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The effects of different levels of β-glucan on yoghurt manufactured with *Lactobacillus plantarum* strains as adjunct culture

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Abstract

This study investigated the effects of β-glucan on regular (full-fat) yoghurts manufactured with probiotic *Lactobacillus plantarum* strains (AB6-25, AC18-82 and AK4-11) combination as adjunct culture. In the present work, production was carried out using full fat milk. Four different experimental yoghurts were produced with 0.25, 0.5, 1.0 and 1.5% β-glucan for treatment groups. All experimental yoghurts were also included probiotic combination and commercial starter yoghurt cultures. In addition, experimental yoghurt groups were compared with three control yoghurts produced with commercial culture (C1), commercial culture and probiotic combination (C2), and commercial culture, *Lactobacillus acidophilus* LMG 11472 and inulin (C3). Viability of lactic acid bacteria (LAB) and probiotic bacteria, physicochemical attributes of yoghurts, production of aroma compounds and organic acids during the storage period of 21 days at 4°C were determined. The addition of β-glucan (0.5 and 1%) enhanced the growth of *L. plantarum* strains ($p<0.05$) in yoghurt at the end of storage period. It was determined that the addition of β-glucan had no observable effect on pH, fat and protein content in yoghurt. In general, yoghurts with β-glucan showed more ($p<0.05$) syneresis compared to other groups. However, this effect was not observed in yoghurts produced with 0.25% β-glucan. Concerning aroma compounds of yoghurt, β-glucan suppressed acetaldehyde formation. With regard to organic acids, the higher β-glucan addition slightly decreased formic and lactic acid formation. It was concluded that the addition of β-glucan (0.25-0.5%) with probiotics enhanced microbiological and physicochemical properties of symbiotic yoghurts which may help to improve the health benefits of consumers.

Key words: β-glucan, yoghurt, probiotic, *Lactobacillus plantarum*.

Introduction

Continuous development of new functional foods is the response of science and industry to the increased consumer awareness regarding health and the role of foods for improving quality of life [1]. The products that contain both prebiotics and probiotics are referred to as 'synbiotics'. Synbiotics are a combination of the effects of probiotics and prebiotics to produce health enhancing functional food ingredients [2]. Prebiotics are defined as non-digestible food ingredients that have a beneficial effect on the health of the host, selectively stimulating growth and/or the activity of a limited number of bacteria in the colon [3]. The beneficial role of dietary fibre in human nutrition has led to a growing demand for incorporation of novel fibres into foods [4].

Oats (*Avena sativa* L.), as a fairly important cereal, has earned increased attention in recent years due to different dietary fibre types, such as β-glucan, arabinoxylans and cellulose, in addition to relatively high levels of protein, lipids (unsaturated fatty acids), vitamins, antioxidants and phenolic compounds [5]. Oat's β-glucan is water-soluble, for this reason water-soluble β-glucan has an important place for human nutrition. Its usage is permitted in many countries and FDA guidelines recommended 0.75 g β-glucan/serving for a health claim [6]. Oat's β-glucan plays a significant role in moderating the effects of hypertension, lowering the total and low-density lipoprotein content, regulating blood glucose and insulin levels, managing weight and promoting gastrointestinal health [7]. It was reported to produce prebiotic effect in the ceca of rats [8]. It was also reported that β-glucan can be used structurally functional additive due to its thickening, stabilizing, and gelling properties [9]. Functional properties of β-glucan have been assessed in numerous preparations and mainly cereal systems and cheeses[10]. Addition of dietary fibers although physiologically beneficial may affect textural characteristics of a product and consequently sensory perception [11].

Yoghurt is a dairy product produced by bacterial fermentation of milk. Yoghurt is traditionally considered to be a healthy food. It can be made functional by the addition of probiotics and prebiotics [2]. In recent years, there has been increasing demand for the use of soluble dietary fibre, and in particular cereal β-glucans, as stabilizers in the manufacture of dairy products such as ice creams and yoghurts [12].

The aim of the present study was undertaken to examine the influence of added probiotic *Lactobacillus plantarum* strains for their potential application as adjunct cultures and different β-glucan concentrations on the microbiological and physicochemical properties, volatile composition and organic acid profiles of yoghurt.

Materials and Methods

L. plantarum AB6-25, AC18-82 and AK4-11 used in this study were previously isolated from human sources [13]. These strains were selected according to the their good probiotic properties such as; resistance to gastric acidity, resistance to 0.4% phenol, production of H_2O_2, adhesion to Caco-2 cell line and antimicrobial activities, and technological properties such as; stability in fermented milk during refrigerated storage, pH changes during cold storage, coagulation of skim milk, proteolytic activities, organic acid and flavor compounds production and survival in β-glucan [14]. The freeze-dried (DVS) starter cultures and the milk were obtained from Çavuşoğulları Dairy Factory (Burdur, Turkey). The probiotic strain *L. acidophilus* LMG 11472 was obtained from the BCCM/LMG culture collection in Gent, Belgium. The commercial oat β-glucan (PROMOAT soluble oat fibre) and inulin (Orafti, HPX) were provided by Güler Kimya Ltd. Şti (Istanbul, Turkey) and Artisan Gida Ltd. (Istanbul, Turkey), respectively.

Yoghurt manufacture: The yoghurt manufacture was carried out using full fat milk containing 3.5% fat. Seven different groups of yoghurt were manufactured. The control 1 (C1) was produced using DVS starter culture (2%), the control 2 (C2) was made using the DVS starter culture (1%) and *L. plantarum* combination, the control 3 (C3) was produced using DVS starter culture (1%), *L. acidophilus* LMG 11472 strain and inulin (2%). Group C3 was added to the study to compare the treatment groups with mostly known synbiotic yoghurt formula. All the treatment groups (T) were produced by using the DVS starter culture (1%) and *L. plantarum* combination. The oat β-glucan were added into the T1, T2, T3 and T4 groups at the levels of 0.25, 0.5, 1 and 1.5%, respectively.

For the yoghurt production, standardized milk was heated at 60–75°C and homogenised at 10,000 rpm for 15 s for solving the β-glucan and inulin. Then the milk was pasteurised at 85°C and cooled at 44°C. The inoculated groups were dispensed into presterilized glass containers, incubated at 42°C until the pH reached 4.50, cooled to 4°C, and then stored for 3 weeks. At time intervals of 1, 7, 14 and 21 days, yoghurt samples were subjected to microbiological and physicochemical analysis. All yoghurt trials were repeated twice.

Microbiological analysis of yoghurts: Viability of the LAB and the total mesophilic aerobic bacteria (TMAB) were assessed during the storage period of 21 days at 4°C. Ten grams of yoghurt were emulsified with 90 ml of sterile 1/4 ringer solution. Decimal dilutions in ringer solution were made and plated on de Man, Rogosa and Sharpe (MRS) (Merck, Germany) and plate count agar (PCA) (Merck, Germany) for the LAB and TMAB counts. MRS and PCA plates were incubated in aerobic conditions for 48 h at 37°C and 30°C, respectively. The number of *L. plantarum* strains were also determined on *L. plantarum* selective agar (LPSM) at 37°C for 48 h [15]. For determining the viability of starter cultures, M17 agar was used for *Streptococcus salivarius* subsp. *thermophilus*. The plates were incubated in aerobic conditions for 48 h at 37°C. *L. delbrueckii* subsp. *bulgaricus* was counted on reinforced clostridia agar (RCA) at pH 5.3 and anaerobic incubation (GasPak System-OXOID) at 42°C for 48 h [16].

Physicochemical analysis of yoghurts: Dry matter (DM) content was determined by gravimetric method using an the oven drying in a laboratory oven BINDER GmbH (Germany), at 105°C (TSI, 1999). Fat were determined by the Gerber method [17]. Total nitrogen was measured by micro-Kjeldahl method [18]. Titratable acidity (TA) was determined by titrating 10 mL sample with 0.1 M NaOH, phenolphthalein was used as an indicator. The extent of syneresis was determined using the methods of Purwandari *et al.* [19]. The degree of syneresis was expressed as a percentage.

Determination of aroma compounds: Aroma compounds analyses were carried out by GC/MS chromatography in yoghurt samples at 1th and 21th days of the storage. Determining the headspace-solid phase microextraction, a fused silica SPME fiber CAR/PDMS was used. Two gram samples were transferred into headspace vials and incubated at 40°C for 15 min, then the fiber was inserted directly into the vial and remained inside the sample for 15 min at 40°C. Then, the fiber was withdrawn and transferred into the injection port of the GC. Desorption time was set to 1 min while the temperature of the injection port was set at 250°C.

GC/MS analysis was performed with GC 17A QP 5050 GC/MS (Shimadzu, Japan). Helium was used as the carrier gas. The MS operating conditions were: Ionization voltage 70 eV, ion source El, ion source temperature 250°C. The following GC operating conditions were used: a 50 m x 0.32 mm I.D., 1.2 µm film thickness column Cp WAX 52 CB; a flow rate of 1.5 ml/min; the injection port set at 250°C the temperature program began at 40°C for 3 min; increased 4°C min^{-1} up to 230°C; stayed at 230°C for 10 min identification of components was carried out by means of commercial libraries (Wiley, Nist and Tutor).

Determination of organic acids: Determination of organic acids were carried out at 1th and 21th days of the storage. Sample preparations were made as described by Alhendawi *et al.* [20]. A twenty µl of the diluted solution was injected into the HPLC system (Shimadzu, Japan) equipped with a Prodigy ODS (2) (250 × 4.6 mm) 5 µm column. The mobile phase consisted of 1% acetonitrile/ 99 % 0.05 M KH_2PO_4 at pH 2.5, and the flow rate was 0.8 ml/min.

Statistical analysis: The complete experiment was repeated twice. Data were subjected to Tukey's test for pairwise comparisons between means by using SPSS 16.0 (SPSS, Chicago, IL, USA). A P value of <0.05 was considered statistically significant.

Results and Discussion

Microbiological analysis of yoghurts: Table 1 shows the viable counts of total LAB, *L. plantarum*, *Str. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in yoghurts during the refrigerated storage. In the 21st days of the storage, it was observed that there were minimal differences in the viability of LAB among groups. While the viability of LAB during storage period in C2, C3, T1 and T3 groups was stable, the viable counts within T2 and T4 groups showed tendency to nonsignificant increase as 0.85 and 0.54 $\log_{10}$ CFU/g, respectively. However, in C1 group which was produced by only bulk culture, LAB counts declined from 7.56 to 6.28 $\log_{10}$ CFU/g ($p<0.05$). In most cases, the population levels of the starter cultures are slightly elevated in yoghurts produced with adjuncts compared with the control [21]. Numerous factors may effect the viability of LAB in yoghurts

Table 1. Effect of storage period on viability of total lactic acid bacteria, *Lactobacilus plantarum* and yoghurt cultures (*L. delbruekii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*) in yoghurt groups (CFU/g)+.

Culture/ Group	Period of storage, day			
	Log_{10} CFU g^{-1}			
	1	7	14	21
LAB				
C1	7.56 ± 0.24^{a}	7.39 ± 0.24^{b}	6.55 ± 0.24^{b}	6.28 ± 0.24^{b}
C2	8.05 ± 0.24^{a}	8.28 ± 0.24^{a}	8.45 ± 0.24^{a}	8.16 ± 0.24^{a}
C3	7.65 ± 0.24^{a}	8.02 ± 0.24^{ab}	7.98 ± 0.24^{a}	7.80 ± 0.24^{a}
T1	8.38 ± 0.24^{a}	8.27 ± 0.24^{a}	8.38 ± 0.24^{a}	8.38 ± 0.24^{a}
T2	7.46 ± 0.24^{a}	7.70 ± 0.24^{ab}	7.65 ± 0.24^{ab}	8.31 ± 0.24^{a}
T3	7.56 ± 0.24^{a}	7.79 ± 0.24^{ab}	8.01 ± 0.24^{a}	7.53 ± 0.24^{a}
T4	7.80 ± 0.24^{a}	8.16 ± 0.24^{ab}	8.27 ± 0.24^{a}	8.34 ± 0.24^{a}
L. plantarum				
C1	< 1	< 1	< 1	< 1
C2	8.21 ± 0.19^{aB}	8.56 ± 0.19^{abAB}	8.58 ± 0.19^{abA}	8.22 ± 0.19^{aAB}
C3	< 1	< 1	< 1	< 1
T1	8.19 ± 0.19^{aB}	8.65 ± 0.19^{aAB}	9.23 ± 0.19^{aA}	8.69 ± 0.19^{aAB}
T2	7.36 ± 0.19^{aB}	8.40 ± 0.19^{abA}	8.36 ± 0.19^{bAB}	8.80 ± 0.19^{aA}
T3	7.29 ± 0.19^{aB}	7.96 ± 0.19^{bAB}	8.31 ± 0.19^{bA}	8.17 ± 0.19^{aA}
T4	8.02 ± 0.19^{aA}	8.56 ± 0.19^{abA}	8.69 ± 0.19^{abA}	8.18 ± 0.19^{aA}
Str. salivarius subsp. *thermophilus*				
C1	8.09 ± 0.1^{aA}	8.21 ± 0.1^{cdA}	8.41 ± 0.1^{aA}	8.27 ± 0.1^{cA}
C2	8.15 ± 0.1^{aB}	8.33 ± 0.1^{abB}	8.41 ± 0.1^{aB}	8.81 ± 0.1^{abA}
C3	7.97 ± 0.1^{aA}	8.16 ± 0.1^{dA}	8.22 ± 0.1^{aA}	8.24 ± 0.1^{cA}
T1	8.35 ± 0.1^{aA}	8.40 ± 0.1^{aA}	8.45 ± 0.1^{aA}	8.78 ± 0.1^{cA}
T2	8.04 ± 0.1^{aB}	8.25 ± 0.1^{bcB}	8.35 ± 0.1^{aB}	8.98 ± 0.1^{aA}
T3	7.72 ± 0.1^{aB}	8.26 ± 0.1^{bcAB}	8.33 ± 0.1^{aA}	8.51 ± 0.1^{bcA}
T4	8.22 ± 0.1^{aB}	8.35 ± 0.1^{aB}	8.41 ± 0.1^{aAB}	8.73 ± 0.1^{abA}
L. delbrueckii subsp. *bulgaricus* *				
C1	6.65 ± 0.27	7.16 ± 0.27	6.58 ± 0.27	6.09 ± 0.27
C2	7.47 ± 0.27	7.08 ± 0.27	6.49 ± 0.27	5.95 ± 0.27
C3	7.19 ± 0.27	7.58 ± 0.27	6.61 ± 0.27	7.50 ± 0.27
T1	7.06 ± 0.27	6.79 ± 0.27	6.69 ± 0.27	6.11 ± 0.27
T2	6.68 ± 0.27	6.79 ± 0.27	6.80 ± 0.27	6.54 ± 0.27
T3	6.58 ± 0.27	6.71 ± 0.27	6.69 ± 0.27	6.54 ± 0.27
T4	6.56 ± 0.27	6.75 ± 0.27	6.61 ± 0.27	6.43 ± 0.27

+ Different small letter superscripts depict the statistical difference within in a column indicate significant difference ($P < 0.05$) among the yoghurt groups for the same bacteria, different capital letter superscripts depict the statistical difference within a row indicate significant difference ($P < 0.05$) for the same yoghurt at different storage days. * No statistical difference among the count of *L. delbrueckii* subsp. *bulgaricus.*

such as pH, presence of dissolved oxygen and hydrogen peroxide, storage temperature, concentration of metabolites such as lactic and acetic acids [22]. Nighswonger *et al.* [23] reported that the viability of the LAB reduced after the storage period in yoghurts manufactured with adjunct cultures *L. acidophilus* and *L. casei*. It is thought that, using probiotic *L. plantarum* strains with prebiotic β-glucan in yoghurt production increase the number of other beneficial microorganisms in the flora [24]. The number of *L. plantarum* was detected below 1 $\log_{10}$ CFU/g in C1 and C3 groups which were manufactured without *L. plantarum* combination. The concentration of *L. plantarum* ranged initially between 7.29 and 8.21 $\log_{10}$ CFU/g with a non–significant difference among batches on the first day after fermentation. The viable counts in general increased slightly during storage after 21 days, however, this increase was non significant in groups C1, T1 and T4 and significant in groups T2 and T3 ($p<0.05$). It is regarded as essential that, the carrier food contains at least 6 $\log_{10}$ CFU/g viable cells of the probiotic microorganism per gram [25]. Furthermore, IDF standards require 7 $\log_{10}$ CFU/g of starter populations [26]. We observed that, both the adjunct *L. plantarum* strains and the starter cultures of yoghurt produced with were present in high levels, above 8 $\log_{10}$ CFU/g, after 3 weeks of storage, thus satisfying the criteria for probiotic bacteria and yoghurt cultures. Nighswonger *et al.* [23] found that, although the viable counts of some of the *L. acidophilus* and *L. casei* used as adjuncts in yoghurt production declined significantly after storage, most probiotic populations were between 5 and 7 $\log_{10}$ CFU/g at the end of the products shelf life. Canganella *et al.* [27] observed the counts of the *L. acidophilus* as 7 $\log_{10}$ CFU/g at the end of 45 days storage in plain yoghurts, manufactured with *L. acidophilus* and *Bifidobacterium infantis* strain. Furthermore, Rönkä *et al*. [28] found that, the viability of an *L. brevis* strain, incorporated into yoghurt before the fermentation process, remained constant and above 6 $\log_{10}$ CFU/g during storage.

Maragkoudakis *et al.* [21] determined the counts of *L. delbrueckii* subsp. *bulgaricus* ACA-DC 84 and *Str. salivarius* subsp. *thermophilus* ACA-DC 6 strains in the 1st day of the storage of yoghurts manufactured with adjunct probiotic cultures and incubated at 42°C as 8.3 and 7.6 $\log_{10}$ CFU/g and 7.2 and 7.0 $\log_{10}$ CFU/g after 14 days, respectively. In our study, the counts of *L. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus* in the C2 group on the 1st day of the storage determined as 7.47 and 8.15 $\log_{10}$CFU/g, respectively. At the 14th day of the storage, the number of the bacteria detected as 6.49 and 8.41 $\log_{10}$CFU/g, respectively. The results showed that, the growth of the *Str. salivarius* subsp. *thermophilus* increased for all groups throughout the storage. The lowest and highest increases were detected in groups C1 and T2 with the levels of 0.18 and 0.94 $\log_{10}$ CFU/g, respectively. The changes in groups C1, C3 and T1 were generally not significant at the end of the 21 day storage; however, it was significant ($p<0.05$) in groups C2, T2, T3 and T4. There were minimal differences in the viability of *L. delbrueckii* subsp. *bulgaricus* among groups and the viable counts decreased slightly during storage, although it was nonsignificant. However, the viability of *L. delbrueckii* subsp. *bulgaricus* was slightly enhanced in C3 group, in the presence of probiotic *L. acidophilus* LMG 11472 and inulin during storage. Ozer *et al.* [29] have also reported that *Str. salivarius* subsp. *thermophilus* was more stable than *L. delbrueckii* subsp. *bulgaricus* during storage of yoghurts for 14 d at < 6°C.

In our study, following 21 days of storage at 4°C, *L. plantarum* strains remained stable or increased. Furthermore, the sum of the yoghurt bacterial strains, *Str. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, were above the minimum requirement of 7 $\log_{10}$CFU/g viable microorganisms per gram [30]. We can suggest that glucan added yoghurts provide a good environment for yoghurt starters and *L. plantarum* strains. Previous study has reported on the ability of probiotic and yoghurt starter cultures to break down and utilise β-glucan and thus, postfermentation addition of this dietary fibre was recommended[8]. Similar to our results, the results of Kearney *et al*. [31] also suggest that β-glucan was not digested and utilised by the yoghurt starter cultures, as viable counts were not significantly different between the β-glucan-containing yoghurt and the control yoghurt.

Physicochemical analysis of yoghurts: Addition of β-glucan and

inulin into regular yoghurts slightly increased the amount of DM% at the end of 21 days of storage. C3 and T4 yoghurt groups contained the highest DM% (12.68% and 12.55, respectively) compared with the other groups. However, this differences were not statistically significant ($p>0.05$) and there was not a significant difference in the amounts of DM % during the storage period ($p>0.05$). In the 1st day of storage, the lowest and highest fat content were determined in group T2 and C2 as 3.15 and 3.95%, respectively. After 21 day storage, the fat content of the inulin added group (C3) showed significant decrease ($p<0.05$). However, the addition of β-glucan in different concentrations did not affect the fat compositions of yoghurts ($p>0.05$).

Protein content of yoghurts is shown in Table 2. Protein content of groups ranged from 2.61-3.17% in the 1st day of the storage and 3.00-3.43% at the end of 21 day storage. While the lowest protein content was determined in group T4 in the 1st day of the storage (2.61%), the highest protein content (3.43%) was also observed in the same group after 21 day storage. No significant differences were observed within the groups during the storage ($p>0.05$). Dave and Shah [32] determined the protein content of yoghurt ranged from 3.55-3.65. The changes in pH and TA% in yoghurts are illustrated in Figs 1 and 2, respectively. The initial TA% was determined between 0.67 and 0.75%. The lowest and highest TA% values were observed in groups T3 and C1, C2, T1, respectively, at 1 d. After 21 d at 4°C, the TA% values showed a tendency to increase in groups C2, T2, T3 and T4. The highest TA% was determined in group T4 (0.80%) and an increase in this group during storage was found to be statisticaly significant ($p<0.05$). This increase might be attributed to the residual fermentation changes [32]. After the inoculation of the cultures, the initial pH of milk decreased to 4.5 within 3 h at 42°C incubation for all groups. Maragkoudakis *et al.* [21] inoculated the milk with 9 $\log_{10}$CFU/ml for *L. delbrueckii* subsp. *bulgaricus* ACADC 84, and 10 $\log_{10}$CFU/ml for *Str. salivarius* subsp. *thermophilus* ACA-DC 6 and *L. paracasei* subsp. *tolerans* ACA-DC 4037 strains and they determined that the fermentation time required for the pH to reach 4.6 was 8-10 h. Finally, they observed that the produced yoghurt had a very sour taste, which was deemed not acceptable. In our study, the decrease in pH between 0.04 and 0.13 units from the set pH of 4.5 was obviously due to continued fermentation during overnight cooling till the temperature of the product reached 4°C. After the initial drop, a gradual decrease in the pH was observed throughout the storage period of 21 d. After 21 d storage, the pH dropped to 4.22 for group T4. The drop in the pH was almost the same (between 4.12-4.16) for all the other groups. The addition of inulin and different amounts of β-glucan did not change the pH significantly during the storage period within the groups. Kearney *et al.* [31] also observed that, pH development during fermentation completed in 7-7.5 h and the pH values during storage were not significantly different between β-glucan containing and control yoghurts. The syneresis levels of yoghurts during the storage period are shown in Fig. 3. In our study, the extent of syneresis was significantly affected by the type of prebiotic. Moreover, all control yoghurts produced without β-glucan showed less ($p>0.05$) syneresis than the β-glucan added groups except T1. In the 1st day of the storage, while the lowest syneresis was determined in group C3 (26.78%), the highest was in group T2 (58.03%). It has been reported that the presence of a long chain polysaccharide likely interfered with a development of a 3-dimensional structure of casein, leading to a weaker gel incapable of retaining water [33]. A slight differences were observed in all groups for syneresis during storage. In C2 and C3 groups of yoghurt, lower syneresis was observed than the all other groups. Similar to our results, Vasiljevic *et al.* [16] determined that, the amount of syneresis increased substantially during 4 weeks of cold storage with values ranging from 31.2 for control to 77.8% for oat β-glucan, as a result of thermodynamic incompatibility between the milk proteins and the added polysaccharides. Thus authors suggested alternative approach such as postfermentation addition of β-glucan to minimize syneresis. Contrary to our results, Kearney *et al.* [31] observed that, the amount of whey removed significantly declined syneresis for the control yoghurt and for the β-glucan-enriched product during storage. On the other hand, Singh *et al.* [6] found

Table 2. Protein content (%) of yoghurts during the storage period.

Groups *	Days			
	1	7	14	21
C1	2.68 ± 0.25	2.89 ± 0.25	3.16 ± 0.25	3.32 ± 0.25
C2	2.91 ± 0.25	3.13 ± 0.25	3.00 ± 0.25	3.27 ± 0.25
C3	2.72 ± 0.25	3.01 ± 0.25	2.74 ± 0.25	3.24 ± 0.25
T1	2.85 ± 0.25	3.04 ± 0.25	2.85 ± 0.25	3.00 ± 0.25
T2	3.17 ± 0.25	2.95 ± 0.25	2.98 ± 0.25	3.27 ± 0.25
T3	3.06 ± 0.25	3.13 ± 0.25	2.96 ± 0.25	3.24 ± 0.25
T4	2.61 ± 0.25	2.93 ± 0.25	3.13 ± 0.25	3.43 ±0.25

* No statistical difference among the protein of groups.

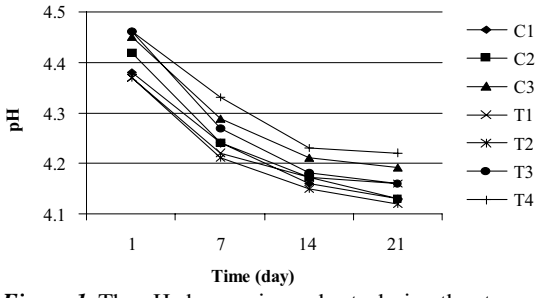

Figure 1. The pH changes in yoghurts during the storage period.

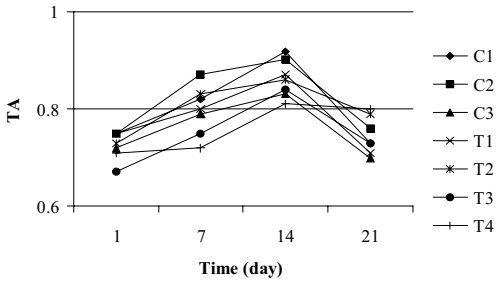

Figure 2. The TA% changes in yoghurts during the storage period.

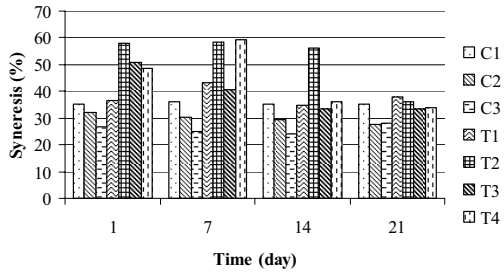

Figure 3. The syneresis (%) levels of the yoghurts during the storage period.

that levels up to 0.3% of purified oat β-glucan resulted in yoghurts with quality characteristics similar to the control yoghurt which was produced without β-glucan.

Determination of aroma compounds: In this study, the highest acetaldehyde content was determined as 4.30 μg/g in C3 group of yoghurt after 21 day of storage. Acetaldehyde was not determined in none of yoghurt samples manufactured with β-glucan addition, except T3 group yoghurt in the 1st d of storage. Oat *et al.* [34] compared the volatiles of traditional acidic, mild and less acidic yoghurts and determined that acetaldehyde formation was reduced in the mild yoghurt.

The major volatile compounds reported responsible for imparting desirable flavor to yoghurt include acetaldehyde, diacetyl, acetone, acetoin (3-hydroxy 2-butanone) and 2-butanone[34]. The characteristic buttery and nutlike flavor of dairy products is attributed to diacetyl and acetoin [35]. Diacetyl contributes to the delicate, full flavor and aroma of yoghurt and is especially important for products that contain low acetaldehyde concentrations [36]. We observed that, the diacetyl concentration increased in all groups except C2 and T3. The highest diacetyl content was found in inulin-added yoghurt (C3) after 21 d storage. Some other researchers also reported that acetaldehyde and diacetyl concentrations decreased in yoghurts during 10 days of storage period [37]. Flavor production is strain dependent and therefore, the composition of a starter culture can greatly influence flavor characteristics of the final product [38].

Changes in concentrations of flavor and other flavor-related compounds during storage are due largely to reactions that result in their formation or conversion to other compounds by bacterial metabolic enzymes, and their losses due to volatilization [39]. Beshkova *et al.* [40] observed that, the mixed cultures stimulated the synthesis of metabolites, which are particularly important for the flavor characteristics of the starter culture. In a previous study, Alonso and Fraga [41] analyzed the volatile flavor compounds in yoghurt, wherein important compounds were acetone and acetic acid. There is a lack of information about the volatile compounds of β-glucan added yoghurts. However, it has been reported that addition of β-glucan, negatively affected the flavor and appearance of soft brined cheese [42]. Even though the importance of acetaldehyde, acetone and diacetyl for yoghurt flavor is well established, it is very difficult to evaluate the contribution of several identified volatile compounds because their sensory perception thresholds vary considerably [43]. We also determined elevated concentrations of acetic acid in all yoghurt groups. Volatile fatty acids are not a principal aroma component in yoghurt but they help balance the flavor of yoghurt, and they also synergistically contribute to flavor with acetaldehyde [44]. Beshkova *et al.* [40] also determined the highest free volatile fatty acid concentration was that of the acetic acid followed by the butyric and caproic acids in yoghurts. We determined several compounds such as aldehydes, ketones, alcohols, lactones and sulfur cornpounds in the yoghurt groups. It was observed that, the ethanol concentration increased in T1, T2 and T4 groups in yoghurts. This maybe due to reduction of acetaldehyde ethanol by the enzymes of *Str. salivarius* subsp. *thermophilus* strains as described by Tamime and Robinson [45].

Table 3. The formic acid, pyruvic acid, lactic acid acetic acid contents obtained from 1st and 21st days of the storage of yoghurt groups+.

Groups	Formic acid		Pyruvic acid		Lactic acid		Acetic acid	
	1.day	21.day	1.day	21.day	1.day	21.day	1.day	21.day
C1	389.2^{bA}	488.5^{aA}	12.2^{aA}	3.4^{bC}	13652.4^{bE}	18949.8^{aB}	282.3^{bF}	312.1^{aG}
C2	281.2^{bF}	402.1^{aC}	5.3^{E}	ND*	13072.2^{bF}	18589.9^{aC}	330.9^{bD}	752.8^{aA}
C3	203.2^{bG}	273.9^{aG}	4.7^{F}	ND*	9021.2^{bG}	15669.9^{aF}	294.7^{bE}	625.5^{aE}
T1	355.5^{bB}	449.8^{aB}	8.3^{aC}	3.6^{bB}	15471.6^{bD}	19271.9^{aA}	414.1^{bA}	695.7^{aB}
T2	342.3^{bC}	350.7^{aD}	7.0^{aD}	3.0^{bE}	23564.5^{aB}	17306.3^{bD}	374.9^{bB}	651.3^{aD}
T3	325.4^{aE}	319.7^{bE}	7.0^{aD}	3.8^{bA}	23986.7^{aA}	17225.3^{bE}	358.9^{bC}	590.3^{aF}
T4	329.8^{aD}	281.3^{bF}	8.5^{aB}	3.3^{bD}	22232.7^{aC}	15255.3^{bG}	268.0^{bG}	661.8^{aC}

+ Different small letter superscripts depict the statistical difference within in a column indicate significant difference ($P < 0.05$) for the same yoghurt at different storage days, different capital letter superscripts depict the statistical difference within a row indicate significant difference ($P < 0.05$) among the yoghurt groups. *ND: Not determined.

Variation in levels of flavor compounds has been reported by using different strains of LAB [46]. However, some authors, have not found acetaldehyde in yoghurt cultures [47]. In our previous studies, we determined the content of the acetaldeyde in pure cultures of *L. plantarum* AB6-25, AK4-11 and AC18-82 as 3.38, 1.68 and 12.52 μg/g, respectively [14]. Yuksekdag *et al.* [48] determined the level of acetaldehyde in yoghurt cultures between 0.18 to 9.7 μg/g. Dana *et al*. [49] also determined the amounts of acetaldehyde by the LAB strains between 2.4 and 4.2 μg/g.

Organic acid profile: Concentrations of formic, pyruvic, lactic and acetic acid in yoghurt samples are presented in Table 3. In general, the production of organic acids increased during storage period. However, this was not a case in pyruvic acid which slightly decreased at the end of storage period. It has been reported that an increase in organic acid concentration limits the numbers of viable cells [32], however, no adverse effect on cell viability was observed in our study (Table 1). Similar to our observation about cell viability was reported previously [16]. The results of our study indicated that lactic acid was the major organic acid in yoghurt. The production of lactic acid was increased during storage in groups of C1, C2, C3 and T1, and decreased in groups of T2, T3 and T4. With respect to other organic acids, an increase in formic and acetic acids and a decrease in pyruvic acid were determined in all yoghurt groups. Moreover, the lowest formic, pyruvic and lactic acid levels were found in group C3, increasing the levels of β-glucan resulted in slightly lower formic and lactic acid formation at the end of storage. The lowest acetic acid concentration was determined in group C1. The higher acetic acid concentrations in other groups may be a results of possible fermentation of β-glucan and inulin as reported by Volikakis *et al.* [42].

Conclusions

The yoghurts manufactured with *L. plantarum* combination as adjunct exhibited good microbiological and physicochemical properties both in yoghurt. The addition of β-glucan (0.5 and 1%) enhanced the growth of *L. plantarum* strains. The results showed that the amount of syneresis was higher in yoghurts with β-glucan (except 0.25%). The addition of β-glucan (0.25-0.5%) with probiotic cultures may enhance physicochemical properties of symbiotic yoghurts and help to improve the health benefits of consumer. However, some process modifications in β-glucan containing yoghurt manufacture may be required to prevent quality defects such as syneresis.

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