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# PRODUCTION OF SYNBIOTIC RECONSTITUTED YOGURT

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## ABSTRACT

Synbiotic products (in both probiotic and prebiotic) have beneficial effects on the health of the consumer. The aim of this study was to investigate the production of synbiotic reconstituted yogurt. The effect of Arabic gum (0.1 %), Tragacanth gum (0.1 %) and mixture of them (each at a concentration of 0.05 %) on the properties of yogurt containing *Lactobacillus acidophilus* was investigated. Then the samples were dried by freeze- drier. Then, samples were kept at 4 $^0$C for three weeks. Physicochemical properties, viability of probiotic bacteria and sensory characteristics of synbiotic reconstituted yogurt samples were analyzed on first, 7th, 14th and 21$^{st}$ days. pH and viscosity of the samples significantly ($p < 0.05$) decreased during storage. Acidity of the samples significantly ($p < 0.05$) increased during storage. The sample containing mixture of two gums on the first day had the highest counts of probiotic bacteria. Counts of probiotic bacteria in the samples except sample containing mixture of two gums significantly ($p < 0.05$) reduced. The sample containing Arabic gum had the highest sensory quality. There were no significant changes in sensory properties of the samples.

**Keywords:** Arabic gum, Freeze drier, *Lactobacillus acidophilus*, Reconstituted Yogurt, Synbiotic, Tragacanth gum

## INTRODUCTION

A food can be considered as functional when apart from its nutritional effects, its beneficial effects on body have been satisfactorily proven. Also, it suitably causes improvement in health and it reduces the disease risks. Probiotic dairy products such as yogurt that contain lactic acid bacteria are one of the successful samples of functional foods (Saarela, 2007).

Different kinds of yogurts include: 1-set, stirred, drinking yogurt (physical state: liquid / viscose) 2-concentrated yogurt (physical state: semi-solid) 3-frozen yogurt (physical state: solid) 4- dried yogurt (physical state: powdered) (Tamime, 2006).

Probiotics are active and live microorganisms that colonize in different parts of body (especially intestine) with enough numbers by improving the intestinal microflora have healthy effects for host (Soccol, 2010).

One of possible way to increase therapeutic and sensory properties of yogurt is producing the drinking yogurt containing probiotic microorganisms (Gonzalez *et al*., 2011).

The main purposes of drying foods are: preserving the foods by decreasing the water activity , reducing the volume of product , changing foods with easier form for maintaining , packaging, transporting and using (Berk, 2009). Also the yogurt powder needs to less expense of packaging and maintaining due to reduction of volume and lack of need to keep cool (Kumar and Mishra, 2004).

In general, there are two ways to dry yogurt: 1-drying by spray drier and 2- drying by freeze drier. Of course second method is preferred because in this way the temperature (-35$^0$C to -25$^0$C) is very lower than spray drying which causes less damage to basic composition of milk and remove taste and flavor milk. Probiotic bacteria have limited useful shelf life in plain yogurt (Tamime and Robinson, 2007). Freeze drying is a process that not only helps to preserve the yogurt, helps to survive enough number of live probiotic bacteria (Rybka and Kailasapathy, 1997).

Of course, the survival of some probiotics during preparation and freezing may be decreased (Rezaei *et al*., 2012). Therefore, use of prebiotic compounds as resource of carbon or nitrogen, that stimulates growth of probiotic bacteria in intestine, can help to their viability during storage. Hydrocolloids or gums can be used as prebiotic in probiotic dairy products. Moreover, they improve sensory properties of these products (Tamime and Robinson, 2007; Issariyachaikul, 2008).

Rybka and Kailasapathy, (1997) reported that yogurt is dried in temperature -40$^0$C for 48 hours. Also they reported that population of yogurt starter bacteria in this method is more than common methods (Kumar and Mishra, 2004).

The aim of this study was to investigate the production of synbiotic reconstituted yogurt.

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**1-Materials**

The DVS pouches of commercial lyophilized starters including YC-X11 yogurt bacteria (mixed culture of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) and *Lactobacillus acidophilus* La-5 were supplied by Chr-Hansen (Denmark). The cultures were maintained according to manufacturer's instructions, at -18°C until used.

Arabic gum and Tragacanth gum were used in this study. Arabic gum was purchased from Mohebbi Co. (Tehran).Tragacanth gum was supplied by herbal shop (Tehran).

**2-Preparation of the synbiotic reconstituted yogurt**

To prepare the yogurt, skim milk powder was mixed with water to reach a dry matter % 20. Milk was heated at $95^0$C for 30 min, then cooled down to $40^0$C. Yogurt starter and probiotic culture (*Lactobacillus acidophilus*) were inoculated to milk. Population of probiotic bacteria was $10^7$cfu/ml on inoculation time.

Arabic gum and Tragacanth gum (each % 0.1) were added to milk separately (0.1 %) and together (each 0.05 %). The samples were incubated at $37^0$C until acidity reached to 80 $^0$D. Then the samples were kept at 4 $^0$C for 24 hours. After that the samples were sent to a set of freeze drier. The samples of probiotic yogurt with diameter 5 mm at $-120^0$C, and pressure 6 mmtor were dried by freeze drier. Some water was added to yogurt powder samples (the ratio of water to yogurt powder was 1:5). Reconstituted yogurt samples have been studied after 10–15 min. The produced samples of this study are shown in Table 1.

Table 1. The produced samples.

| Samples | |
|---|---|
| A1 | Reconstituted yogurt containing *L. acidophilus* - No gum (Control sample) |
| A2 | Reconstituted yogurt containing *L. acidophilus* + Arabic gum (%0.01) |
| A3 | Reconstituted yogurt containing *L. acidophilus*+Tragacanth gum (%0.01) |
| A4 | Reconstituted yogurt containing *L. acidophilus* + Mixture of two gum (0.05% each) |

**3-Physicochemical analysis**

pH was measured by a pH meter (Adwa, Romany). Titratable acidity and dry matter were determined by AOAC method (AOAC, 2002). Viscosity was measured by Brookfield viscometer that used shear rate 46 and spindle 4. Syneresis was determined by centrifuging yogurt at 4500 rpm for 30 min at 10 $^0$C and it was expressed as volume of separated whey per 100 ml of yogurt (Sahan *et al*., 2008).

**4-Microbiological analysis**

MRS-bile agar (MRS agar from Merck, Germany and bile from Sigma, USA) 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 (Tharmaraj, 2003).

**5-Sensory evaluation**

All samples were evaluated by 11 trained panelists. The sensory attributes were appearance, aroma, taste and texture. The acceptability values were scored on 5 (very good), 4 (good), 3 (moderate), 2 (bad) and 1 (very bad).

**6-Statistical analysis**

Experiments were performed in triplicate. The data were analyzed using One-way Analysis of Variance (ANOVA) and Duncan test by SPSS 18.0.

**RESULTS AND DISCUSSION**

**1-Physicochemical properties of probiotic reconstituted yogurt**

The amounts of pH of probiotic reconstituted yogurt samples are shown in Fig.**1**. Sample A1 on first day had the highest pH. The lowest pH was related to sample A2 on 21$^{st}$ day.

Results show that pH in probiotic reconstituted yogurt samples has been significantly decreased ($p<0.05$) during cold storage. Also no significant deference was showed in pH of probiotic reconstituted yogurt samples between first and 7$^{th}$ days, and between 14$^{th}$ and 21$^{st}$ days.

In other research yogurt produced by *Lactobacillus casei* and *L. acidophilus* has been studied. Results showed that primary pH was 4.5-5 that decreased to 4.2- 4.4 during storage (Nighswonger *et al*., 1996).

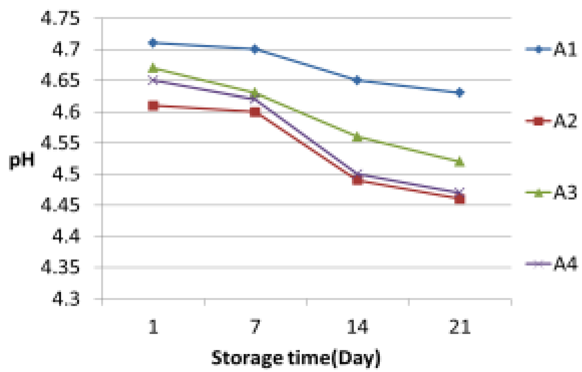


Fig.1. The amounts of pH of probiotic reconstituted yogurt samples during cold storage.

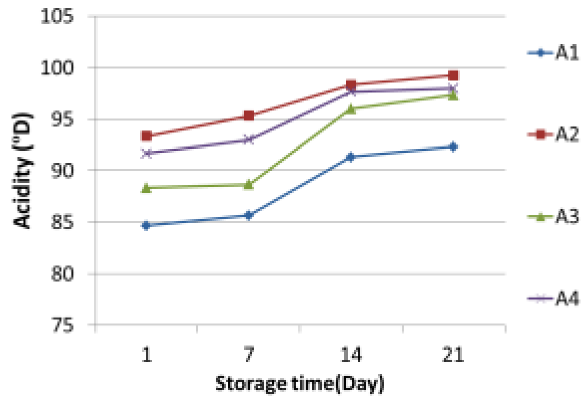


Fig. 2. Acidity of probiotic reconstituted yogurt samples during storage.

Ozer *et al*., (1998) reported that amount of pH in concentrated yogurt (dry matter %16) in the end of 240 minutes reached to 4.3 while amount of pH decrease in yogurt with higher dry matter has been slower duo to higher buffering capacity of concentrated milks.

Acidity of probiotic reconstituted yogurt samples is shown in Fig. 2. There is a negative correlation between pH and acidity level in probiotic reconstituted yogurt samples. Sample A2 on 21$^{st}$ day had the highest acidity. The lowest acidity was related to sample A1 on first day. Results showed that acidity level has been significantly increased ($p<0.05$) during cold storage. Also no significant difference was showed in acidity of probiotic reconstituted yogurt samples between first and 7$^{th}$ days, and between 14$^{th}$ and 21$^{st}$ days.

In similar survey by Antunes *et al*., (2005) the increasing of acidity of drinking yogurt was attributed to activity of starters during storage. Also post acidification in yogurt in period of cold storage was due to producing lactic acid by *Lactobacillus bulgaricus*.

Also the results of many researchers have been showed the significantly increasing in acidity of yogurt in period of storage (Vahcic and Hruskar, 2000).

The amounts of dry matter of probiotic reconstituted yogurt samples are shown in Fig. 3. Results show that dry matter amounts in probiotic reconstituted yogurt samples have been decreased during storage but this decrease was not significant ($p>0.05$).

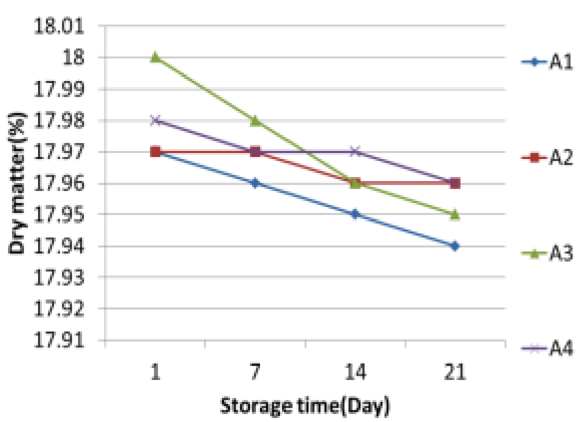


Fig. 3. The amounts of dry matter of probiotic reconstituted yogurt samples during storage.

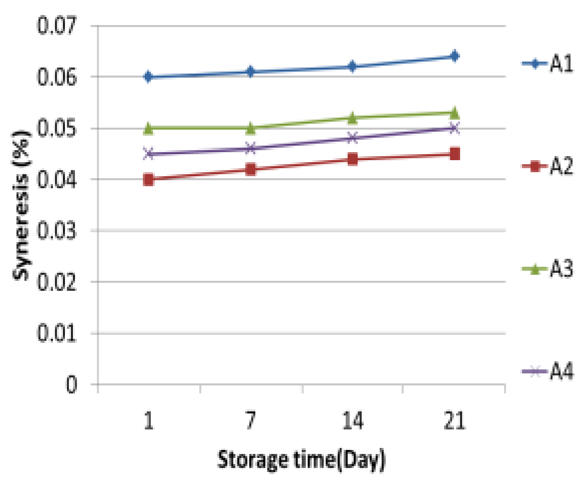


Fig. 4. The amounts of syneresis of probiotic reconstituted yogurt samples during storage.

Robinson (1977) reported that if dry matter of product is downer than 20 %, the product does not have suitable consistency and taste. Also if dry matter is higher than 25 %, product is gummy and has bitter taste.

The amounts of syneresis of probiotic reconstituted yogurt samples are shown in Fig. 4. Results show that syneresis level in probiotic reconstituted yogurt samples has been decreased during storage but this decrease was not significant (p>0.05). Sample A1 on 21st day had the highest syneresis. The lowest syneresis was related to sample A2 on first day.

Farooq and Haque (1992) reported that amounts of yogurt syneresis increase during two weeks.
Other researchers reported that in general to increase time of storage in yogurt increase syneresis but the trend of increase is very slow in yogurt containing prebiotic compounds (Aryana and Mcgrew, 2007).

The amounts of viscosity of probiotic reconstituted yogurt samples are shown in Fig. 5. Sample A3 on first day had the highest viscosity. The lowest viscosity was related to sample A1 on 21st day. Results show that viscosity level in probiotic reconstituted yogurt samples has been significantly decreased (p<0.05) in period of storage from first day till 21st day. Also, there was no significant difference in viscosity of samples between first and 7th day.

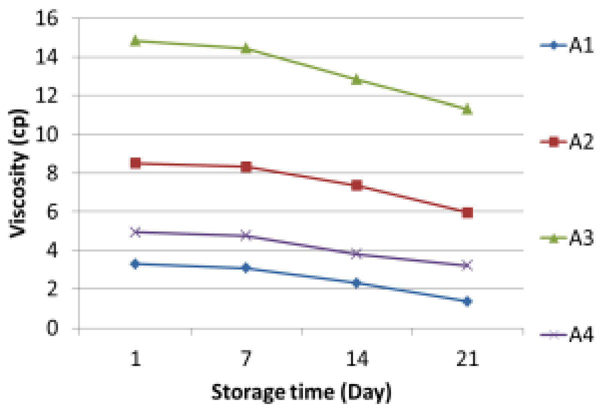


Fig. 5. The amounts of viscosity of probiotic reconstituted yogurt samples during storage.

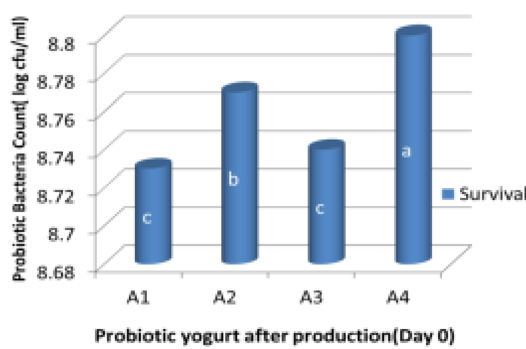


Fig. 6. The population of probiotic bacteria in yogurt samples immediately after production.

In other research, effect of guar gum (0, 0.1, 0.2, 0.3%) and Arabic gum (0, 0.1, 0.3, and 0.5%) has been studied on rheological properties of frozen yogurt. Frozen yogurt samples showed shear- thinning behavior. Most of samples follow up power law model (Rezaei *et al*., 2011).

**2-Counts of probiotic bacteria**

The population of probiotic bacteria in yogurt samples immediately after production is shown in Fig. 6. The population of probiotic bacteria in the samples is more than $10^8$ CFU/mL. The highest number of probiotic bacteria was related to sample A4. Samples A1 and A2 had the lowest number of probiotic bacteria.

The number of probiotic bacteria in probiotic reconstituted yogurt samples during storage is shown in Fig. 7.

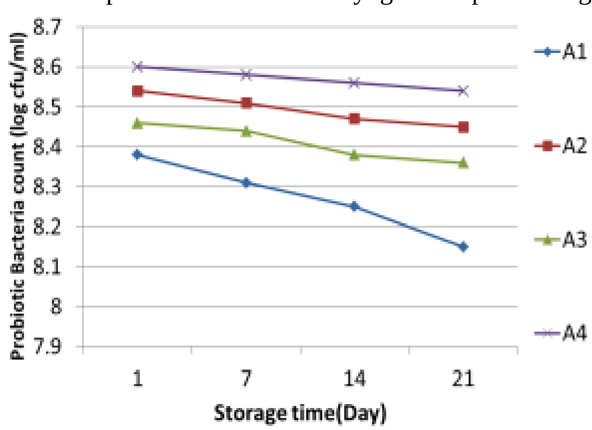


Fig.7. The population of probiotic bacteria in probiotic reconstituted yogurt samples during storage.

Sample A4 on first day had the highest probiotic bacteria count. The least probiotic bacterial count was related to sample A1 on 21th day. Results show that number of probiotic bacteria in the samples except A4 decreased significantly (p<0.05) during storage. Also, results of this research show that number of probiotic bacteria in reconstituted yogurt samples containing both of two gums is more than sample containing Arabic gum. Moreover, counts of probiotic bacteria in sample containing Arabic gum is more than sample containing tragacanth gum and population of probiotic bacteria in sample lacking gum is less than other samples.

Rezaei *et al.*, (2012) evaluated the effects of Arabic gum and guar gum on counts of *Lactobacillus acidophilus* and *Bifidio bacterium* in frozen yogurt during 60 days at -18$^0$C. These gums improved viability of probiotic bacteria. However, counts of probiotic bacteria in frozen yogurt decreased during storage but all the samples contained enough number of probiotics ($10^7$ CFU/mL).

In a similar study, Baati *et al*., (2000) investigated the effect of prebiotics on viability of probiotic bacteria in freeze-dried yogurt during three months. They reported that viability of probiotic bacteria in the samples lacking prebiotics was less than samples containing prebiotic compounds.

**3-Sensory characteristics**

Taste score of probiotic reconstituted yogurt samples is shown in Table 2. Sample A1 on first day had the highest taste score. The lowest taste score was related to sample A4 on 21$^{st}$ day. Changes of taste in the samples were similar together during storage and there was no significant difference between them (p>0.05).

Table 2. Taste score of probiotic reconstituted yogurt samples during storage (Mean ± SD).

| Flavour / sample | First day | 7$^{th}$ day | 14$^{th}$ day | 21$^{st}$ day |
|---|---|---|---|---|
| A1 | 4.89 ± 0.52$^a$ | 4.45 ± 0.50$^a$ | 4.18 ± 0.40$^a$ | 3.95 ± 0.40$^a$ |
| A2 | 4.72 ± 0.46$^a$ | 4.62 ± 0.40$^a$ | 4.45 ± 0.30$^a$ | 3.87 ± 0.10$^a$ |
| A3 | 4.80 ± 0.42$^a$ | 4.68 ± 0.38$^a$ | 4.56 ± 0.44$^a$ | 3.96 ± 0.30$^a$ |
| A4 | 3.81 ± 0.50$^a$ | 3.78 ± 0.46$^a$ | 3.72 ± 0.40$^a$ | 3.36 ± 0.40$^a$ |

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

Some researchers reported that use of high amounts of prebiotic compounds can decrease the sensory properties especially taste in yogurt (Mattila-Sandholm and Saarela, 2003).
Mouthfeel score of probiotic reconstituted yogurt samples is shown in Table 3.

Table 3. Mouth feel score of probiotic reconstituted yogurt samples during storage (Mean ± SD).

| Mouth feel / sample | First day | 7$^{th}$ day | 14$^{th}$ day | 21$^{st}$ day |
|---|---|---|---|---|
| A1 | 4.45 ± 0.82$^a$ | 4.18 ± 0.94$^a$ | 3.90 ± 0.87$^a$ | 3.81 ± 0.68$^a$ |
| A2 | 4.54 ± 0.50$^a$ | 4.50 ± 0.52$^a$ | 4.45 ± 0.52$^a$ | 4.36 ± 0.52$^a$ |
| A3 | 2.54 ± 0.90$^a$ | 2.45 ± 0.80$^a$ | 2.20 ± 0.68$^a$ | 2.16 ± 0.82$^a$ |
| A4 | 3.81 ± 0.68$^a$ | 3.72 ± 0.46$^a$ | 3.62 ± 0.46$^a$ | 3.48 ± 0.40$^a$ |

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

Sample A2 on first day had the highest mouth feel score. The lowest mouth feel score was related to sample A3 on 21th day. Changes of mouth feel in the samples were similar together during storage and there was no significant difference between them (p>0.05).

Other researchers reported that the addition of some prebiotic compounds to dairy products can increase the acceptability and improve the consistency and mouth feel (Golob *et al*., 2004).

Non-oral sense score of probiotic reconstituted yogurt samples is shown in Table 4.

Table 4. Non-oral sense score of probiotic reconstituted yogurt samples during storage (Mean ± SD).

| non-oral sense / sample | First day | 7th day | 14th day | 21st day |
|---|---|---|---|---|
| A1 | 3.54 ± 0.80[a] | 2.72 ± 0.89[a] | 2.36 ± 0.78[a] | 2.00 ± 0.68[a] |
| A2 | 4.83 ± 0.50[a] | 4.62 ± 0.52[a] | 4.45 ± 0.52[a] | 4.36 ± 0.50[a] |
| A3 | 4.81 ± 0.48[a] | 4.75 ± 0.45[a] | 4.70 ± 0.50[a] | 4.63 ± 0.40[a] |
| A4 | 4.00 ± 0.52[a] | 3.81 ± 0.40[a] | 3.72 ± 0.40[a] | 3.63 ± 0.44[a] |

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

Sample A2 on first day had the highest score of non-oral sense. The lowest score of non-oral sense was related to sample A1 on 21th day. Changes of non-oral sense in the samples were similar together during storage and there was no significant difference between them ($p>0.05$). Reduction of non-oral sense of probiotic reconstituted yogurt samples can be related to increasing syneresis during storage.

Gelation, syneresis and producing bitter peptides due to protein breakdown in yogurt structure can decrease sensory quality of dairy products (Mohebbi and Ghoddusi, 2008).

Table 5. Color score of probiotic reconstituted yogurt samples during storage (Mean ± SD).

| Color / sample | First day | 7th day | 14th day | 21st day |
|---|---|---|---|---|
| A1 | 4.90 ± 0.52[a] | 4.82 ± 0.38[a] | 4.74 ± 0.30[a] | 4.50 ± 0.40[a] |
| A2 | 4.38 ± 0.40[a] | 4.25 ± 0.40[a] | 4.18 ± 0.30[a] | 4.09 ± 0.50[a] |
| A3 | 4.27 ± 0.30[a] | 4.21 ± 0.30[a] | 4.18 ± 0.30[a] | 4.06 ± 0.46[a] |
| A4 | 4.36 ± 0.40[a] | 4.27 ± 0.40[a] | 4.21 ± 0.46[a] | 4.18 ± 0.50[a] |

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

Color score of probiotic reconstituted yogurt samples is shown in Table 5.

Sample A1 on first day had the highest color score. The lowest color score was related to sample A3 on 21th day. Changes of color in the samples were similar together during storage and there was no significant difference between them ($p>0.05$).

General acceptability score of probiotic reconstituted yogurt samples is shown in Table 6.

Table 6. General acceptability score of probiotic reconstituted yogurt samples during storage (Mean ± SD).

| General acceptability / sample | First day | 7th day | 14th day | 21st day |
|---|---|---|---|---|
| A1 | 3.72 ± 0.68[a] | 3.54 ± 0.52[a] | 3.45 ± 0.52[a] | 3.36 ± 0.46[a] |
| A2 | 4.81 ± 0.50[a] | 4.78 ± 0.46[a] | 4.72 ± 0.40[a] | 4.63 ± 0.46[a] |
| A3 | 3.36 ± 0.42[a] | 3.33 ± 0.49[a] | 3.20 ± 0.50[a] | 3.18 ± 0.40[a] |
| A4 | 3.81 ± 0.40[a] | 3.80 ± 0.40[a] | 3.75 ± 0.46[a] | 3.72 ± 0.46[a] |

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

Sample A2 on first day had the highest general acceptability score. The lowest general acceptability score was related to sample A3 on 21th day. Changes of general acceptability in the samples were similar together during storage and there was no significant difference between them ($p>0.05$).

Other researchers reported that general acceptability of probiotic yogurt decreased during storage (Cruz *et al*., 2010). Moreover, Matijevic *et al*., (2009) reported that acceptability of probiotic yogurt containing prebiotics is more than sample lacking prebiotic compounds.

In a similar study (Rezaei *et al*., 2011), the effect of Guar gum (%0,%0.1,%0.2, %0.3 ) and Arabic gum (%0.1, %0.3, %0.5 ) on sensory quality of frozen yogurt has been investigated. The sore of acceptability of samples containing gum was more than control sample (without gum).

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