fermented milk products such as yogurt (outwehant, 2007). Lactulose consists of galactose and fructose produced from lactose through heat processing or alkaline isomerization of milk (thammarutwasik *et al*., 2009).Inulin and oligofructose are indigestible fermentable fructans resulting in increased calcium absorption, consequently improved bone density (Bosscher *et al*., 2006), reduced serum cholestrerol level (lopez–Molina *et al*., 2005), increased bioavailability and stimulated growth and activity of probiotics (Gibson, 2004). Various studies suggest the effective role of prebiotics in formulations of food products (Roller *et al*., 2004).The results of some research have shown that fructooligosaccarides predominantly stimulated lactobacilli growth, while lactulose may result in increased number of bifidabacteria (kosin & Rakshit, 2006). A food product containing both probiotic bacteria and prebiotics is called synbiotic. Synbiotic products have more beneficial effects on the health of consumers, in addition, in these products, the viability of probiotic bacteria increases over storage and passage through digestive system (Yeganehzad *et al*., 2007). The aim of this study is to evaluate the effect of oligafructos, lactolose and inulin mixtures as probiotic on physicochemical properties of symbiotic yogurt.

Materials and Methods

Crude milk containing 2.5% fat was purchased from a dairy farm, kamalshahr, karaj. Microbial strains consisting of combined culture of yoghurt YC-x11 containing *lactobacillus delbrueckii subsp bulgaricus* and *streptococcus thermoplilus* and probiotic mono-strain culture of *lactobacillus casei LC-01*, both freeze-dried and of DVS, were procured from CHR Hansen, Denmark. Prebiotics including lactulose, inulin and oligofructose were purchased from Buffalo, US; Flocca, Swiss; and Mellaleosa, US, respectively.

- *Primary culture preparation*

To prepare the primary culture, 2L of crude milk was heated at 80-85^{0}C for 15-20 min. The heated milk was transferred to two 1-L flasks, and then culture powder (50 unit) containing yoghurt starters were added to one of the flasks and the powder (25g) containing prebiotic bacterium *lactobacillus casei Lc-01* was added then incubated at 4^{0}C for 12h and finally at the end they were refrigerated (Aghajani *et al*., 2011).

- Synbiotic yoghurt production

To produce synbiotic yoghurt, 250-mL sterile containers containing pasteurized milk (2.5% fat) and dried skimmed milk (1.5% fat) were inoculated simultaneously with 120 µl of the starters and 140µl of prebiotic bacterium. In the next stage, prebiotics (1.5%) were separately added and then incubated at 4^0C. When the pH value of the samples reached 4.5 – 4.7, they were refrigerated. It should be noted that the control samples were inoculated with the starters and prebiotic bacterium at the above – mentioned ratios, but it contained prebiotic compounds (Aghajani *et al*., 2011).

- Treatments

Treatments LI, LO, IO, LIO, and C represent yogurt containing lactulose and inulin, lactulose and oligofructose, inulin and oligofructose and the mixture of lactulose,inulin and oligofructose and the control (without prebiotics). The fermented samples were refrigerated at 4C and then tested at 1 (following overnight), 7, 14, and 21 days.

- Experimental factors:

-pH measurement

pH value of the samples were measured using pH meter (Swiss, Metrohm 632) at 25^0C (AOAC 2002: 981.12).

- Acidity measurement

Acidity was measured based on Dornic degree using 1.9N solution of sodium hydroxide and phenolphthalein indicator as reagents (AOAC 2002: 947.05).

- Syneresis or serum separation measurement

To measure syneresis, 25g of yoghurt is weighed in a centrifuge tube, and the tube is centrifuged at 350 r G and 10^0C for 30 min. The top liquid is separated from the sample and is removed and the tube is re-weighed. Syneresis rate was expressed as lost water per 100g of yoghurt (Gonzalez–Martinez *et al*., 2002).

- Statistical analysis

All the experiments were conducted in triplicate order. The results were statically analyzed by one-way analysis of variance (ANOVA). The mean comparison was carried out with Duncan’s multiple range tests using SPSS for Windows version 18.0. Significant levels were defined using the value $p<0.05$.

Results and Discussion

Mean pH values of synbiotic yogurt samples once fermented are presented in Figure 1.

As shown in Figure 1, C and IO samples had the lowest and the highest pH values respectively. In contrast LI and C samples had the lowest and the highest acidity. It is clear from figure 1 that the pH values have declined slowly until the end of the 1st week but thereafter reduced significantly. For example, IO and LIO samples declined faster than the others, where as it was slower in LO sample. At 1st day, only pH value of IO was significantly different from the others including the control ($p<0.05$) and there were no significant differences among the samples. At 7th day LI and LIO did not show any significant differences from the control sample, while LO and IO samples were significantly different from each other and from the control sample ($P < 0.05$). LO and LIO had the highest and the lowest mean pH values, respectively. At 14th day, there were no significant differences between LO and the control samples, while the other samples had significant differences from the control ($p<0.05$). LIO had the lowest mean pH value. At 21th day, there were no significant differences between LI

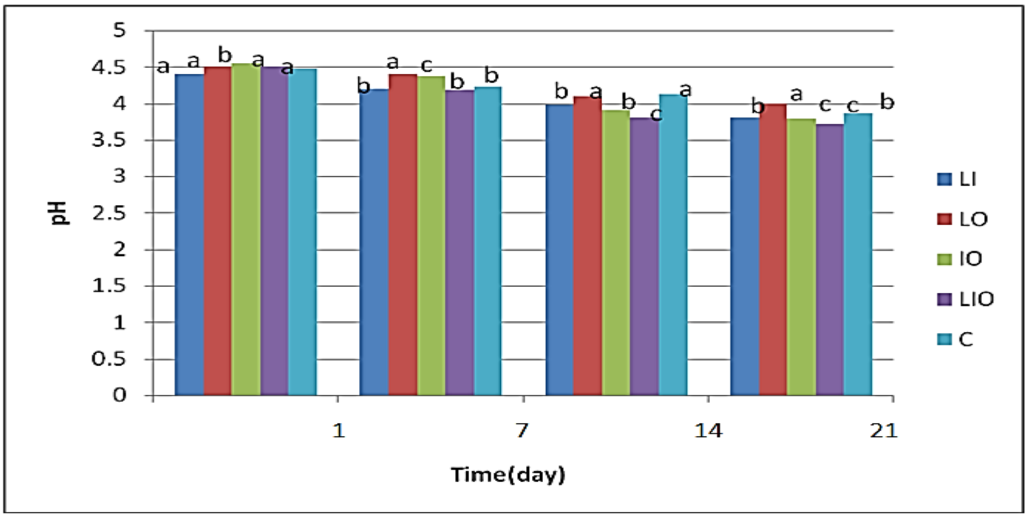

Fig. 1. pH value of synbiotic yogurt samples over storage

and control as well as IO and LIO. At the end of storage, LIO and LO showed the lowest and the highest mean pH values.

It has been proved that when probiotic microorganisms are added to the fermented products after fermentation, the rate of growth and reproduction of bacteria are reduced. In this study, probiotic microorganisms were added prior to the fermentation allowing them to become more compatible to milk environment. Among the samples of produced yogurt the mixture of lactulose inulin and oligofructose showed the lowest pH values. Vraious investigations have shown that the activity of starter bacteria resulted in significant decline of pH over 21days storage (Yeganehzad *et al*., 2007). The research has revealed that lactulose does not influence acidification and pH declining while a mixture of lactulose – inulin significantly reduced pH value. The reason is that inulin stimulates growth and activity of starter as well as probiotic bacteria resulting in remarkable acid production (Tabatabaei & Mortazavi, 2008). pH declining in consentrated milk using permeate in the presence of lactulose – inulin has been reported by some researchers. The investigations showed that inulin added to probiotic yogurt caused an increase in lactic acid production (Donkor, 2007).Acidity variations of the samples over storage are presented in Figure 2.

In contrast to pH, acidity of synbiotic yogurt samples significantly increased over time. For example at the 1st week of storage the acidity of IO sample was lower than the other samples whereas at the end of the storage its acidity (128.00±0.029) showed the highest (except for LIO) suggesting a significant difference from control ($p<0.05$), At 1st day IO and LI samples showed the lowest and the highest acidity respectively, with a significant difference from the control ($p<0.05$). LIO had the highest acidity at the end of the storage, showing a significant difference from the control ($P<0.05$). At 21st day LI sample had the lowest acidity. Increased acidity of probiotic yogurt was the result of two starters and probiotic bacteria growth over the storage. The results of some researchers have proven a significant increase in acidity of probiotic yogurt over storage (Vahic & Hruskar, 2000). The other investigations have also shown the activity of bacteria until the end of storage period (Aklain *et al*., 2004).On the other hand, some compounds such as prebiotics might relatively increase acidity. In a study reduced time of fermentation and increased acidification in yogurt in the presesence of inulin was reported (oliveira *et al*., 2009).

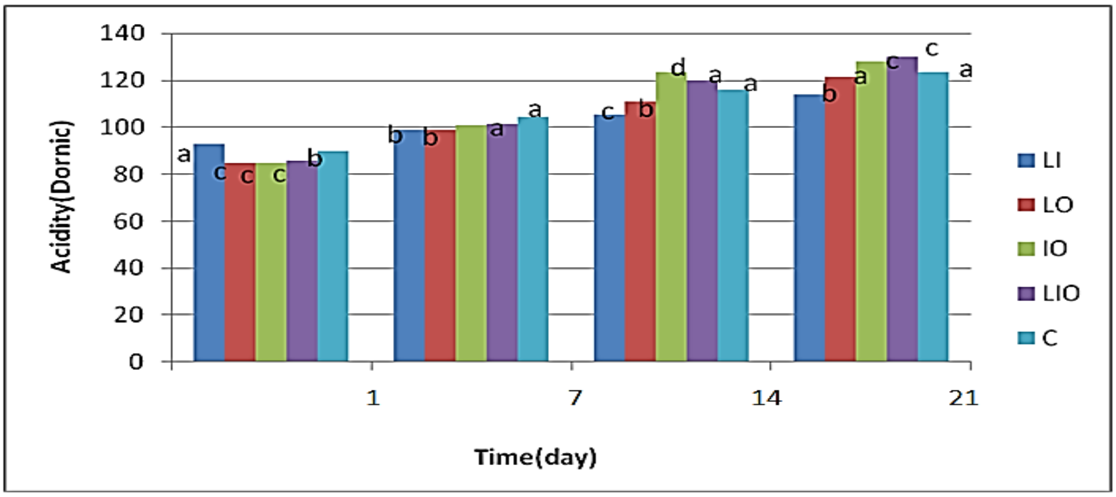

Fig. 2. Acidity of synbiotic yogurt samples over storage

When selecting starter and probiotic bacteria to produce fermented milk products the ability of producing acceptable level of acid with a minimum incubation time is the most important consideration.

One of the best recommended ways for reducing the time of fermentation and increasing acidification is using an auxiliary culture such as starters along with probiotic bacteria which also is considered in this study. In some research commercially available cultures were applied and the properties of acidification of yogurt samples at 6C for 22 week were avaluated. The results suggested the increased titratable acidity of the sample up to 3.22% (kneifel *et al*., 1993). The results of this study also showed that extending the storage period resulted in increased acidity. LIO sample had the lowest pH value and the highest acidity i.e. it was the most acidic sample at the end of storage. A reason for this increase might be the stimulated growth and activity of lactobacillus casei by three prebiotic compounds contained in LIO sample.

The results of evaluating syneresis of synbiotic yogurt samples over the storage are presented in Figure 3.

As shown in figure 3, the trend of synersis was rising over the time till the end of 21st day. At the end of 1st week, IO sample had the highest percentage of syneresis showing a significant difference from the control sample (P<0.05). At the 1st and the 7th days, control sample had the lowest percentage of synersis while at the 14th days this sample showed the highest percentage. At the 21st days, LI and LIO samples showed the lowest and the highest percentages of syneresis respectively.

Synersis or whey separation and its transferring to the surface of yogurt is the main quality problem of this product however, it is possible to reduce this by increasing solid matters of milk to 15% (Shah, 2003), using stabilizers, prebiotics or starters producing exopolysaccarides (Amatayakul *et al*., 2006). This is caused by a unstable gel network with a continuous changing arrangement. This results in weak traping of serum phase in the gel network and consequence separation of serum phase (Tamime & Robinson,1999). Synersis phenomenon is directly related to other factors such as the level of physical disturbance, careless milk ripening including uncontrolled temperature during incubation and very low pH, resulting in disturbed protein micells (Donkor, 2007; Moller & Vrese, 2004).

As indicated in Figure 3, LI sample had the lowest percentage of syneresis showing a

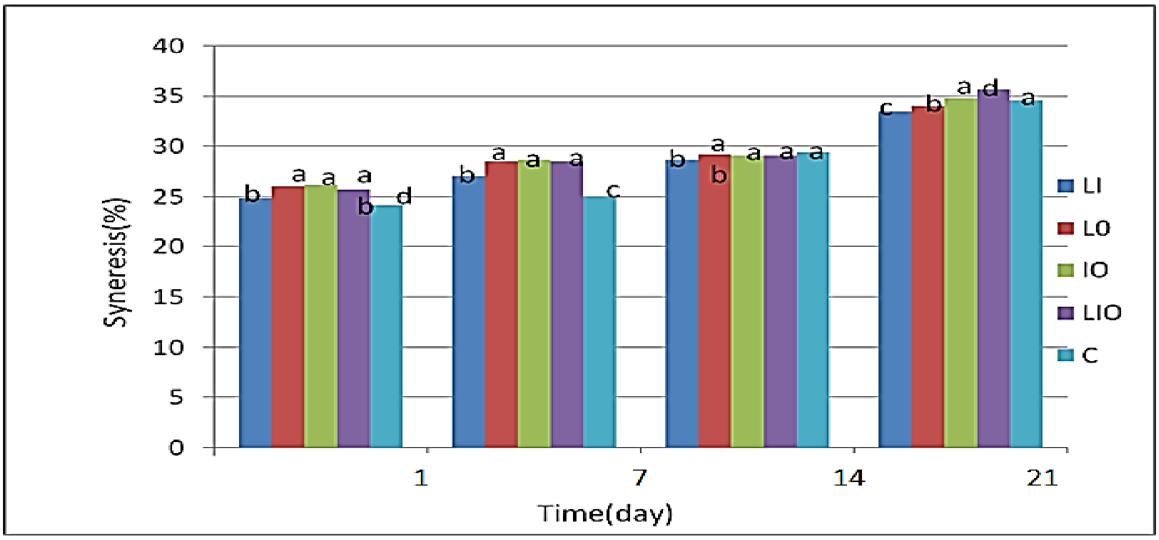

Fig. 3. Syneresis percentage of synbiotic yogurt samples over storage

significant difference from the control ($p<0.05$). In general, as the storage period of ordinary and probiotic yogurt increases the percentage of syneresis tends to rise. However, according to the results of studies, the increase in the percentage in synbiotic yogurt is less than ordinary various yogurt because of the effective role of prebiotics in increasing water holding capacity in the texture (Reid *et al.*, 2003). The results of some studies showed that using prebiotic compounds such as inulin and lactulose at optimum concentrations might reduce the percentage of syneresis.

Conclusion

In this study it was shown that the acidity and the percentage of syneresis have increased with regard to the growth and the activity of starter and probiotic bacteria. The addition of different percentages of prebiotics resulted in slowed trend of variations and improved the quality of the product. The sample containing the mixture of lactulose and inulin was selected as the best synbiotic yogurt sample at the end of storage period.

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