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Influence of Fortification with Inulin and Hi-maize on Acidity and Viability of Starter Culture in Probiotic Yoghurt

Inulin ve Hi-maize ile Zenginleştirmenin Probiyotik Yoğurdun Asitliğine ve Starter Kültür Canlılığına Etkisi

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Key Words:

Probiotic yoghurt, inulin, Hi-maize, acidity, viability

ABSTRACT

In this study, the effect of fortification with inulin and resistant starch (Hi-maize) on yoghurt starter and probiotic bacteria survival, as well as acidity properties of set-type probiotic yoghurt containing *Lactobacillus acidophilus* were investigated. Milk was fortified with inulin or Hi-maize at 2% and 4% ratios whereas control group had no supplement. Samples fortified with Hi-maize had higher lactic acid value than those fortified with same ratio of inulin. Lactic acid percentages increased significantly in all probiotic yoghurts at the end of storage in comparison to the first day ($p<0.05$). The highest viable counts of *Streptococcus thermophilus* were enumerated in the sample fortified with 2% Hi-maize during 14 days of storage. The control sample had as high viable counts of *Lactobacillus bulgaricus* as the sample fortified with 4% Hi-maize, except first day of the storage. The highest viability of *L. acidophilus* was detected in control and the sample fortified with 4% Hi-maize on 7th and 14th day whereas the highest value was reported for the control sample at the end of the storage. *L.acidophilus* populations ranged from 6.30 to 7.95 log cfu/g in probiotic yoghurts during 21 days of refrigerated storage. Therefore, all experimental yoghurts presented probiotic viability above the minimum recommended level of 6 log cfu/g suggested for beneficial health effects.

Anahtar Sözcükler:

Probiyotik yoğurt, inulin, Hi-maize, asitlik, canlılık

ÖZET

Bu çalışmada, inulin ve dirençli nişasta (Hi-maize) ile zenginleştirmenin *Lactobacillus acidophilus* içeren set tipi probiyotik yoğurdun yoğurt bakterileri ile probiyotik bakteri canlılığı ve asitlik özellikleri üzerine etkisi araştırılmıştır. Süt %2 ve %4 oranında inulin veya Hi-maize ile zenginleştirilirken kontrol grubuna herhangi bir ilave yapılmamıştır. Hi-maze ile zenginleştirilen örneklerin aynı oranda inulin katılan örneklere göre daha yüksek laktik asit değerine sahip oldukları görülmüştür. Tüm probiyotik yoğurtlarda laktik asit yüzdelerinin depolama sonunda ilk gün ile kıyaslandığında önemli derecede arttığı görülmüştür ($p<0.05$). En yüksek *Streptococcus thermophilus* sayısı depolamanın 14 günü boyunca % 2 Hi-maize ile zenginleştirilen örnekte tespit edilmiştir. Kontrol örneği depolamanın ilk günü dışında % 4 Hi-maize ile zenginleştirilen örnek kadar yüksek sayıda *Lactobacillus bulgaricus* içermiştir. Depolamanın 7. ve 14. günlerinde en yüksek *L. acidophilus* sayısı kontrol örneği ile % 4 Hi-maize ile zenginleştirilen örnekte tespit edilirken, depolama sonunda en yüksek sayı kontrol örneğinde belirlenmiştir. 21 günlük buzdolabı koşullarında depolama boyunca *L. acidophilus* sayıları 6.30 -7.95 log kob/g arasında değişiklik göstermiştir. Sonuç olarak, tüm yoğurt örneklerinin yararlı sağlık etkileri bakımından tavsiye edilen minimum probiyotik canlılık seviyesi olan 6 log kob/g miktarını sağladığı görülmüştür.

INTRODUCTION

Yoghurt is consumed widely around the world owing to its high nutritional value. Good-quality yoghurt can be obtained by adding ingredients to increase the total solids of the yoghurt mix to a desired level. There are many factors that affect yoghurt quality, including the chemical composition of the milk and the methods of fortification used (Guzman-Gonzalez et al. 1999). The addition of probiotic bacteria to yoghurt improves its functionality and health effects. Probiotics are living microorganisms that have a beneficial effect on the health of the host. The strains called probiotics beneficially restore microbial balance in the gut flora of the host. Over the years, probiotics have been associated with the improvement of lactose intolerance, increase in natural resistance to infectious disease, suppression of cancer, as well as reduction in serum cholesterol level. Prebiotics are non-digestible complex carbohydrates that selectively stimulate the growth or bioactivity of beneficial microorganisms, so beneficially effect the host. The term synbiotic refers to products that contain both probiotics and prebiotics (Liong and Shah, 2005; Donkor et al., 2007; Olveira and Gonzales-Molero, 2016). Within various probiotic microorganisms, *Lactobacillus acidophilus* has been used as probiotic bacteria in the manufacture of synbiotic dairy products in many studies (Akalın and Ünal, 2010; de Souza Oliveira et al., 2011; Heydari et al., 2011; Hasani et al., 2016). In order to have a beneficial effect of probiotics on the gut, it has been recommended that they should be viable and ingested in numbers ≥ 10^6 cells per gram (Vasiljevic and Shah, 2008). This level is now recommended by Turkish Food Codex (1997). Among the different process parameters, such as milk base composition, heat treatment, fermentation, and storage conditions, starter culture also plays a determinative role in gel structure formation of yoghurt. As probiotic bacteria grow slowly in milk because of the lack of proteolytic enzymes, they contribute poor sensory and textural characteristics to the product (Klaver et al., 1993; Dave and Shah, 1998a, b). Therefore, the practice is to blend these organisms with yoghurt starter culture (Damin et al., 2008; Marafon et al., 2011). In addition, in the concept of functional foods, probiotics are added to yoghurt in the prevention of disease and maintenance of health and well-being as a natural way of enhancing functionality.

Skim milk powder (SMP) is traditionally used to fortify yoghurt milk. However, in recent years, manufacturers are more interested in other ingredients because of their promoting effects on viability and textural characteristics of yoghurt and nutraceutical attributes. Among these ingredients both inulin and high maize resistant starch which are carbohydrate-derived prebiotics, are used for these purposes (Mohammadi and Mortazavian, 2011). Inulin which is produced from several fruits and vegetables, is a soluble and fermentable fiber named fructan that reaches the large intestine practically intact, is then hydrolyzed in the uppersection of the intestine and is fermented by bacteria (Tamime, 2005). Resistant starch is a small starch fraction resistant to digestion, and it can be fermented by the healthy microflora in the large intestine (Homayouni et al., 2013). Resistant starch has also been suggested for use in probiotic compositions to promote the growth of such beneficial microorganisms (Fuentes-Zaragoza et al., 2010). Although both inulin and Hi-maize have been used in the manufacture of different dairy products, their effects on probiotic yoghurt containing *L. acidophilus* have not been investigated.

The objective of this research was to investigate the acidity and microbiological characteristics of set-type probiotic yoghurt fortified with inulin and Hi-maize at two different ratios (2% and 4% w/w).

MATERIAL and METHODS

Starter culture and ingredients

The commercial yoghurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lactoferm YO-195) and *Lactobacillus acidophilus* (Lactoferm LA) (Biochem S.r.l., Via Fratelli Rosselli, 38 00015 Monterotondo, Rome, Italy) were in a freeze-dried direct vat set (DVS) form. Each culture was added at a content of 0.04g/L, respectively, and was used according to the recommendation of the manufacturer. Dairy ingredients that were used included skim milk powder (SMP) (Pınar Dairy Products, Izmir, Turkey), inulin (Fibruline Instant, Cosucra, Belgium) and Hi-maize (Hi-maize® 260, high amylose maize resistant starch, Ingredion, Hamburg, Germany).

Probiotic yoghurt manufacture

Probiotic yoghurt samples were manufactured in the laboratory of Ege University Faculty of Agriculture Department of Dairy Technology. Flow diagram for manufacture of probiotic yoghurts was shown in Figure 1. A commercial pasteurized milk having 11.6% total solid, 3% milk fat and 3.1% protein was used in the manufacture of probiotic yoghurt samples. pH value of milk was 6.6 while it has a titratable acidity of 0.154%. Set-type probiotic yoghurt was prepared using milk that was standardized with skim milk powder to obtain 110 g/L of nonfat milk solids. The milk was divided into five lots. The control group had no supplement. The other four groups were supplemented with 2% w/w and 4% w/w inulin and Hi-maize. After they were mixed properly, each milk base was heated at 85°C for 30 min by circulation in a hot water bath. The mixtures were then cooled to 43°C in an ice bath and cultures were added according to the manufacturer's instructions. The mixtures were then put into plastic containers and incubated at 42°C until a pH 4.70 was reached. After fermentation, the yoghurt samples were cooled and transferred to a refrigerator and stored at 4°C for 21 days for analyses.

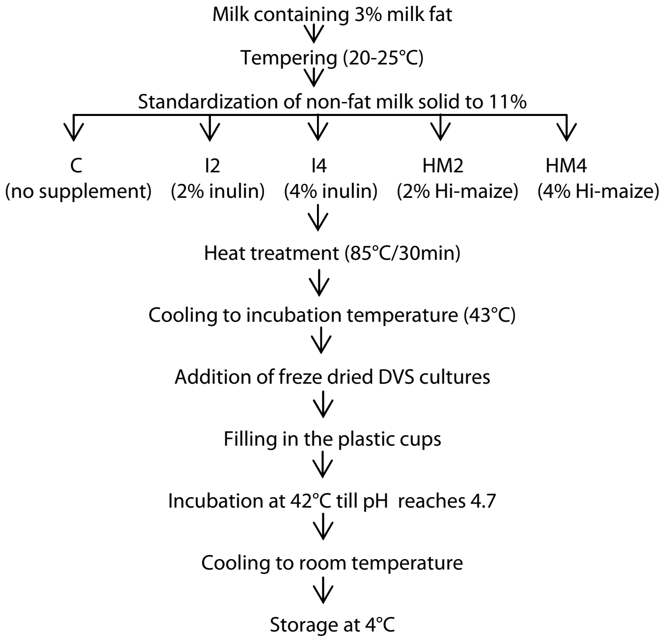

Figure 1. Flow diagram for the preparation of probiotic yoghurts

Biochemical analyses

The total solids were determined by drying samples at 110 °C for 2 h (AOAC, 1995). The fat content was analyzed by the Gerber method (Renner, 1993). These chemical analyses were done after 24 h of product storage at 4°C. The pH of the yoghurts was determined using a pH meter (Hanna Instruments Model pH: 211; Woonsocket, RI, USA). Titratable acidity was expressed as g of lactic acid/100 g after mixing 10 g of yoghurt sample with 10 mL of hot distilled water and titrating with 0.1 N NaOH using 0.5% phenolphthalein indicator (AOAC, 1995). The pH and titratable acidity of the samples were determined every week during 21 d of storage. Analysis was performed in triplicate.

Microbiological analyses

Microbiological analyses were done according to Ünal and Akalın (2013). The counts of *S. thermophilus* were enumerated on an M-17 agar (Merck, Darmstadt, Germany) after incubating the plates aerobically at 37 °C for 48 h while MRS agar (Merck, Darmstadt, Germany) adjusted to a pH 5.2 along with anaerobic incubation at 42 °C for 72 h was used for the enumeration of *L.bulgaricus*. The counts of *L.acidophilus* were enumerated on MRS Agar added D-Sorbitol (Sigma-Aldrich, USA) at a concentration of 10g/L, anaerobically at 37 °C for 72 h. Plates containing 25–250 colonies were enumerated and recorded as cfu/g of sample (Dave and Shah, 1996).

Statistical analysis

The experiments were performed in twice with three parallel. Six values for each sample were averaged (n=6). The data obtained was processed by one-way ANOVA using the general linear model procedure of the SPSS version 11.05 (SPSS Inc., Chicago, IL, USA). The means were compared with the Duncan test at p<0.05 level.

RESULTS and DISCUSSION

Biochemical characteristics

There were significant differences (p<0.05) in total solid values of all probiotic yoghurts. Total solid content of probiotic yoghurt samples ranged between 13.18±0.03% and 16.43±0.03% while all experimental samples had a milk fat content of 3±0.00% as expected (data not shown).

The changes in pH and titratable acidity in probiotic yoghurts during 21 d of storage at 4°C were given in Figures 2A and 2B. In general, the pH was lower in yoghurts fortified with Hi-maize when compared with inulin for the same addition rate (p<0.05). The addition rate of inulin did not affect the pH values of the samples on the 1st and 21st day of storage. Guven et al. (2005) also reported that inulin concentration does not influence the pH level in set-type yoghurt. In another study, inulin addition (at a ratio of 1%, 2% or 3%) did not also significantly alter the pH values of low-fat set yoghurt with probiotic-cultured banana purée on the first day of storage (Srisuvor et al., 2013). On the other hand, the fortification ratio of Hi-maize generally affected pH values of probiotic yoghurts (p<0.05).

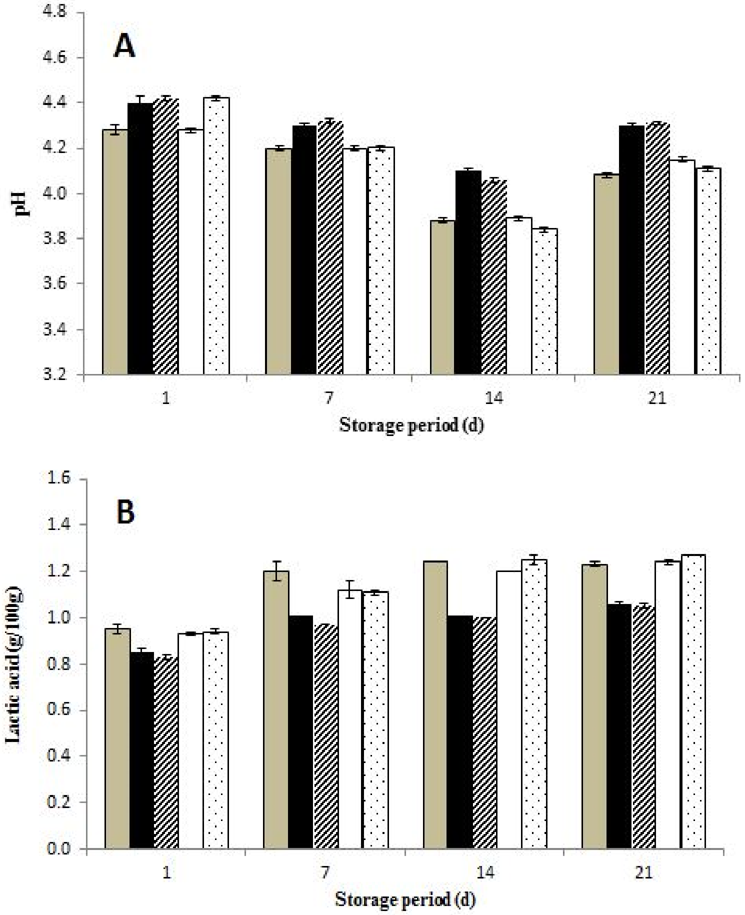

Figure 2. pH (A) and lactic acid (g/100g) (B) in probiotic yoghurts during storage. C: probiotic yoghurt with no supplement (gray bar), I2: probiotic yoghurt fortified with 2% inulin (black bar), I4: probiotic yoghurt fortified with 4% inulin (hashed bar), HM2: probiotic yoghurt fortified with 2% Hi-maize (white bar), HM4: probiotic yoghurt fortified with 4% Hi-maize (dotted bar), Error bars indicate standart deviations.

Some fluctuations were observed for the pH values in all samples during storage however a significant reduction was determined at the end of the storage when compared to the 1st day in all experimental yoghurts. On the other hand, pH values of all probiotic yoghurts showed an increase ($p<0.05$) on 21st day when compared to 14th day which might be explained by alcaline compounds formed as a result of proteolytic degradation and the ability of *S.thermophilus* to produce some basic metabolites during the later stage of storage (Tinson et al., 1982). Similar increase in pH values was also observed in probiotic yoghurts of some other studies (Ramchandran and Shah, 2010; Akalın et al., 2012). Kavaz and Bakırcı (2014) also detected a similar increase on 21st day for the pH values of control probiotic yoghurt and sample added with 2% inulin and 1% demineralized whey powder. In contrast, Stijepic et al. (2013) did not detect any significant change in pH values of probiotic fermented dairy products added with inulin during the storage at 4°C when compared to control sample. This disagreement with our study can be caused by the difference in product type, starter culture and the supplementation ratio of inulin.

Samples fortified with Hi-maize had higher lactic acid value than those fortified with same ratio of inulin ($p<0.05$). No significant differences were noted in lactic acid values between samples fortified with 2% inulin and 4% inulin whereas the addition rate of Hi-maize significantly affected the lactic acid contents. Srisuvor et al. (2013) also did not determine any significant difference in lactic acid values of low-fat set type yoghurt with probiotic-cultured banana purée which was fortified with different ratios (1%, 2% or 3%) of inulin. Probiotic yoghurts supplemented with 2% inulin had lower lactic acid content than control sample. Similar results were also obtained by Balthazar et al. (2015) for ovine milk yoghurt.

Moreover, higher lactic acid content of the control sample can be attributed to the generally higher viable counts of starter culture bacteria.

Lactic acid percentages increased significantly in all probiotic yoghurts at the end of storage in comparison to the first day ($p<0.05$). Lactic acid contents of probiotic yoghurts have also significantly increased throughout the storage period in some other studies (Özer et al., 2005; Hasani et al., 2016).

Viability of *S. thermophilus, L. delbrueckii* subsp. *bulgaricus, L.acidophilus*

The changes in the viable counts of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus acidophilus* in probiotic yoghurts during refrigerated storage are presented in Table 1. The counts of *S.thermophilus* were generally found above 7 log cfu/g throughout the storage period. There were significant differences ($p<0.05$) in the viability of *S.thermophilus* among yoghurt types. In general, the highest viable counts of *S.thermophilus* were enumerated in the sample fortified with 2% Hi-maize during 14 days of storage. The counts of *S.thermophilus* significantly increased on 7th day in control and samples added with inulin which coincided with the pH decrease at day 7 of storage. Özer et al. (2005) also found similar results for the viable counts of *S.thermophilus* in probiotic yoghurts containing different ratios of inulin.

Table 1: Changes in the viable counts of *S. themophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* during refrigerated storage of yoghurts (log cfu/g)

	Storage days			
Product	1	7	14	21
	S. thermophilus			
C	7.25 ± 0.05^{Dc}	7.52 ± 0.09^{Cb}	7.38 ± 0.23^{Cbc}	7.93 ± 0.04^{Aa}
I2	7.07 ± 0.02^{Ec}	8.12 ± 0.13^{Aa}	7.46 ± 0.18^{BCb}	7.53 ± 0.15^{Bb}
I4	7.61 ± 0.14^{Cb}	7.88 ± 0.17^{Ba}	7.65 ± 0.06^{ABb}	7.56 ± 0.00^{Bb}
HM2	8.11 ± 0.12^{Aa}	8.20 ± 0.10^{Aa}	7.76 ± 0.06^{Ab}	7.18 ± 0.16^{Dc}
HM4	7.90 ± 0.16^{Ba}	7.81 ± 0.00^{Ba}	7.15 ± 0.10^{Db}	6.64 ± 0.27^{Ec}
	L. delbrueckii* subsp. *bulgaricus			
C	6.00 ± 0.00^{Ed}	7.66 ± 0.03^{ABc}	7.93 ± 0.04^{Aa}	7.86 ± 0.03^{Ab}
I2	6.30 ± 0.00^{Dc}	7.28 ± 0.03^{Da}	7.26 ± 0.09^{Ca}	6.90 ± 0.07^{Cb}
I4	6.73 ± 0.17^{Cb}	7.42 ± 0.13^{Ca}	7.25 ± 0.11^{Ca}	6.65 ± 0.30^{Db}
HM2	7.11 ± 0.25^{Bb}	7.63 ± 0.13^{Ba}	7.50 ± 0.11^{Ba}	7.60 ± 0.05^{Ba}
HM4	7.59 ± 0.11^{Ac}	7.77 ± 0.05^{Ab}	7.88 ± 0.02^{Aa}	7.65 ± 0.05^{ABc}
	L. acidophilus			
C	6.30 ± 0.00^{Dd}	7.65 ± 0.02^{Ac}	7.89 ± 0.05^{Aa}	7.78 ± 0.03^{Ab}
I2	6.42 ± 0.29^{Dc}	7.28 ± 0.02^{Ca}	7.09 ± 0.10^{Da}	6.82 ± 0.17^{Cb}
I4	6.72 ± 0.17^{Cb}	7.48 ± 0.02^{Ba}	7.26 ± 0.20^{Ca}	6.88 ± 0.16^{Cb}
HM2	7.28 ± 0.08^{Bc}	7.60 ± 0.05^{Aa}	7.48 ± 0.09^{Bb}	7.59 ± 0.05^{Ba}
HM4	7.88 ± 0.07^{Aa}	7.64 ± 0.11^{Ab}	7.88 ± 0.04^{Aa}	7.59 ± 0.05^{Bb}

$^{a\text{-}d}$Means ± standart deviations in the same row with different superscript lowercase letters are significantly different ($P < 0.05$)

$^{A\text{-}E}$Means ± standart deviations in the same column with different superscript uppercase letters are significantly different ($P < 0.05$)

C :Control probiotic yoghurt containing no supplement; I2: probiotic yoghurt fortified with 2% inulin, I4 : probiotic yoghurt fortified with 4% inulin, HM2 : probiotic yoghurt fortified with 2% Hi – maize, HM4 : probiotic yoghurt fortified with 4% Hi – maize

The control sample had as high viable counts of *L. bulgaricus* similar to the sample fortified with 4% Hi-maize. On the other hand, probiotic yoghurts supplemented with inulin showed lower viability of *L. bulgaricus* than those with Hi-maize ($p<0.05$). The highest values for *L. bulgaricus* were obtained on 14th day of storage in all experimental yoghurts. Paseephol and Sherkat (2009) reported that the addition of inulin powder did not influence the survival of yoghurt bacteria in yoghurt. Balthazar et al. (2015) found similar results with our study for yoghurt starter bacteria in yoghurt with different ratio of inulin and concluded that inulin does not positively affect the viability of reported bacteria.

Probiotic yoghurt samples presented the populations of *L.acidophilus* ranging from 6.30 to 7.95 log cfu/g during 21 days of refrigerated storage. Product containing probiotic microorganisms should have a minimum population of viable bacteria, throughout its shelf life, which has shown to be efficacious (generally 10^6-10^8 cfu/g per day) (Champagne et al., 2011). Therefore, all experimental yoghurts presented probiotic viability above the minimum recommended level of 6 log cfu/g suggested for beneficial health effects. Similarly, Barat and Özcan (2016) detected minimum suggested level for *L.acidophilus* and *Bifidobacterium lactis* viability in probiotic fermented milk drinks enriched with prebiotic additives.

Control probiotic yoghurt had the lowest viable count of *L.acidophilus* than the samples supplemented with inulin and Hi-maize on the first day. Heydari et al. (2011) also found similar relationship between control yoghurt and yoghurts supplemented with 1.5 % inulin or Hi-maize at the beginning of the storage. In another study, the incorporation of inulin resulted in an improvement of the viability of *L.acidophilus* in yoghurt when compared to *L.acidophilus* in yoghurt without inulin (Iltar et al., 2012). However, the addition of inulin did not significantly increase the viable counts of *L.acidophilus* for the rest of the storage period in our study. Bozanic et al. (2002) found similar results for *L. acidophilus*, and reported that inulin did not support the growth and survival of *L. acidophilus* in probiotic yoghurt. Juhkam et al. (2007) found lower viability of *L.acidophilus* for control yoghurt when compared to samples containing inulin. This contrariety might be due to the higher addition rate of inulin when compared to the addition rate of our study.

The highest viability was detected in control and the sample fortified with 4% Hi-maize on 7th and 14th day whereas the highest value was reported for the control sample at the end of the storage. In the study of Nobakhti et al. (2009), the viability of *L.acidophilus* was highest in control treatment and the lowest in treatment with 3% Hi-maize in synbiotic fermented milk drink. On the other hand, Heydari et al. (2011) did not detect significant differences in the viability of *L.acidophilus* between control probiotic yoghurt and yoghurt supplemented with 1.5% Hi-maize during 21 days of storage except the first day.

The highest *L.acidophilus* values were generally obtained on 14th day in all yoghurt samples whereas the viability decreased at the end of the storage. Similarly, Juhkam et al. (2007) observed a stable decrease in the counts of *L.acidophilus* for yoghurts containing 2 to 5% inulin throughout the storage period.

CONCLUSIONS

Hi-maize showed better growth stimulant for both *L. acidophilus* and *L. delbrueckii* subsp*. bulgaricus* at the same ratios, throughout the storage periods. Furthermore, the highest viability for *L. acidophilus* was detected in control and the sample fortified with 4% Hi-maize on 7th and 14th day of the storage. Although significant changes were observed among yoghurt types, the highest viable counts of *S.thermophilus* were enumerated in the sample fortified with 2% Hi-maize during 14 days of storage. At the end of storage significantly higher lactic acid contents were determined in all probiotic yoghurts in comparison to the first day ($p<0.05$). No significant differences were noticed in lactic acid values between samples fortified with different ratios of inulin, whereas the addition rate of Hi-maize significantly affected the lactic acid contents. In conclusion, the fortification of probiotic yoghurt, containing *L. acidophilus*, with Hi-maize seems to be a good alternative in comparison to inulin.

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