PRODUCTION OF NOVEL FUNCTIONAL WHITE SOFT CHEESE

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ABSTRACT

The increasing side effects and the cost of allopathic medicines make the consumer incline towards alternative therapeutic agents. As dietary supplements are the most consumers acceptable therapeutic agents the present study was carried out to develop a symbiotic soft cheese with new Lactobacillus strains. Functional white soft cheese was manufactured using starter containing Streptococcus thermophilus as a main strain mixed (1:1) with one of the selected Lactobacillus strains (Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus johnsonii NRRL B-2178, Lactobacillus hilgardii NRRL B-1843 and Lactobacillus curvatus NBIMCC-3452) with adding 3% of one of the selected prebiotics (dextrin and litesse). The moisture content, the pH values, acidity, total and soluble nitrogen % as well as the bacteriological and organoleptic properties during storage at 7°C for 30 days of soft cheeses were evaluated. The results revealed that, there was significant difference (p<0.05) in all studied properties of white soft cheese. Symbiotic white soft cheese manufactured with probiotic Lb.curvatus NBIMCC-3452 and 3% of prebiotics (dextrin or litesse) had the highest viability of these strains. The highest average sensory evaluation points were recorded in cheeses made with Str. thermophilus plus Lb. johnsonii NRRL B- 2178 (1:1) and 3% dextrin; and Str. thermophilus plus Lb. curvatus NBIMC 3452 (1:1) and 3% litesse at 20 days of refrigeration storage. It could be recommended that combination among dextrin or litesse as
prebiotics with Lactobacillus strains (Lb. hilgardii NRRL B-1843, Lb johnsonii NRRL B-2178 and Lb. curvatus NBIMCC-3452) as probiotics for manufacturing symbiotic white soft cheeses with high quality and healthy properties.

Keywords: Functional cheese, probiotics, prebiotics, Lactobacillus

INTRODUCTION

Nowadays, the current consumer's interest towards functional products that contribute to decrease risks of diseases so, there is a growing market for foods containing probiotic bacteria, and sales have increased from 7 to 32% each year as a function of products and geographical regions (Helene et al., 2011). Commercial interest in functional food containing probiotic strains has consistently increased due to the awareness of the benefits for gut health, disease prevention and therapy (Chapman et al., 2011). However, Modern consumers expect their food to be healthy and to prevent illness as they are increasingly interested in their personal health (Kailasapathy, 2009). This explains the reason for a rising interest in probiotic health-based products.

In fact, probiotic products are important functional foods as they represent about 65% of the world functional food market (Agrawal, 2005). Probiotic bacteria have been incorporated into a wide range of foods, including dairy products (such as yogurt, cheese, ice cream, dairy desserts) but also in non-dairy products (such as chocolate, cereals, juices) (Anal and Singh, 2007). Foods containing probiotic bacteria are categorized as “functional foods” and such products are gaining widespread popularity and acceptance throughout the developed world.

A number of health benefits for product containing live probiotic bacteria have been claimed including alleviation of symptoms of lactose intolerance, treatment of diarrhea, anticarcinogenic properties, and reduction in blood cholesterol and improvement in immunity (Vasiljevic and Shah, 2008). In order to exert their beneficial effect, probiotic bacteria need, firstly, to survive during the manufacturing food-process and then in the upper gastrointestinal (GI) ecosystem.

The ability of probiotic strains to survive passage through the GI tract can be mainly attributed to their acid and bile tolerance. These are intrinsic characteristics of the strain,
which can be improved by the protective action of carrier foods (Charalampopoulos et al., 2003) and/or by the presence of nutrients such as prebiotics (Corcoran et al., 2005).

The most common food matrices used as probiotic vehicles are dairy products, which are able to enhance the transit tolerance of bacteria. Cheese is a dairy product which has a good potential for delivery of probiotic microorganisms into the human intestine due to its specific chemical and physical characteristics compared to fermented milks (higher pH value and lower titratable acidity, higher buffering capacity, greater fat content, higher nutrient availability, lower oxygen content and denser matrix of the texture). To be considered to offer probiotic health benefits, probiotics must remain viable in food products above a threshold level (e.g., $10^6$ CFU/g) until the time of consumption (Karimi et al., 2011).

In fact, cheese provides a valuable alternative to fermented milks and yogurts as a food vehicle for probiotic delivery, due to certain potential advantages. It creates a buffer against the high acidic environment in the gastrointestinal tract and thus creates a more favourable environment for probiotic survival throughout the gastric transit, due to higher pH. Moreover, the dense matrix and relatively high fat content of cheese may offer additional protection to probiotic bacteria in the stomach (Bergamini et al., 2005 and Sharp et al., 2008).

The presence of the prebiotics were described to promote increased growth rates of bifidobacteria and lactobacilli, besides increased lactate and short chain fatty acids production in petit-suisse cheese supplemented with these microorganisms and submitted to batch culture fermentation with human faecal slurry (Cardarelli et al., 2007).

Fresh cheese appears to be ideally suited to serve as a carrier for probiotic bacteria as it is an unripened cheese, during storage it is submitted to refrigeration temperatures, and its shelf life is rather limited (Heller et al., 2003). So, many researches were incorporated different probiotic (Lactobacillus and Bifidobacterium) or and prebiotics during manufacturing to produce functional dairy products, such as soft cheeses (Effat, 2000 and Mehanna et al., 2002).

Therefore, the main objective of this research was to analyze the potential effect of prebiotic ingredients (as a 3 % dextrin or litesse) on growth and survival of some probiotic lactobacilli cultures in actual functional white soft cheese and to study the influence of probiotic cultures on properties of cheese.
MATERIALS AND METHODS

Materials

Fresh whole raw buffalo's milk (6% fat and 8.94% SNF) was used in this study and obtained from the herd of Faculty of Agriculture, Cairo University. Hannilase rennet powder (CHY- Max powder extra) was purchased from Chr. Hansen's Lab., Denmark. Commercial fine grade salt of El-Nasr Salines Company, Egypt and calcium chloride from Sigma Chemical Company, Str. Louis, USA, were used for manufacturing synbiotic white soft cheese.

Bacterial strains

The microorganisms used in this study were as follows: *Streptococcus thermophilus* St-20 and *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb-12 were obtained from Dairy Microbiology Laboratory, National Research Centre. *Lactobacillus johnsonii* NRRL B-2178 and *Lactobacillus hilgardii* NRRL B -1843 were provided from Northern Regional Research Laboratory, Illinois, USA. *Lactobacillus curvatus* NBIMC 3452 was supplied by National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria.

All strains had previously been shown to possess properties required of probiotic microorganisms including bile salt and low pH tolerance, various antibiotic resistances and antagonistic activity (*Mabrouk et al.*, 2007).

Prebiotics

Litesse™ (Poly dextrose) was obtained from Danisco Sweeteners, OY, and KOTKA, Finland and Dextrin was obtained from population of Corn Products Company, 10th of Ramadan City, Egypt.

Functional white soft cheese manufacturing

In this research, seven groups of functional white soft cheese were manufactured according to the method adopted by *Fahmi and Sharara* (1950). Fresh buffaloes' milk was standardized to contain 6% fat and 8.84% SNF, heat treated to 80°C for 10 min and then
cooled to 40°C. Milk was divided into 7 equal portions for the addition of 5 different starter cultures. Five different starter cultures freshly prepared in 12% sterile reconstituted skim milk powder (RSMP) and separately supplemented with 3% dextrin and litesse were added to the seven milk portions at the level of 2%.

Experimental procedures were implanted in the following treatments:

Control: *Str. thermophilus* + *Lb. delbrueckii* ssp. *bulgaricus* (1:1).

T1: *Str. thermophilus* + *Lb. johnsonii* NRRL B-2178 (1:1) + 3% litesse.

T2: *Str. thermophilus* + *Lb. johnsonii* NRRL B-2178 (1:1) + 3% dextrin.

T3: *Str. thermophilus* + *Lb. hilgardii* NRRL B-1843 (1:1) + 3% litesse.

T4: *Str. thermophilus* + *Lb. hilgardii* NRRL B-1843 (1:1) + 3% dextrin

T5: *Str. thermophilus* + *Lb. curvatus* NBIMC 3452 (1:1) + 3% litesse.

T6: *Str. thermophilus* + *Lb. curvatus* NBIMC 3452 (1:1) + 3% dextrin.

The inoculated milk portions were allowed for *Lactobacillus* strains propagation and acid production for 1 h, and then calcium chloride solution 40% (0.02%) and salt (2.5%) were added to each portion and stirred well. The rennet was added at the rate of 1.5 g / 100 kg milk and left to complete coagulation. The curd was ladled in rectangular frames (20x20cm) lined with cloth and the drained whey was collected to be used a brine for cheese storage and maturation.

The resulting functional white soft cheeses were cut into cubs and packaged into plastic containers (capacity 150 cc), which filled with cooled whey of the same lot of the resulting cheeses and stored at 7 ± 2 °C for 30 days. The experiments were carried out in triplicate. Samples of each functional white soft cheese were withdrawn when fresh and after 10, 20 and 30 days of storage for chemical, bacteriological and organoleptic analysis. Data were reported as the average of three independent trials.

**Chemical analyses**

Titratable acidity, moisture, salt content, total nitrogen (TN) and soluble nitrogen (SN) (using semi - microkjeldahl method) contents of all functional white soft cheese samples were determined according to the methods described by Ling (1963). The pH values of all cheese samples were also measured by using a pH meter (HNNA, Model 211).
Bacteriological analyses

Samples of all functional white soft cheeses were prepared for bacteriological analysis according to the method described in the Standard Methods for the Examination of Dairy Products (Marshall, 1992).

Total bacterial count

Total viable bacterial counts of all functional white soft cheeses samples were determined by using plate count agar medium (Oxoid) according to Houghtby et al. (1992). The plates were incubated at 32 ± 1 °C for 48 h.

Lactobacillus count

Viability of probiotic cultures were assessed during production and during refrigeration storage at 7°C for 30 days. Lactobacillus bacterial counts was determined using MRS agar medium (Oxoid) according to De Man et al. (1960). All plates were anaerobically incubated at 37°C for 48 h.

Streptococcus thermophilus count

Streptococcus thermophilus count was enumerated using M17 agar medium (Oxoid) according to International Dairy Federation (1995). The plates were anaerobically incubated at 37°C for 48 h.

Sensory evaluation

Samples of functional soft cheese were cut into approximately 5x5 cm pieces and placed on white plates. Samples were tempered at ambient temperature (20 ± 2 °C) and then presented to the panelists in a random order. The cheeses were evaluated organoleptically after zero, 10, 20 and 30 days of ripening in Dairy Science Department, National Research Center by ten members of laboratory staff familiar with soft cheese. Panelists evaluated cheese for appearance (10 points) , body and texture (40 points) and flavor (50 points). Scores were obtained for the three sensory attributes.
Statistical analyses

All achieved data were statistically analyzed using the general linear models procedure of the Statistical Analysis System SAS (1998). Significance of difference was defined at \( p<0.05 \). All experiments as well as the related analysis results were repeated three times. Also, all obtained data are expressed as average.

RESULTS AND DISCUSSION

Chemical analysis

Moisture content (%)

![Figure 1](image)

**Figure 1** Changes in moisture (%) of functional white soft cheeses, during refrigeration storage at 7°C for 30 days

The moisture contents of functional white soft cheeses made with adding one of the 3 probiotic *Lactobacillus* strains and the 2 prebiotics (dextrin or litesse) was slightly higher as compared with the control white soft cheese (figure 1). Also, significant differences in
moisture content (p < 0.05) were found between all functional white soft cheese treatments. Treatment made with *Lb. curvatus* NBIMCC 3452 and *Str. thermophilus* (1:1) with 3% dextrin (T5) had the highest moisture content at the end of storage period. Generally, the data reveal that there was a gradual loss in the moisture content of all functional white soft cheeses through the refrigeration period. This might be due to the shrinkage of the curd as a result of acid development which helps to expel the whey from the cheese mass (*Gafour, 2005*).

**Salt content (%)**

Data presented in figure 2 showed that, salt content (%) of functional white soft cheese were nearly the same values when fresh in all cheese treatments. There were significant differences (p<0.05) between functional and control white soft cheeses in salt content at the end of the refrigeration period (7°C/30 days). Besides, it could be seen that the salt contents (%) of functional white soft cheeses were gradually increased by slight values up till the end of the refrigeration period. These findings of salt content are in harmony with those obtained by *Shehata et al. (2001)* and *Mehanna et al. (2002)*.

![Salt content (%) of functional white soft cheeses, during refrigeration storage at 7°C for 30 days](image)

**Figure 2** Salt content (%) of functional white soft cheeses, during refrigeration storage at 7°C for 30 days

**Changes of pH values and titratable acidity**

As shown in figure 3 there were significant differences (p <0.05) between functional and control white soft cheeses in pH values when fresh and during the refrigeration period (7°C/30 days). Also, the results show that the pH values of functional white soft cheeses made with 3 probiotic *Lactobacillus* strains in the presence of 3 % of different prebiotics were
significantly lower than the control white soft cheese made with the traditional starter (Str. thermophilus and Lb. delbrueckii ssp. bulgaricus) and without adding prebiotics, either when fresh or during the refrigeration period (7°C/30 days). Moreover, functional white soft cheeses manufactured with Lb. curvatus NBIMC 3452 plus Str. thermophilus (1:1) and added 3 % dextrin had the lowest pH value, especially at the end of storage period. In addition it could be notice that the pH values of all soft cheeses gradually decreased during the refrigeration period (7°C/30 days). The obtained results are in harmony with those obtained by Magdoub et al. (1995); they reported that the decrease in pH values may be due to the convert of residual lactose in cheese to lactic acid and free fatty acid which had developed in the cheese at the end of storage period. Besides, Fooks et al. (1999) reported that the decrease in pH values may be due to short chain fatty acids which produced in varying quantities as metabolic end product of the probiotic bacteria.

![Figure 3](image)

**Figure 3** Change in pH values of functional white soft cheeses, during refrigeration storage at 7°C for 30 days

Titratable acidity (%) of functional white soft cheeses when fresh and during refrigeration storage (7°C/30 days) are illustrated in figure 4. The changes in titratable acidity of functional soft cheese followed an opposite trend to pH. Titratable acidity (%) of functional white soft cheese was significantly higher; especially at the end of refrigeration period; as compared with the control cheese. Moreover, statistical analysis revealed that the titratable acidity of synbiotic white soft cheeses was significantly affected (p<0.05) by the refrigeration period and variation among the six different treatments. However, functional white soft
cheeses made with *Str. thermophilus* plus *Lb. johnsonii* NRRL B-2178 and *Lb. hilgardii* NRRL B-1843 (1:1) and added 3% litesse had the highest values of titratable acidity (0.73%) at the end of the refrigeration period. Mehanna *et al.* 2002 and Elewa *et al.* 2009) mentioned that the development of acidity during the refrigeration period is a direct response for converting the residual lactose in cheese into lactic acid by the available micro-flora.

![Figure 4](image)

**Figure 4** Acidity developments (%) of functional white soft cheeses, during refrigeration storage at 7°C for 30 days

**Ripening parameters**

**Change in total nitrogen (%)**

Figure 5 illustrates that, change in total nitrogen (TN) (%) of synbiotic white soft cheeses during refrigeration storage at 7°C for 30 days. Statistical analysis indicated that the TN content of all cheese treatments were significantly affect (p <0.05) by the refrigeration period (7°C/30 days). Besides, it could be seen that, during the refrigeration period (7°C/30 days), the control cheese had lower values of TN as compared with those synbiotic white soft cheese made with the 3 probiotic *Lactobacillus* strains in addition to 3 % prebiotics (dextrin and litesse). Also, it could be observed that, the TN % reached the lowest values at the end of the refrigeration period. These results are in harmony with those obtained by El- Zayat and Osman (2001), they mentioned that the decrease in the TN content during refrigeration in all
treatments could be attributed to the protein degradation into SN and subsequently partial loss into the pickling solution.

2.1

2.1.2

2.1.4

2.1.6

2.1.8

2.2

2.2.2

2.2.4

2.2.6

2.2.8

Control  T1  T2  T3  T4  T5  T6

Figure 5 Change in total nitrogen (%) of functional white soft cheeses, during refrigeration storage at 7°C for 30 days

Change in soluble nitrogen (%)

Figure 6 show changes in soluble nitrogen (SN) (%) of functional white soft cheeses separately made with 3 probiotic Lactobacillus strains and 2 prebiotics separately added (3% dextrin and litesse). These data indicate slightly higher in SN% of the functional cheeses than cheese control at the end of the refrigeration period (7°C/30 days). Besides, it could be noticed gradual increase in the SN% in all cheeses up till the end the refrigeration period. Statistical analysis proved that variation in different cheese treatments and the refrigeration period were not significantly affect (p>0.05) the SN %. These results coincide with those obtained by Elewa et al., (2009), who reported that the SN contents of white soft cheeses made with probiotics show an increase at the end of storage period. On similar trend, Shehata et al. (2001) mentioned that the increase of SN (%) of soft cheese could be due to the enzymes released by starter cultures (Lb. casei, Lb. rhamnosus and Lb. delbrueckii ssp. bulgaricus) during the pickling.
Figure 6 Change in soluble nitrogen (%) of functional white soft cheeses, during refrigeration storage at 7°C for 30 days

Bacteriological analysis

Total viable bacterial counts

Figure 7 show that, there were significant differences (p <0.05) in total viable bacterial counts among the control and functional white soft cheeses during refrigeration storage (7°C/30days). Besides, functional cheeses had slightly higher total viable bacterial counts when fresh and during refrigeration (7°C/30days) comparatively with the control cheese. Also, it could be shown that the total viable bacterial counts of all functional cheeses were increased during the first 10 days of the refrigeration period then declined reaching the lowest counts at the end of the refrigeration period. In addition, it could be seen that the refrigeration period had significantly affected (p <0.05) the total viable bacterial counts of all functional cheeses. Decrease in the total viable bacterial counts during refrigeration storage could be attributed to lactic acid, in addition to some metabolites, such as H$_2$O$_2$ released during the lactic starter growth. The achieved results consentient with Mehanna et al. 2002 and Degheidi et al., 2009.
Lactobacilli and Streptococci counts

Survivals of *Lactobacillus* and *Streptococcus* strains (Log cfu/g) in functional white soft cheeses made with 3% dextrin during refrigeration storage (7°C/30days), are shown in Tab. 1. Lactobacilli and Streptococci counts in all cheese treatments were significantly affected (p<0.05), either when fresh or during the refrigeration storage period (7°C/30days). Higher counts of *Lactobacillus* strains could be attributed to the ability of genus *Lactobacillus* to survive at high acidity and the presence of 3% dextrin as compared with counts of Streptococci genus. The highest counts at the end of the refrigeration period were recorded by each of *Lactobacillus curvatus* NBIMCC-3452 and *Str. thermophilus*. Besides, it could be noticed that cheese made with *Lb. hilgardii* NRRL B-1843 had higher Lactobacilli counts, but lower counts of *Str. thermophilus* as compared with the corresponding counts of *Lb. johnsonii* NRRL B-2178 and *Str. thermophilus*. Moreover, at the end of the refrigeration period, counts of *Str. thermophilus* of all functional cheeses were higher than that in the control treatment. It could be also seen that counts of all lactic acid strains (*Lb. delbrueckii* ssp. *bulgaricus*, *Lb. johnsonii* NRRL B-2178, *Lb. hilgardii* NRRL B-1843, *Lactobacillus curvatus* NBIMCC-3452 and *Str. thermophilus*) increased during the refrigeration period reaching the maximum counts after 10 days, and then decreased with prolonging the storage period. These results were in
agreement with those obtained by Elewa et al., (2009), who reported that addition of prebiotics most probably improved the growth and viable counts of probiotics.

Table 1 Survival of Lactobacillus and Streptococcus strains (Log cfu/g) in functional white soft cheeses made with 3% dextrin, during refrigeration storage at 7°C for 30 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Strains</th>
<th>Refrigeration period (days)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Str. thermophilus</td>
<td>8.30^{bA}</td>
</tr>
<tr>
<td></td>
<td>Lb. delbrueckii ssp. bulgaricus</td>
<td>8.52^{bB}</td>
</tr>
<tr>
<td>T2</td>
<td>Lb. johnsonii NRRL B-2178</td>
<td>8.39^{bcC}</td>
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<tr>
<td></td>
<td>Str. thermophilus</td>
<td>8.12^{bdD}</td>
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<tr>
<td>T4</td>
<td>Lb. hilgardii NRRL B-1843</td>
<td>8.71^{bcE}</td>
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<tr>
<td></td>
<td>Str. thermophilus</td>
<td>8.30^{baA}</td>
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<tr>
<td>T6</td>
<td>Lb. curvatus NBIMCC – 3452</td>
<td>8.68^{cF}</td>
</tr>
<tr>
<td></td>
<td>Str. thermophilus</td>
<td>8.41^{bgG}</td>
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Different small letters within the same row are significantly different (P < 0.05). Different capital letters within the same column are significantly different (P < 0.05).

Data shown in Tab. 2 indicate that, during the first 10 days of the refrigeration period (7°C/30days) it could be perceive that all Lactobacillus strains (Lb. delbrueckii ssp. bulgaricus, Lb. johnsonii NRRL B-2178, Lb. hilgardii NRRL B-1843 and Lb. curvatus NBIMCC-3452) and Str. thermophilus increased, then followed by gradual decline reaching the minimum counts at the end of the refrigeration period. Furthermore, Statistical analysis revealed that type of strain and / or the refrigeration period significantly affected (p<0.05) Lactobacilli and Streptococci counts of all functional cheeses. Lactobacillus hilgardii NRRL B-1843 and Str. thermophilus in the cheese treatment recorded the highest counts at the end of the refrigeration period.
According to the fore-mentioned results, the highest counts (Log cfu/g) of probiotic *Lactobacillus* strains were observed in functional white soft cheese made with *Lb. hilgardii* NRRL B-1843 and 3% dextrin, followed by made with *Lb. curvatus* NBIMCC-3452 and 3% litesse. Besides, it could be noticed that the numbers of *Str. thermophilus* in the in functional cheeses were higher than that in the control cheese. This could be revealing the stimulating effect of the added prebiotics on the growth behavior of all lactic acid strains (Elewa et al. 2009). These results coincide with those obtained by Sharaf et al. (2003); they reported that the use of dextrin could be improving the viability of *Lactobacillus* strains.

**Sensory evaluation**

Data shown in Tab. 3 indicate that, sensory evaluation of functional white soft cheese behaved the same trend in all cheese treatments, as gradual enhancement was noticed during the first 20 days of the refrigeration period. However, continuous production of lactic acid and other organic acids lead to fragile cheese showing gradual decrease in body and texture and appearance scores recorded for all cheese treatments up till the end of the refrigeration period. Statistical analysis revealed that type of strain and/or the refrigeration period (7°C/30days) significantly affected (P<0.05) total scores of sensory evaluation of all cheeses. Moreover, it could be observed that the maximum total scores were recorded at the end of the refrigeration period for functional cheese containing probiotic *Lb. curvatus* NBIMCC-3452 with 3% litesse (T5). These results coincide with those obtained by Stanton et al. (1998) Buriti et al. (2005).

**Table 2** Survival of *Lactobacillus* and *Streptococcus* strains (Log cfu/g) in functional white soft cheeses made with 3 % litesse, during refrigeration storage at 7°C for 30 days

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<tr>
<td>Control</td>
<td><em>Str. thermophilus</em></td>
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<tr>
<td></td>
<td><em>Lb. delbrueckii</em> ssp.</td>
<td>8.52&lt;sup&gt;bB&lt;/sup&gt;</td>
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<td></td>
<td><em>bulgaricus</em></td>
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<td><em>Lb. johnsonii</em> NRRL</td>
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<td>B-2178</td>
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<td>Treatment</td>
<td>Storage period (days)</td>
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Different small letters within the same row are significantly different (P < 0.05). Different capital letters within the same column are significantly different (P < 0.05 %).

**Table 3** Sensory evaluation of synbiotic white soft cheeses, during refrigeration storage at 7°C for 30 days.
CONCLUSION

The results of the present study demonstrate that combination among dextrin or litesse as prebiotics with *Lactobacillus* strains (*Lb. hilgardii* NRRL B-1843, *Lb. johnsonii* NRRL B-2178 and *Lb. curvatus* NBIMCC-3452) as probiotics can be used for manufacturing functional white soft cheeses with high quality and with potential health benefits.

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