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Changes in Sensory Properties of Probiotic Yogurt Containing Free or Microencapsulated Lactobacillus Plantarum during the Storage Period

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ABSTRACT

Background: The study of sensory properties during the storage period allows us to produce a high quality food product and to estimate the shelf life. **Objective:** In this research, the effect of free and microencapsulated *Lactobacillus plantarum* on sensory properties of yogurt was studied during the 8 weeks storage period. Probiotic yogurt samples containing 8.23 log CFU/ml of free L. plantarum (F) and microencapsulated *L. plantarum* (M) were industrially produced and were compared with plain yogurt samples (C) as control. Mixture of alginate and resistant starch gel was used to prepare microcapsules by emulsification technique. The organoleptical characteristics of yogurt samples including color, taste, flavor, texture and overall acceptability were evaluated based on the Hedonic test. Data obtained by triplicate trial were submitted to the SPSS software and were analyzed by the use of statistical methods of analysis of variance (ANOVA) and T-test. **Results:** After 8 weeks of storage at 4˚C, color and taste of M sample were improved, However, taste and flavor of F and C samples were declined, but not significantly ($p>0.05$). Texture and consistency were non-significantly improved in F samples, and declined in M samples ($p>0.05$), and significantly declined in C samples ($p<0.05$). Total acceptability scores in C samples were dropped, while in F and M samples were non-significantly improved ($p>0.05$). It was indicated that *L. plantarum* could improve some sensory properties of yogurt in both free and microencapsulated forms during the 8 weeks of the storage period. **Conclusion:** It was concluded that production of probiotic yogurt containing free or encapsulated *L. plantarum* is possible by maintaining the sensory characteristics similar to regular yogurt or even better than regular yogurt.

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INTRODUCTION

Evaluation of sensory characteristics of food is important in producing a high quality products and production of high quality food regardless of these characteristics is impossible. The study of sensory properties of a food product during the storage period allows us to estimate the shelf life of the product (IFTS, 1993; Kilcast and Subramaniam, 2000).

Yogurt is one of the most consumed fermented milk products and has the positive impact on human health and especially important in the human diet due to the high nutritional values (Tamime and Robinson, 2007). In recent years the production of this product has been growing in Iran. So that, the rate of production from about 26 thousand tons in 1997, has risen to almost 52 thousand tons in 2001. Besides, probiotic yogurt with a variety of different brands such as Biomas and Promas are manufactured by different companies and the consumption of these products are also rising (Statistics letters of Agriculture, 2001).

Recently numerous probiotic products including yogurt, ice cream, cheese, kefir produced and many valuable researches have been done in this regard. The World Health Organization's 2001 definition of probiotics is "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host". Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used as probiotic microorganisms (FAO/WHO, 2006). *Lactobacillus plantarum* has several strains which indicated as probiotic bacteria (Shafiei and Razavilar, 2013), and the numerous health benefits were attributed to them such as anti-pathogenic effects, cholesterol lowering activity, reducing fibrinogen concentration in blood, and decreasing abdominal bloating in irritable bowel syndrome (Shafiei *et al*., 2012). Despite a variety of dairy products have

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been used as carriers of probiotics however, probiotic yogurt and acidophilus milks are considered the main probiotic carriers (Champagne *et al*., 2005; Saad *et al*., 2008a, 2008b).

Yogurt in addition to containing valuable nutrients as protein, calcium and phosphorus may also be considered as a probiotic product. It is indicated that yogurt starter bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) have probiotic properties such as improvement of lactose digestion and boost the immune system, however they do not me*et al*l the criteria for a probiotic and they are sensitive to acidic conditions of the stomach. So they can not reach the intestine alive and at an acceptable level. Hence the fact that these bacteria can be considered as probiotic is suspicious (Tejada *et al*., 1999; Pestka *et al*., 2001).

In order to reinforce probiotic properties of yogurt and its functional feature, probiotic strains such as *Lactobacillus* and *Bifidobacterium* are added to it. Add probiotic bacteria may interfere with the starter bacteria and may change the sensory properties of the product. Therefore probiotic yogurt may be different from regular yogurt based on these features. Using technique of microencapsulation in addition to improve the survival of probiotic bacteria in difficult environment, it can also prevent interference between starter bacteria and probiotic bacteria. However it should be considered that the sensory characteristics of the product not adversely changed. So the study on sensory properties of probiotic yogurt containing free or microencapsulated probiotic bacteria during the storage period seems to be necessary.

Resistant starch used in microencapsulation is a prebiotic substance which has beneficial health effects to the consumer by enhancing the growth of probiotic bacteria (Mattila-Sandholm *et al*., 2002; Mohammed Akhiar, 2010). Also, resistant starch can reduce the risk of intestinal cancer as a dietary fiber (Dimantov *et al*., 2003).

The main purpose of this study was to produce probiotic or symbiotic yogurt containing free or co-encapsulated *L. plantarum* PTCC 1058 with acceptable sensory and organoleptic properties by emulsion techniques of microencapsulation and evaluation of the changes carried out in sensory properties of yogurt samples during the 8 weeks storage period at 4˚C.

MATERIALS AND METHODS

Manufacture of yogurt samples:

Yogurt samples were manufactured in Tabriz PEGAH dairy plant (Iran Dairy Industries Co.) and based on the method proposed by Tamime and Robinson (1999). The milk was pasteurized at 90˚C for 4 min. then cooled to 42-45˚C and inoculated with 2-3% w/w of yogurt starter culture (YoFlex® Express, Christian Hansen, Denmark). Then the inoculated milk was conducted to the filling machine (Caspian, Iran) to fill in the 100 ml content poly styrene (PS) containers. To prepare probiotic yogurt samples, 1.7×10^9 CFU/ml of free or encapsulated probiotic bacteria was added into the containers in the sterile condition before filling. Then the containers were sealed with aluminum foil and conducted to the 43˚C incubator for 3-4 h until their pH reach to 4.6, then they were transmitted to 4˚C refrigerator and stored at this temperature until testing. Three types of yogurt samples were produced in this research as follow:

a) Regular yogurt without *L. plantarum* as control samples (C)
b) Probiotic yogurt samples containing free *L. plantarum* (F)
c) Probiotic or synbiotic yogurt samples containing encapsulated *L. plantarum* (M)

Encapsulation procedure:

L. plantarum PTCC 1058 was prepared from Pasteur Institute (Tehran, Iran) and were activated by inoculating in the MRS-broth (de Man-Rogosa-Sharpe) at 37˚C for 24-48 h. One ml of cultured MRS broth was transmitted into the each of sterile 1.5 ml graduated plastic tubes and centrifuged at 11000 × g relative centrifugal force (RCF) for 10 min at 4 ^{o}C for settling of bacterial mass. Then bacterial mass washed with sterile 0.9 % saline and used in the mcroencapsulation process (Shafiei *et al*., 2012b).

Resistant starch (starch mais, Acros, New Jersey, USA), alginic acid sodium salt (sodium alginate, Sigma Chemical Co., USA), was used for microencapsulation of *L. plantarum*. Emulsion method was applied for microencapsulation of bacteria. Mixture of sodium alginate (2% W/V) and resistant starch (2% W/V) in distilled water was prepared containing 1% V/V probiotic bacterial mass (Khalida *et al*., 2000). The gel solution was sterilized at 121˚C for 15 minutes before contained bacterial mass. One part of this mixture was added drop wise to 5 parts of vegetable oil (sterilized before) containing 0.2% Tween 80 (Merck, Germany) as an emulsifier and stirred (Sheu and Marshall, 1993) at 500 rpm for 15 minute until the mixture absolutely emulsified in the oil phase. Then 0.1 M calcium chloride solution (sterilized before) was added to this emulsion and was allowed to stand until the water-in-oil emulsion was completely broken and formed calcium alginate beads were separated at the bottom of beaker with water phase (Calcium chloride solution). Oil was drained and beads were collected and stored at 4˚C after washing with sterile distilled water.

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Sensory evaluation:

Sensory properties of yogurt samples were evaluated by 30 untrained taste panelists based on the Hedonic Test. The total score was considered 5 for color, taste and texture and 20 for overall acceptability (Watts *et al*., 1987). The panelists received enough size of samples so that they could retest the product if they desired. They were prepared with mineral water for changing the taste before trying other sample. The panelists were seated separately and were not allowed to have verbal communication during the evaluation process to ensure accurate data collection (Shafiei, 2011).

Statistical analysis:

All analyses were performed using Software-SPSS version 20 (SPSS, Chicago, Illinois, USA). Significant differences between the means of cell counts were determined using Independent T-test. The mean values and the standard error were calculated from the data obtained with triplicate trials. Statistical significance was set at $p<0.05$. Graphs were plotted by Microsoft Office Excel 2003 software.

RESULTS AND DISCUSSION

Color and Appearance:

Changes in color of yogurt samples during 8 weeks storage at 4˚C were shown in Fig. 1A. There were not any significant differences between color score of 3 yogurt samples until week 4. In week 4 the color score of C and F samples was significantly higher than M. In week 5 the differences was not significant. In week 6 color of F and M was better than C and the differences between F and C was significant. In week 7 color score of M samples was higher than F and significantly higher than C samples. In week 8 the color of F was significantly better than M and it was better than C.

After 24 h of storage at 4℃, the color and appearance score of F samples was non-significantly higher than M samples and lower than C samples ($p>0.05$). There was not significant differences between color and appearance of M and C samples ($p>0.05$). After 8 weeks, color and appearance of M samples was improved, however the color and appearance score of F and C samples was declined, non-significantly ($p>0.05$). The score of color and appearance of M samples were non-significantly better than C samples and they were better than F samples, after week 8 ($p>0.05$).

Results of this research are in agreement with findings of Kailasapathy (2006) who reported that probiotic yogurt containing encapsulated probiotic bacteria with alginate and resistant starch had non-significantly better color and appearance than control normal yogurt. Although it appears that the addition of alginate beads in yogurt may be slightly change the color, but use of resistant starch in combination with alginate cause to prepared microcapsules have a white color Kailasapathy (2006). Thus, there were not sensible changes in color of M samples compared to C samples, even caused to improve the color of M samples, with the passage of time. The present results are in agreement with the findings of Hussain *et al* (2009) who indicated that the color score of probiotic yogurt containing free and non- microencapsulated probiotic bacteria was lower than the control yogurt samples.

Taste and flavor:

Changes in taste of yogurt samples during 8 weeks storage at 4˚C were shown in Fig. 1B. After 24 h storage at 4℃, taste and flavor of F samples was significantly better than M samples ($p<0.05$). However, there were not any significant differences between the taste score of 3 yogurt samples until week 4. In week 4 the taste score of C samples was significantly higher than M. In week 5 the differences was not significant. In week 6 taste of M was significantly better than F and C. In week 7 taste score of F samples was higher than M and significantly higher than C samples. In week 8 the taste of M was significantly better than F and C. After 24 h of storage at 4℃, no significant difference was observed between the taste and flavor of M and C yogurt samples ($p>0.05$).

Taste and flavor of F samples was significantly better than M samples ($p<0.05$), and non-significantly better than C samples ($p>0.05$). Thus it can be concluded that addition of *L. plantarum* as free and non-encapsulated improves the taste and flavor of yogurt.

The results of this research are in agreement with the findings of Hussain *et al* (2009) who reported that the taste and flavor of probiotic yogurts were better than regular yogurt samples. Also present findings are in accordance with the results of Kailasapathy (2006) as he reported that the probiotic yogurt containing free cells of *L. acidophilus* DD910 and *B. lactis* DD920 have non-significantly better taste and flavor compared with control normal yogurt and probiotic yogurt containing encapsulated bacterial cells.

Taste and flavor of M yogurt samples was non -significantly improved after 8 weeks of storage at 4℃ ($p>0.05$). However, this factor was non-significantly declined in F and C samples ($p>0.05$). This could be due to the gradual release of microencapsulated *L. plantarum* cells and their influence on taste and flavor of yogurt.

After 8 weeks of storage at mentioned temperature, there was not any significant differences between taste and flavor of three yogurt samples ($p>0.05$). So, *L. plantarum* PTCC 1058 could be used in the manufacture of probiotic yogurts as a beneficial probiotic species.

Texture and consistency:

Changes in texture of yogurt samples during 8 weeks storage at 4˚C were shown in Fig. 1C. There were not any significant differences between texture score of 3 yogurt samples until week 3. In week 3 the texture score of C samples was significantly higher than M. In week 4, texture of F and C was significantly better than M. In week 5 and 6 the differences was not significant. In week 6 texture of M was significantly better than F and C. In week 7 texture score of M and F samples was significantly higher than C. In week 8 the texture of F was significantly better than C and it was significantly better than M.

After 24 h of storage at 4℃, the texture and consistency score of M samples were significantly lower than C samples ($p<0.05$). The texture and consistency score of F samples were higher than M samples and lower than C samples, but not significantly ($p>0.05$).

After 8 weeks of storage the texture and consistency of F samples were non-significantly improved ($p>0.05$). But, this factor were dropped in M and C samples with significant decrease in C samples ($p<0.05$). After week 8, the texture and consistency score of F samples were significantly better than M samples ($p<0.05$) and non-significantly better than C samples ($p>0.05$).

These findings suggest that the addition of *L. plantarum* to the yogurt in free form can improve the texture and consistency of the product during the storage period. It is reported that exopolysaccharide-producing bacteria increase the viscosity and texture of yogurt and enhance the resistance of yogurt gel against the mechanical damages (Tamime and Deeth, 1980; Griffin *et al*., 1996). It have been shown that yogurt starter bacteria have the ability to produce exopolysaccharides in a lesser amount, vary depending on the strain used (Kailasapathy, 2006).

Despite, it was thought that adding the microcapsules containing alginate and starch improves the texture, but it has not been observed in practice. Reduce of the texture feature of M samples may be due to the disruption of yogurt gel structure by added microcapsules. However, because the small size of prepared microcapsules (about 20 μm), no grittiness were sense in the mouth.

Results of this research are in contrast with findings of Kailasapathy (2006) who showed that probiotic yogurt containing alginate and resistant starch microcapsules had better texture than probiotic yogurt containing free probiotic bacteria and plain yogurt. It is indicated that sodium alginate used for microencapsulation of probiotic bacteria can form gel by cations exist in yogurt such as calcium. Prepared microcapsules have certain gaps and traps some part of the calcium ions in yogurt gel (Kailasapathy, 1996).

Starch was used in this study was the resistant starch with a high amylose content. This type of starch can absorb water and swell, but can not be fully gelatinized. So, swollen starch can increase the viscosity and stiffness of yogurt gel. Porous structure of sodium alginate microcapsules may allow the starch granules get out of the microcapsules and enter the yogurt gel matrix (Kailasapathy, 2006). Williams *et al*. (2004) reported that the addition of starch into the stirred yogurt can improve the textural characteristics.

Total acceptability:

Total acceptability of yogurt samples during 8 weeks storage at 4˚C were shown in Fig. 1D. After 24 h of storage at 4℃, total acceptability score of F samples were higher than M and C samples, but not significantly ($p>0.05$). After one week, the score of F and C was significantly higher than M. There were not any significant differences between the score of three yogurt samples in week 2 and 3. In week 4 the score of C samples was significantly higher than M. But in week 5 the results were reversed and the score of M samples was significantly higher than C. In week 6 the differences was not significant. In week 7, the score of F samples was significantly higher than C and M. In week 8 the score of F and M was significantly better than C.

After 24 h of storage at 4℃, total acceptability score of F samples were higher than M and C samples, but not significantly ($p>0.05$). Also there were not any significant differences between total acceptability of M and C samples ($p>0.05$). After 8 weeks, the total acceptability of F and M samples were improved, however this factor was dropped in C samples, non-significantly ($p>0.05$). After week 8, the scores of total acceptability in F and M samples were non-significantly better than C samples ($p>0.05$).

The results are in agreement with Hussain *et al*. (2009) who reported that the total acceptability of probiotic yogurt was higher than plain yogurt. Although, our results are in agreement with Kailasapathy (2006) who indicated that there were not significant differences between overall acceptance of probiotic yogurt containing free and microencapsulated bacteria and plain yogurt.

Adding free *L. plantarum* to yogurt could improve the taste and flavor and adding alginate/resistant starch encapsulated *L. plantarum* could improve the color of yogurt. Because the factors of color, taste and flavor are important factors in the acceptance or rejection of a product by the consumer (Hussain *et al*., 2009), so the overall acceptance of the probiotic yogurts was obtained higher than plain yogurt. It was found that adding *L.*

plantarum as free or alginate/resistant starch microencapsulated form can cause minor loss in some sensory features, but the overall acceptance of our probiotic yogurt samples were higher than plain yogurt samples.

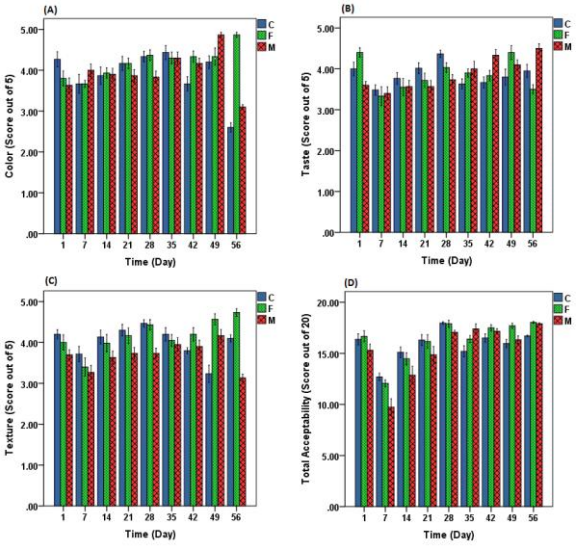

Fig. 1: Sensory properties of yogurt samples during 8 weeks storage at 4˚C. (A) Color, (B) Taste, (C) Texture, (D) Total acceptability. F: probiotic yogurt contained free *L. plantarum*, M: probiotic yogurt contained microencapsulated *L. plantarum*, C: plain yogurt (Control). Mean ± 1 SE (n=30).

Conclusion:

It was indicated that *L. plantarum* could improve some sensory properties of yogurt in both free and microencapsulated forms during the 8 weeks storage period. So, it was concluded that production of probiotic yogurt containing free or encapsulated *L. plantarum* is possible by maintaining the sensory properties similar to or even better than regular yogurt.

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