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Evaluation of Physicochemical Changes and Survival of Probiotic Bacteria in Synbiotic Yoghurt

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ABSTRACT: Yoghurt is a popular healthy food, consumed by many people. The popularity of this product made it possible to use it as a base in order to produce probiotic preparations. Prebiotics are used for better growth and survival of probiotic bacteria as well as to improve organoleptic, rheological and technological properties of probiotic yoghurt. The aim of this research was to study physicochemical changes and survival of probiotic bacteria in synbiotic yoghurt. In this study, prebiotics including Lactulose, Inulin and Oligofructose were used separately and probiotic bacterium *Lactobacillus casei* was used to prepare the yoghurt. Samples were stored at 4°C for 21 days and during this time pH, acidity, syneresis and probiotic counts were investigated and compared to the control (probiotic yoghurt without prebiotics). The results showed that the highest pH, the most probiotic counts and the highest taste and texture scores were related to the sample which had Inulin. In contrast the control sample had the least probiotic count and the lowest score concerned with taste and texture. This sample had the least percent of syneresis which wasn't significantly different from others. The most and the least acidity were related to the control sample and the yoghurt containing Lactulose, respectively. The results suggested positive effect of synbiotic in probiotic yoghurt. Based on this study, since prebiotics improve physicochemical properties and enhance probiotic bacteria survival they can be incorporated in yoghurt formulation. The results show that Inulin has a better effect on physicochemical changes and probiotic bacteria survival as compared to other prebiotics.

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

Introduction

Among all the fermented dairy products, yoghurt is the most popular one and has more acceptability worldwide. This product is produced through lactic fermentation by two starter bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Hussain *et al*., 2009).

Probiotics have been introduced as microorganisms which in sufficient amounts have health benefits for the host (Donkor *et al*., 2007). The most important health benefits of probiotics include promoting immune system function (Cross, 2002), reducing serum cholesterol level (Yeganehzed *et al*., 2007), improving lactose digestion, improving calcium absorption, proteins and vitamins synthesis (Heenan *et al*., 2004), preventing different types of cancer especially colon, preventing growth and activity of pathogenic microbes (Tamime, 2005), improving nutritional value, and synthesizing bacteriocines (Mageed & Prakash, 2007). The most common probiotic bacteria belong to genera Lactobacillus and Bifidobacterium.

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Lactobacillus casei is a gram-positive and non-producing spore bacterium. Anti-oxidant activity, high stability in fermented dairy products and anti-microbial activity (Saide & Cilliand, 2005) are the most important properties of this bacteria.

Prebiotics are indigestible nutrients having health promoting benefits for the host through promoting growth or activity one or more bacteria in the colon (Donkor *et al*., 2007). Lactulose, inulin and oligofructose are among the most important prebiotics used in foods products specially fermented dairy products such as yoghurt (Paseephol, 2008). Lactulose is composed of galactose and fructose which is produced from lactose through heat processing of milk or alkaline isomerization (Hussain *et al*., 2009).

Inulin and oligofructose are indigestible and fermentable fructans which promote Ca^{++} absorption resulting in improved bone density, reduced cholesterol level, increased bioavailabity of probiotics and promoting their growth and activity (Thammarutwasik *et al* , 2009; Mattila – Sandholm & Saarela, 2003). Various studies suggest the important role of prebiotics in the formulation of the products (Roller *et al*., 2004). This study is concerned with physicochemical changes and survivability of probiotic bacteria in synbiotic yoghurt.

Materials and Methods

-Materials

Crude milk containing about 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 purchased 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 heated at 80-85°C for 15-20 min. The heated milk is transferred to two 1-L erlene – meyer flasks, then culture powder (50 unit) containing yoghurt starters added to one of the erlene-meyer flasks and the powder (25g) containing probiotic bacterium *Lactobacillus casei Lc-01* was added to were incubated at 4°C for 12h. At the end they were refrigerated.

- Synbiotic yoghurt production

To produce synbiotic yoghurt, 250-mL sterile containers containing pasteurized milk (2.5% fat) and dried skim milk (1.5% fat) were inoculated simultaneously with 120 µl of the starters and 140µl of probiotic bacterium. In the next stage, prebiotics (1.5%) were separately added and then incubated at 4°C.

When pH value of the sample reached 4.5 – 4.7, they were refrigerated. It should be noted that control samples were also inoculated with the starters and probiotic bacterium at the above – mentioned ratios, but it contained no prebiotic compounds.

- Experimental factors

- pH measurement

pH value of samples was measured using pH-meter (Swiss, Metrohm 632) at 25°C (AOAC 2002: 981.12).

- Acidity measurement

Acidity was measured based on Dornic degree (AOAC 2002: 947.05).

- Syneresis or serum separation measurement

To measure syneresis, at first, 25g of yoghurt weighed in centrifuge tubes, then the tubes were centrifuged in 350 G at 10°C for 30 min. The separated liquid from the sample that collected in the top of tube was removed and the tubes were re-weighed.

Syneresis rate was expressed as lost water per 100g of yoghurt (Gonzalez – Martinez *et al*., 2002).

- *Microbial test*

Microbial test consisted of sample culture in MRS vancomycin agar using pour plate method according to the standard. In order to provide this, proper dilutions of the samples were made in sterile ringer solution and the plates were incubated at 37°C following the culture preparation. Colony counts were measured following 72h incubation period (Tharmaraj & Shah, 2003).

- *Sensory evaluation*

Yoghurt samples were evaluated using 5-score Hedonic test. The samples were evaluated by 9 panelists regarding the organoleptic attributes including taste and texture (Fadela *et al*., 2009).

Results and Discussion

Table 1 shows the physicochemical characteristics of probiotic yoghurt samples the day after production (according to mean± standard deviation).

As it shows, the highest pH value was measured in control sample which had no significant difference from the other samples. The sample containing inulin had the lowest pH value. Concerning the acidity, control sample had the highest acidity value and showed a significant difference ($p<0.05$), while the lowest acidity value was obtained in the sample containing lactulose. In respect of, there was a significant difference between control and the other samples ($p<0.05$). The sample containing lactulose had the highest percentage of synersis and control sample showed the least amount. There was no significant difference between samples containing lactulose and oligofructose (Table 1). The result of probiotic bacterium count (PBC) are presented in Table 2, which indicates that the sample containing inulin as well as control sample had the highest PBC. There was no significant difference between the samples containing lactulose and oligofructose, while the difference between these samples and the control was significant ($p<0.05$) (Table 2).

Table1. The physicochemical characteristics of probiotic yoghurt samples after production (mean ± deviation)

Probiotic yoghurt samples	Prebiotic	pH	Acidity (◦D)	Syneresis(%)
1	L	4.38 ± 0.160^{a}	83.50 ± 0.229^{b}	26.35 ± 0.026^{b}
2	I	4.17 ± 0.168^{a}	85.33 ± 0.289^{c}	24.53 ± 0.076^{a}
3	O	4.38 ± 0.168^{a}	84.36 ± 0.228^{b}	26.11 ± 0.018^{b}
4	C	4.38 ± 0.168^{a}	89.66 ± 0.289^{a}	24.15 ± 0.076^{a}

*L,I,o and C are samples contain of Lactulose, Inulin and Oligofructose and Control sample respectively.
**The means shown with different in a row are significantly different (p<o.o5).

Table 2. Probiotic bacterium count in probiotic yoghurt samples after production (mean ± deviation)

Probiotic yoghurt samples	Prebiotic	Probiotic yoghurt (logcfu/ml)
1	L	8.18 ± 0.001^{b}
2	I	8.27 ± 0.013^{a}
3	O	8.17 ± 0.001^{b}
4	C	8.27 ± 0.013^{a}

*The means shown with difference in a row are significantly different (p<0.05).

Figure 1 shows the variation curve of pH value of probiotic yoghurt samples during refrigeration. The variation trend was descending and it declined significantly during 3 wk storage ($p<0.05$). For example, the decline for the samples containing lactulose and oligofructose was more following the 2nd week, in contrast, it was slower for the samples containing inulin during the first two weeks. The samples containing lactulose and oligofructose showed higher variation curve level, while after 2nd week a significant decline was observed ($p<0.05$) (Figure 1).

Variation curve of acidity of probiotic yoghurt samples during refrigeration presented in Figure 2.

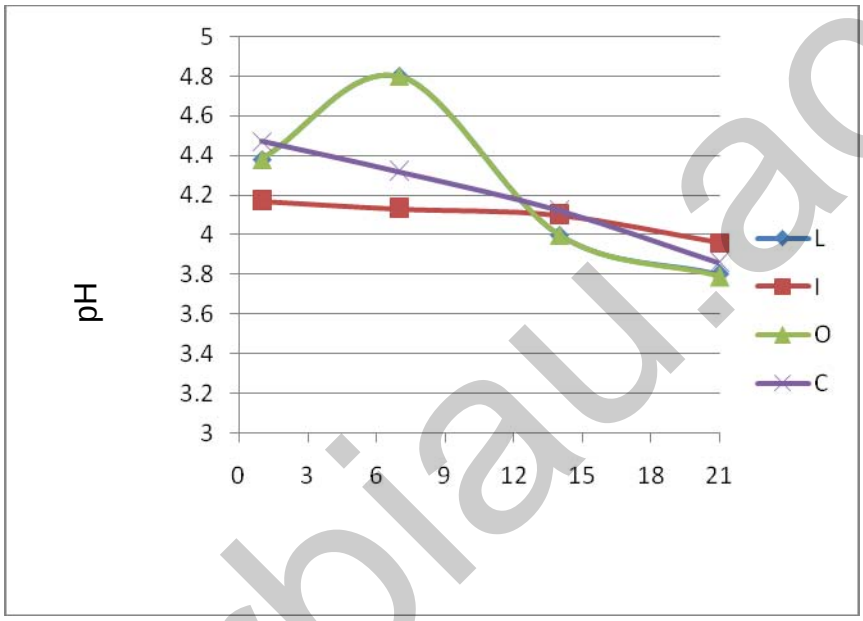

Fig. 1. Variation curve of pH value during refrigeration

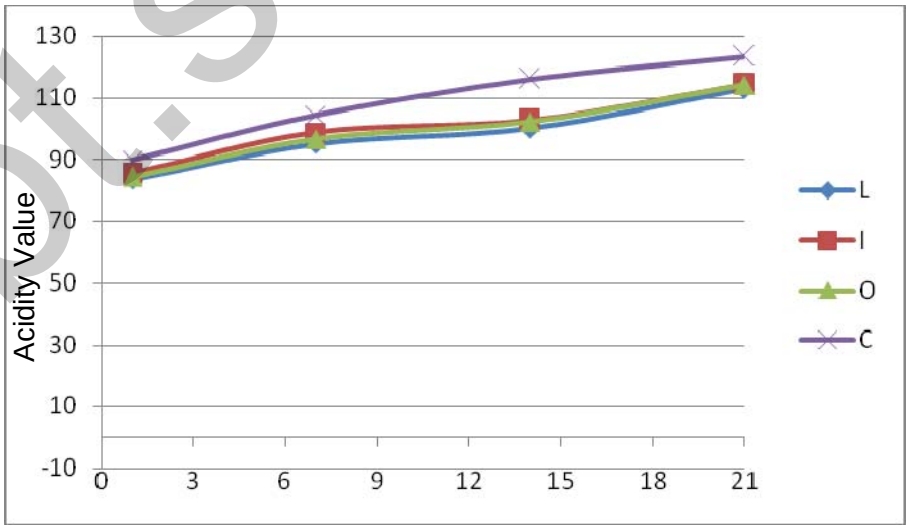

Fig. 2. Variation curve of acidity value during refrigeration

The ascending trend of the acidity of the samples is evident and in contrast to pH, the acidity of probiotic samples showed significant increase ($p<0.05$) during refrigeration. Control sample had the highest acidity at the beginning and at the end of the storage period and showed the highest level of variation curve in acidity. The lowest acidity (112.80 ± 0.913) was observed in the sample containing lactulose at the end of the storage period. The variation trend of acidity of yoghurt samples had significant difference from the control sample ($p<0.05$) (Figure 2).

The results of syneresis measurement of probiotic samples during storage presented in Figure 3.

Ascending trend of synersis is evident in the Figure 3. Over time, synersis of the samples showed significant increase ($p<0.05$). For example, in the first week of storage, syneresis curve of control sample had lower level as compared to the other samples while syneresis percentage then increased significantly. At the end the sample containing oligofructose showed the highest syneresis percentage (34.64 ± 0.084) which had no significant difference from the control sample (34.56 ± 0.084). There was no significant difference between syneresis percentage of control sample and the other test samples (Figure 3).

Figure 4 shows the result of probiotic bacteria count during refrigeration. As it is shown, probiotic bacteria count showed an ascending trend by 14d and bacteria count decreased significantly from 2nd wk($p<0.05$). Control sample lacking the prebiotics showed the lowest bacteria count (logcfu/ml 5.78 ± 0.009) at 21d. Adding prebiotics resulted in an increased probiotic bacteria at the end of 2nd wk and there was a significant decrease ($p<0.05$) in bacteria count notably in control sample from 14d. At the end of storage, the sample containing inulin had the highest probiotic bacteria count (6.20± 0.009) which had a significant difference from control sample ($p<0.05$). In addition, there was no significant difference in the sample containing lactulose and oligofructose (Figure 4).

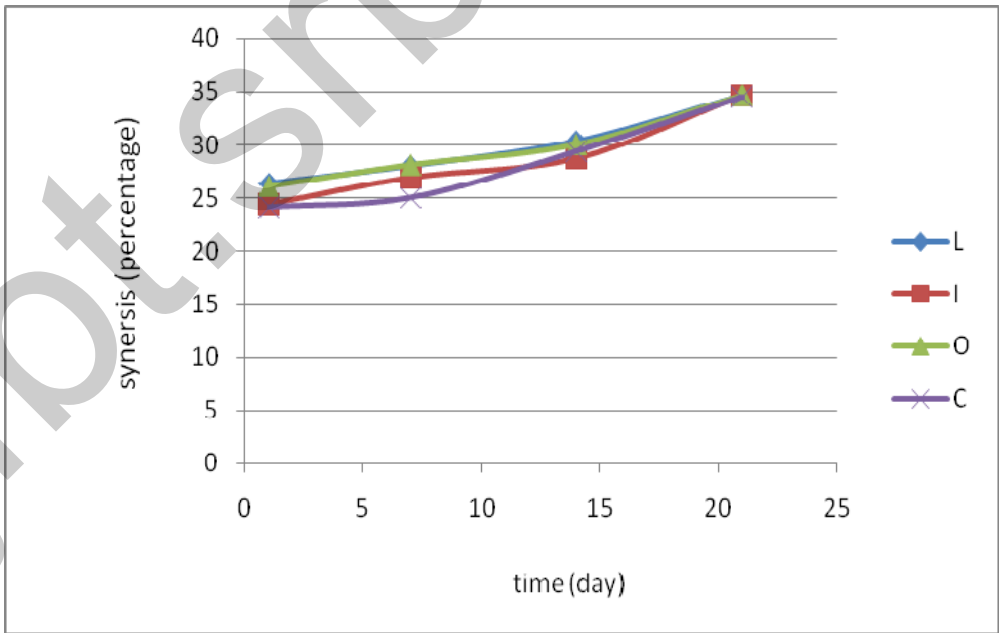

Fig. 3. Variation curve of syneresis measurement during refrigeration

Table 3 shows flavour evaluation scores of probiotic yoghurt samples during refrigeration. As it is shown in the first day the sample containing inulin showed the highest scores which had no significant difference from the control sample. There was also no significant difference between the samples at 7d. In the 2nd week the sample containing inulin had the highest score showing significant difference ($p<0.05$) from the other samples. Control sample had the lowest score. There was no significant difference among the samples containing inulin, lactulos and oligofructose (Table 3).

The scores resulted from the texture evaluation of probiotic samples during refrigeration are presented in Table4.As it is shown, the sample containing inulin and control sample had the highest scores in the first day. The samples containing lactulose and oligofructose had significant difference ($p<0.05$) from the control sample. There was no significant difference among the samples at 7d. The sample containing inulin had the highest score at 14d showing significant difference ($p<0.05$) from the control sample. Control sample had the lowest score. The sample containing inulin had the highest score at 21d showing significant difference ($p<0.05$) from the other samples. Control sample showed the lowest score (Table 4).

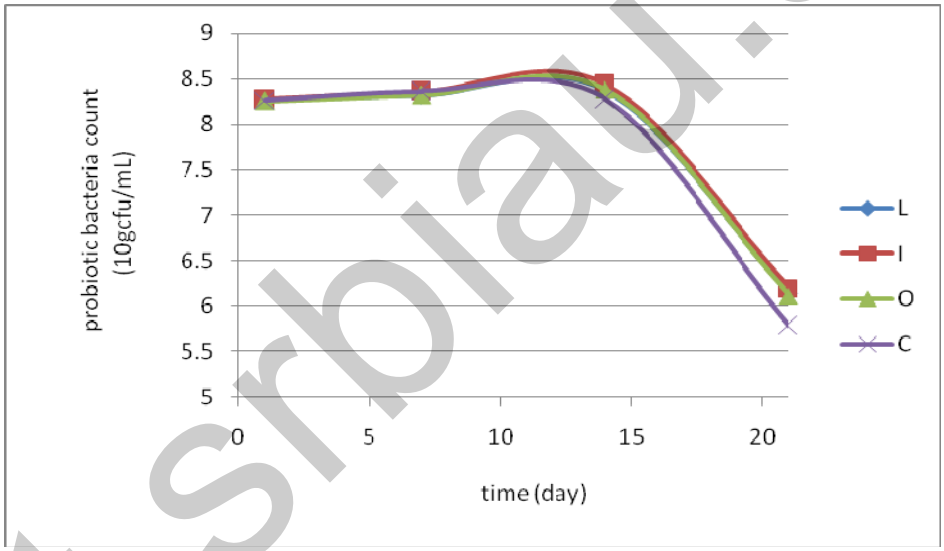

Fig. 4. Variation curve of probiotic bacteria count during refrigeration

Table 3. Scores of flavor evaluation for probiotic yoghurt samples during refrigeration

Period/ yoghurt samples	1th day	7th day	14th day	21th day
L	4.30 ± 0.113^{b}	4.62 ± 0.129^{a}	3.33 ± 0.221^{b}	2.93 ± 0.204^{a}
I	4.70 ± 0.108^{a}	4.63 ± 0.129^{a}	3.37 ± 0.221^{a}	2.96 ± 0.204^{a}
O	4.11 ± 0.195^{b}	4.59 ± 0.129^{a}	3.32 ± 0.221^{a}	2.96 ± 0.204^{a}
C	4.59 ± 0.108^{a}	4.59 ± 0.129^{a}	3.30 ± 0.221^{b}	2.96 ± 0.204^{a}

*The means shown with difference in a row are significantly different ($p<0.05$).

Table 4. Scores of texture evaluation for the probiotic yoghurt samples during refrigeration

Period/ yoghurt samples	1th day	7th day	14th day	21th day
L	2.89 ± 0.165[b]	3.98 ± 0.024 [a]	3.00 ± 0.217[b]	3.20 ± 0.119[b]
I	3.81 ± 0.101[a]	4.00 ± 0.024[a]	4.03 ± 0.217[a]	4.20 ± 0.119[a]
O	2.74 ± 0.165[b]	3.94 ± 0.024[a]	2.90 ± 0.217[b]	2.16 ± 0.119[b]
C	3.81 ± 0.101[a]	3.92 ± 0.024[a]	2.03 ± 0.271[c]	2.48 ± 0.119[c]

*The means shown in a row are significantly different (p<o.o5).

The results obtained in this study indicate that increased storage duration by 21d resulted in a significant reduction in pH value of probiotic yoghurt. Various studies have shown that the growth rate, when bacteria added to the fermented dairy products following the fermentation stage, is reduced and the reduction is increased during refrigeration. In this study, probiotic bacteria is added prior to fermentation to improve the adaptability to the milk environment. The sample containing oligfructose had the lowest pH value. Various investigations indicated that the activity of starter bacteria of yoghurt resulted in significant decrease in pH during refrigeration (Ozer *et al*., 2007). Some studies supported the role of oligofructose in decreasing pH value of yoghurt (Hilliam, 2003). It has been also reported that prebiotics stimulate the growth and the activity of probiotic bacteria while stimulating acid production by starters, resulting in a reduced pH value of the product over the time (Tabatabaie & Mortazavi, 2008). One of the suggested method to reduce the time of fermentation and to increase the acid production is to use a co-culture such as yoghurt starter as well as probiotic bacteria (Farnworth, 2005).

In this study, control sample showed the highest value for the acidity during storage, emphasizing the significant role of co-culture along with probiotics. The results of some studies have also shown significant increase in acidity of probiotic yoghurt during storage (Vahicic & Hruskar, 2000). The results of this study suggested an increase in acidity over the time. Syneresis means separation of aqueous phase from continuous phase or gel network, which is undesirable in yoghurt production. This is common in low – fat yoghurt because of low solid content. The use of compounds such as gelatin, pectin, starch and prebiotics has been suggested to reduce syneresis (Harte *et al*., 2003; Amaya-liao *et al*., 2008). In this study, the lowest percentage of syneresis was obtained in control sample showing no significant difference from the other samples. The results of some studies have suggested that using prebiotics may reduce syneresis percentage (Paseephol, 2008). The most important factor for food products especially yoghurt having an acidic environment is survival of probiotics. Some important factors affecting the survivability of probiotics in fermented dairy products are culture conditions, the used specific strain, final acidity, inoculation level, fermentation time and the nutrients (Lourens – Hattingh & Viljoen, 2001). Various reports have suggested the minimum live probiotic population when using the probiotic product, (Shah, 2001; Bari *et al*., 2009). As indicated in diagram 4, the sample containing inulin showed the highest survival of probiotic bacteria following 21d of storage, showing a

significant difference ($p<0.05$) from the control sample with the lowest probiotic bacteria count. One of the most important reasons for more survival of bacteria is the presence of prebiotics because of stimulating growth and activity of probiotics. The production of high acid level by yoghurt starter bacteria, on one hand, and lack of stimulating growth agents such as prebiotics, on the other hand are the reasons for significant reduction of probiotic bacteria count in control sample. Various reports on more survivability of probiotic bacteria in the presence of prebiotics in yoghurt have been presented (Stanton *et al*., 2005). The most important factor for of yoghurt popularity is its sensory properties (Mattila – Sandholm & Saarela, 2003).

Many investigations have supported the fermented dairy products such as yoghurt (Boehm & Stahl, 2003; Matjevic *et al*., 2009). The results of this study showed that the samples containing inulin had the best sensory qualities (taste and texture) and the control sample showed the lowest score on taste suggesting that the total acceptability of synbiotic yoghurt is more than probiotic and plain yoghurt. This suggests the significant role of prebiotics in improving sensory attributes of the final product (Hussain *et al*., 2009). Inulin may improve consistency and firmness of the yoghurt and sensory properties of the fermented product (Paseephol, 2008). One of the reasons for firm texture of this product is the stimulation of exopolysaccarides production by starter bacteria (Saarela *et al*., 2009).

Conclusion

It might be concluded that the use of prebiotics has resulted in improved sensory and rheological attributes in synbiotic yoghurt during storage.

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