EFFECTS OF SHORT-CHAIN AND LONG-CHAIN INULIN ON THE QUALITY OF PROBIOTIC YOGURT CONTAINING *LACTOBACILLUS RHAMNOSUS*

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ABSTRACT

This study investigated the effects of adding short-chain and long-chain inulins on the growth of *Lactobacillus rhamnosus* and the physicochemical and sensory properties (appearance, structure-texture, odor and taste) of probiotic yogurt during cold storage. The inulin-supplemented yogurt batches were compared with control batches of unsupplemented yogurt and *L. rhamnosus*-inoculated yogurt. The inulins were incorporated at 2% w/w in the yogurt mixtures that were inoculated with *L. rhamnosus* and mixed cultures of *Streptococcus thermophilus* and *L. delbrueckii* ssp. *bulgaricus*. The contents of organic acids (lactic, citric, acetic, propionic, formic and butyric) profile were determined using high-performance liquid chromatography. The results indicated that the viability of *L. rhamnosus* (6.71 $\log_{10}$ cfu/g) in probiotic yogurt was enhanced by the presence of short-chain inulin (RSI). Consequently, yogurt with short-chain inulin (RSI) received higher scores for odor, taste and overall acceptance than did yogurt containing long-chain inulin (RLI). However, the chain lengths of the inulins did not significantly affect the consistency, product appearance or the organic acid profile.

PRACTICAL APPLICATIONS

Inulin is a natural component of some fruits and vegetables and widely used in functional foods for its nutritional benefits. In addition to its health promoting properties, as a dietetic fibre and as a prebiotic ingredient inulin has technological properties, as a low-calorie sweetener, fat substitute and texture modifier. Inulin also promotes healthy probiotic bacteria grown in the colon and enhances calcium absorption and immune functions. This study provided information on the effects of short-chain and long-chain inulin on the properties of probiotic yogurt containing *Lactobacillus rhamnosus*.

INTRODUCTION

An increasing interest in functional dairy products that have a positive effect on human health and nutrition has developed recently. Probiotics can be defined as living microorganisms that have proven beneficial effects on the health of the host and that improve the intestinal microbial balance and also reduce disease risk and symptoms (Salminen *et al.* 1999; Sip and Grajek 2010). Probiotics have been used for the treatment of various types of diarrhea (Wullt *et al.* 2003; Szymanski *et al.* 2006), hepatic encephalopathy (Ozcan-Yilsay and Yilmaz 2007), urogenital infections (Reid *et al.* 2003) and gastrointestinal diseases such as Crohn's disease (Bousvaros *et al.* 2005) and pouchitis (Kuehbacher *et al.* 2006; Gionchetti *et al.* 2012). Moreover, the positive effects associated with probiotics include antihypertensive and anti-tumor activities, cholesterol reduction, improvement of lactose tolerance and stimulation of the immune system (Rastall *et al.* 2000; Roberfroid 2000; Beylot 2005).

Lactic acid bacteria, including *Lactobacilli*, *Streptococci*, *Lactococci* and *Bifidobacteria*, are the most common bacterial species considered as potential probiotics (Lin 2003; Ranadheera *et al.* 2010). *Lactobacillus rhamnosus and* the human-derived *L. rhamnosus* GG, which is one of the widely used commercial probiotic strains with recognized health benefits, are frequently used in infant's formulas and children's food because of their preventive and curative effects on diarrhea, dental caries and allergies (Marteau *et al.* 2001; Szajewska *et al.* 2001; Canbulat and Ozcan

2007). Mixtures of probiotics and prebiotics, which favorably modify the gut flora and its metabolism by increasing the survival of probiotic bacteria, are described as synbiotics (Bharti *et al.* 2012). Several authors have suggested that a concentration of 10^6–10^9 viable cells/g of a functional food product is the minimum necessary to for its ingestion to have a beneficial effect (Schrezenmeier and de Vrese 2001; Ouwehand *et al.* 2002).

Inulin is a prebiotic food ingredient that increases the activity of *L. rhamnosus*, increases calcium absorption and is a good source of fermentable dietary fiber. Various *in vitro* and *in vivo* studies have shown that a diet supplemented with inulin stimulates the growth of probiotic bacteria in the intestine. Inulin is found in many vegetable products, among which chicory roots are considered the most suitable for industrial applications (Schneeman 1999; Flamm *et al.* 2001; Paseephol *et al.* 2008). The properties of inulin are linked to the degree of polymerization (DP). The naturally occurring inulins have fructose polymerization degrees varying from 2 to >60 units. Long-chain inulins (DP between 23 und 25 units) are thermally more stable, less soluble and more viscous than are the short-chain inulins (average DP of 11 units; Moerman *et al.* 2004; Wada *et al.* 2005). The DP of inulin and the probiotic bacteria strain affect the growth and viability of probiotic cultures in probiotic fermented dairy products that contain inulin (Gibson and Wang 1994; Kaur and Gupta 2002). Moreover, inulin can mimic fat in water-based foods such as dairy products when used to replace fat, providing a fat-like mouthfeel and texture (Izzo and Franck 1998; Zimeri and Kokini 2003). Inulin provides the health benefits that are normally associated with dietary fiber; namely, it aids the digestion of high-protein diets, decreases fat absorption, provides roughage and thereby prevents constipation while helping the body eliminate toxins, remains in the digestive tract and thereby provides a sense of fullness without extra calories, lowers the levels of cholesterol and triglycerides in the blood, facilitates the control of blood glucose for diabetics, and decreases the incidence of colon cancer (Williams 1999; Causey *et al.* 2000; Franck 2002).

The viability of probiotic bacteria in dairy products containing inulin and the impact of inulin on their chemical, textural and sensorial characteristics have been examined (Hardi and Slacanac 2000; Dello Staffolo *et al.* 2004; Guven *et al.* 2005; Akin and Kirmaci 2007; Aryana *et al.* 2007; Paseephol 2008; Mazloomi *et al.* 2011). However, information on the effect of inulin on the growth of *L. rhamnosus* during storage is very limited (Sadek *et al.* 2004). Therefore, the objectives of this study were to determine the effects of inulins of various chain lengths (short and long) on the survival of *L. rhamnosus* during yogurt fermentation and on the physicochemical and sensory characteristics of fat-free plain yogurt during 28 days storage.

MATERIALS AND METHODS

Materials

Milk and Inulins. Fresh standardized, pasteurized, nonfat milk samples were procured from the Sutas dairy plant (Bursa, Turkey). Inulin, long-chain inulin with a DP (>23 fructose or glucose units) and short-chain inulin with a DP of 8, all with a purity of 99.5, were supplied by Sensus (Roosendaal, Netherlands).

Starter Cultures and Probiotic Strains. The starter cultures used for direct vat inoculation were (Chr. Hansen, Copenhagen, Denmark): YFL-702, containing *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and FRO (Danisco, Niebull, Germany), containing *L. rhamnosus*. The working cultures were prepared by activating the frozen cultures. Sufficient amounts of the pure spray-dried strains were weighed to obtain initial counts of 8 $\log_{10}$ colony-forming units (cfu)/g.

Precultures were prepared by dissolving each of the dried cultures in 25 mL of reconstituted sterilized skim milk (10.7% [w/v] total solids) that had been tempered at 42C for 15 min. Afterwards, the rehydrated *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* starter culture was used for the production of a control yogurt, as described by (Ozcan *et al.* 2008), and for a co-culture of the rehydrated yogurt starter culture mixed with a single rehydrated strain of *L. rhamnosus*. To facilitate the activation of *L. rhamnosus*, 0.05% L-Cys-HCl was added to diminish the oxidation-reduction potential of the medium. The *L. rhamnosus* cultures were incubated at 40 ± 1C for 18 h under anaerobic conditions in an Anaerogen Anaerobic System (Oxoid, Basingstoke, U.K.; Tharmaraj and Shah 2003). The amount of inoculum necessary to provide approximately 6 or 7 $\log_{10}$/cfu in the probiotic yogurt was calculated.

Yogurt Manufacture. The set style yogurts were produced in the pilot dairy plant of the Sutas Co. (Bursa, Turkey). The fat content of the raw milk was analyzed using a Gerber instrument (Gerber Instruments AG, Effretikon, Sweden) and standardized to 0 ± 003% ratio of fat and 12% w/v of nonfat dry matter. The inulins (2% w/v) were dissolved and mixed in cold milk (5C) using a staff mixer (6,000 rpm). The four different fat-free yogurt products produced per day were probiotic yogurt batches containing short-chain inulin (RSI) or long-chain inulin (RLI), unsupplemented yogurt (C) and yogurt containing *L. rhamnosus* (R). The milk mixtures were homogenized (20 MPa, 60C) and then heated at 90C for 10 min and cooled to 40C prior to inoculation with the bacterial cultures. The control milk was inoculated with 2% (wt/wt) of

the working culture. The other milk samples were inoculated with a probiotic single culture (FRO, containing *L. rhamnosus*) or YFL-702 culture containing a mixture of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* at a 1:1 ratio. The samples were incubated at 40C until pH 4.7 was reached and then they were transferred to 4C. Three trials of each type of yogurt were conducted.

Analyses

Enumeration of Microorganisms. The number of viable probiotic bacteria in the yogurt prepared with the mixed culture was enumerated after 1, 7, 14, 21 and 28 days of storage at <10C. Samples (1 mL) were added to 9 mL of sterile peptone diluent (0.1 g/L; Merck, Darmstadt, Germany) and appropriate dilutions were prepared. Enumeration was conducted using the pour plate technique on *Lactobacilli* MRS pH 6.2 agar (Merck; acidified to pH 6.8 with 1 M HCl) plus vancomycin (MRS V), as previously described (Tharmaraj and Shah 2003). The vancomycin stock solution (100 mg vancomycin hydrochloride/5 mL of distilled water [Sigma, St. Louis, MO]) was filter-sterilized (Millipore Corp., Bedford, MA) and 50 μL of this stock solution was added to 100 mL of melted sterile MRS agar just before pouring the plates (at 40C). The number of viable *L. rhamnosus* in the probiotic yogurt was enumerated after anaerobic incubation at 37C for 72 h. Anaerobic conditions were created using Anaerogen (Oxoid). The colonies on plates containing 20 to 200 colonies were counted and the counts were expressed as $\log_{10}$ cfu/g of the yogurt product. The selectivity of the growth conditions was confirmed by microscopic examination.

Kinetics of Acidification. The pH of the samples was monitored during the fermentation period and during 28 days of storage at +4C using a pH meter (Model 691, Metrohm AG, Herisau, Switzerland). The titratable acidity of the probiotic yogurts was determined according to AOAC methods No. 947.05 (AOAC 2000). The titratable acidity was calculated based on lactic acid being the predominant acid and was expressed as g of lactic acid/100 mL of product. The temperature of the samples was 25C for these analyses.

Physicochemical Analysis. Syneresis (released whey) was measured by placing a 25-g yogurt sample on a cheese cloth placed on top of a funnel. After 2 h of drainage at +4C, the volume of whey collected in a graduated cylinder was measured and used as the index of syneresis. Syneresis (%) was expressed as the volume of drained whey (mL)/25 g of sample (Wacher-Rodarte *et al.* 1993). The consistency of yogurts was determined at +4C using a Bostwick consistometer (CSC Scientific Company, Seoul, Korea) from the distance (cm) rum during 30 s in the consistometer (Cavallini and Rossi 2009).

Organic Acid Profile. The organic acid contents in the probiotic yogurt that was stored for 28 days were determined by high-performance liquid chromatography (HPLC) using the technique described by Bevilacqua and Califano (1989) and Akalin *et al.* (2002).

Sample Preparation. Seven mL of sample was added to 40 mL of buffer-acetonitrile mobile phase (0.5% [w/v] $(NH_4)2HPO_4$ [0.038 M] – 0.4% [v/v] acetonitrile [0.049 M], adjusted to pH 2.24 with H_3PO_4), extracted for 1 h in an orbital shaker and centrifuged at 6,000 × g for 5 min, according to a modification of the method of Bevilacqua and Califano (1989). The supernatant was collected and filtered once through Whatman no. 1 filter paper, twice through a 0.45-μm membrane filter, and then used directly for the HPLC analysis. Triplicate analyses were performed on all the samples.

HPLC Analysis. A Jasco liquid chromatograph (Dionex ICS 3000, LC-900 Series, Dionex Corp., Sunnyvale, CA) equipped with a model H-980-01 holder that accepts Rheodyne valves, a 7124 injector fitted with a 20-μL sample loop, a Jasco PU-980 solvent delivery system, and an ICS series UV-visible/variable wavelength detector (Dionex VWD). The detector was set at 210 nm. The operating conditions used were the following: mobile phase, aqueous 0.5% (w/v) $(NH_4)2HPO_4$ (0.038 M) – 0.2% (v/v) acetonitrile (0.049 M) adjusted to pH 2.24 with H_3PO_4; flow rate, 0.6 mL/min; ambient column temperature; and chart speed, 0.8 cm/min. A reversed-phase Acclaim Organic Acid (250 × 4 mm) column (Dionex Corp.) was used. The mobile phase was prepared with analytical grade $(NH_4)2HPO_4$ dissolved in distilled water and HPLC-grade acetonitrile and H_3PO_4. HPLC-grade reagents were used as standards (Sigma Chemical Co.). The solvents were degassed under vacuum. Both the solvents and the standards solutions were filtered through 0.45-μm membrane filter (Sartorious SM 11606).

Quantification was based on the external standard method (Bevilacqua and Califano 1989). The percent recovery was determined with three replicates of yogurt samples by adding a known amount of each organic acid standard to the 7-g sample during extraction. The amounts added were approximately 50% of the actual concentration of the samples. All determinations were performed using three separate extracts of each sample and each was injected in duplicate.

The organic acid standards were prepared in the mobile phase solution, and the chromatograph was calibrated using these standard solutions. The peaks obtained from the

samples were identified by comparison of their retention times with those of the standard compounds and the relative percentage of each organic acid was calculated from the peak area. The concentration of each organic acid (lactic, citric, acetic, propionic, formic and butyric acids) in the product was then determined. The method was tested using a yogurt matrix and then validated by determining the recoveries of organic acids from the yogurt samples. High yields of all of the components were obtained.

Sensory Analysis. The sensory evaluation of the yogurt samples throughout the 28 days of cold storage was conducted by 11 members of an expert panel from food engineering laboratory who were trained in examining dairy products using the method of Martin-Diana *et al.* (2003) as modified by Dello Staffolo *et al.* (2004).

The appearance, structure-texture, odor, taste and overall acceptability of the samples were scored on a hedonic scale of 1 to 5 (1 = unacceptable and 5 = excellent). Sample defects and descriptors such as acid, sweet, bitter, acetaldehyde taste, milky, creamy, grainy, off-flavor, ropy and gritty texture, whey syneresis, and lumpiness were recorded (Cheng 2010). The samples were provided to the panelists in plastic cups coded with three-digit random numbers. Water was provided to the panelists for rinsing their palates between testing samples.

Statistical Analysis. The data from each experiment were analyzed by an analysis of variance using SAS software (2004) and the differences observed among the treatments were determined by the Fisher's least significant difference (LSD) test at $P < 0.01$.

RESULTS AND DISCUSSION

Cell Viability

The viability of *L. rhamnosus* in the probiotic yogurt prepared in this study was enhanced by using short-chain inulin during storage. Supplementation with inulin with an increased DP led to a decreased rate of consumption of inulin by *L. rhamnosus*. The presence of long-chain inulin did not enhance the viability *L. rhamnosus* bacteria during yogurt storage as much as short-chain inulin (Fig. 1). In general, probiotics are added to food as adjunct cultures in concentrations of 10^7–10^8 cfu/mL or /g and do not participate in the fermentative process and their concentration does not change during the development of the product. As for the starter bacteria, they can reach a concentration of between 10^8 and 10^9 cfu/mL or /g at the end of the fermentative process. However, the content of viable probiotic microorganisms decreases during storage and the rate of this loss depends on the type of yogurt and whether a lactic starter culture was used (Vinderola *et al.* 2002; Vinderola and Reinheimer 2003). Therefore, it has been suggested that fermented dairy products require probiotic bacteria at a concentration of at least 10^7 cfu/mL to have healthful effects on the gastrointestinal tract when the products are consumed (Ouwehand and Salminen 1998). The minimum levels should be present for probiotic bacteria in yoghurt ranging from 10^5 to 10^6 cfu/mL (Gueimonde *et al.* 2004). Even though there is no worldwide agreement on viability of probiotics in food products, the values 10^6 cfu and 10^7–10^8 cfu/mL or /g are the minimum and the satisfactory

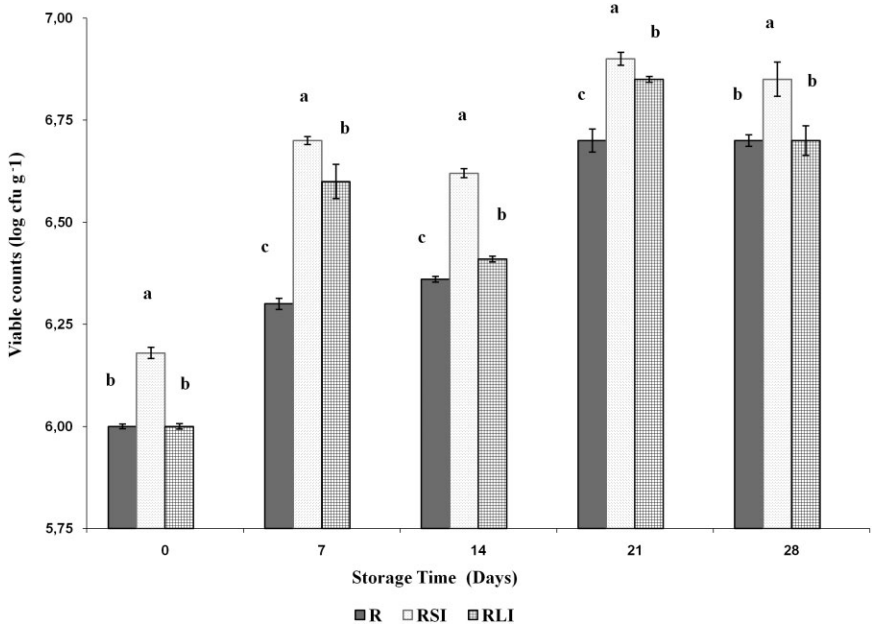

FIG. 1. VIABILITY OF *LACTOBACILLUS RHAMNOSUS* IN PROBIOTIC YOGURT DURING STORAGE
R: yogurt containing *Lb. rhamnosus*, RSI: yogurt containing *Lb. rhamnosus* and short chain inulin, RLI: yogurt containing *Lb. rhamnosus* and long chain inulin. Means of triplicates; error bars indicate SD. [a-c] bars with different letters are significantly different ($P < 0.01$).

levels generally accepted respectively (Vinderola *et al.* 2000). The viability of *L. rhamnosus* increased as the storage time increased and was higher in yogurt containing short-chain inulin throughout the experimental period (Fig. 1; $P < 0.01$). At 1, 7, 14, 21 and 28 days of refrigerated storage, the yogurts contained stable viable populations of *L. rhamnosus* (at least 10^{6-7} cfu/mL when the products are consumed). The growth of *L. rhamnosus* was initially slower in yogurt containing long-chain inulin (6.65 $\log_{10}$ cfu/g) than in yogurt containing short-chain inulin (6.71 $\log_{10}$ cfu/g). This was expected because most *L. rhamnosus* strains do not grow well in yogurt containing inulin with a high DP.

Increasing the viability of probiotic bacteria during fermentation or refrigerated storage by adding inulin has been reported (Akin and Kirmaci 2007). Concentrations of 1.5% inulin (w/v) were found to be sufficient to stimulate growth and retain the viability of probiotic microorganisms in yogurt (Aryana and McGrew 2007; Aryana *et al.* 2007). Wada *et al.* (2005) reported a marked difference in the polydispersity of inulins based on their chain lengths, with the ability of long-chain inulin lower than that of short-chain inulin. Sadek *et al.* (2004) reported that the viability of lactic acid bacteria in fermented milk was increased by the presence of inulin and *L. acidophilus* and *L. rhamnosus* growth was enhanced by 2% inulin.

Acidity Measurements

The mean pH and titratable acidity values of the stored products are presented in Table 1. There was no significant difference between the pH values of the probiotic and control yogurts during storage ($P > 0.01$). An increased titratable acidity was observed in the probiotic yogurt containing short-chain inulin. The titratable acidity of yogurt containing short-chain inulin (RSI) (1.14 ± 0.01% La) was higher than that of yogurt containing long-chain inulin (RLI) or yogurt containing *L. rhamnosus* (R; 1.13 ± 0.01% La; $P < 0.01$). These results might be explained by the differential viability of *L. rhamnosus* in these yogurts. The titratable acidity was increased and the pH values were decreased on the 28th day of storage (data not shown). The effect of inulin on the physicochemical characteristics of fermented products has been reported. Hardi and Slacanac (2000) reported that the rate of pH decrease of fermented milk products was increased by the presence of inulin and the type of starter culture during storage. However, Guven *et al.* (2005) reported that inulin did not affect the pH, titratable acidity or acetaldehyde content of fat-free yogurt and Dello Staffolo *et al.* 2004) found that yogurt containing inulin had a stable pH, similar to that of other fiber-containing yogurts during storage.

TABLE 1. PHYSICOCHEMICAL PROPERTIES OF STORED YOGURT

	pH	Titratable acidity (La%)	Syneresis (25 g/mL)	Consistency (%)	Lactic acid	Citric acid	Organic acetic acid	Acids propionic acid	Formic acid	Butyric acid
C	4.41 ± 0.07^{a}	1.11 ± 0.06^{c}	6.48 ± 0.18^{b}	11.20 ± 0.10^{a}	10.82 ± 0.18^{d}	1.94 ± 0.13^{b}	<0.042	<0.0024	0.068 ± 0.01^{a}	$<0.010^{d}$
R	4.40 ± 0.07^{a}	1.13 ± 0.04^{b}	5.77 ± 0.17^{c}	10.68 ± 0.18^{b}	11.06 ± 0.13^{c}	2.07 ± 0.04^{a}	<0.042	<0.0024	0.062 ± 0.01^{c}	0.032 ± 0.01^{a}
RSI	4.38 ± 0.06^{a}	1.14 ± 0.03^{a}	6.88 ± 0.13^{a}	9.80 ± 0.08^{c}	11.46 ± 0.22^{b}	2.02 ± 0.06^{a}	<0.042	<0.0024	0.064 ± 0.01^{b}	0.028 ± 0.01^{b}
RLI	4.39 ± 0.08^{a}	1.13 ± 0.05^{b}	5.89 ± 0.10^{c}	9.94 ± 0.12^{c}	11.62 ± 0.09^{a}	2.08 ± 0.05^{a}	<0.042	<0.0024	0.068 ± 0.01^{a}	0.023 ± 0.01^{c}
Significance	ns	**	**	**	**	**	ns	ns	**	**

Values are the mean values ± SD, and the statistical comparison of the mean values: mean values in the same column with different superscripted letters are significantly different $P < 0.01$(**); ns, non-significant.
C, control yogurt; ns, not significant; R, yogurt containing *L. rhamnosus*; RLI, yogurt containing *L. rhamnosus* and long-chain inulin; RSI, yogurt containing *L. rhamnosus* and short-chain inulin.

Physicochemical Properties

The physical attributes of yogurts, including the lack of visible whey separation and perceived viscosity, are crucial aspects of the quality and overall consumer acceptance of yogurts (Lucey and Singh 1998). Thus, the texture of the yogurt is an important sensory attribute influencing its acceptability. The viscosity measurements obtained using a consistometer, in which flowing a longer distance indicates lower viscosity, are shown in Table 1. The consistency of yogurts supplemented with inulin were lower than that of the control (C) yogurt and yogurt prepared with *L. rhamnosus* (R; $P < 0.01$) throughout the storage period. The syneresis (released whey) values were increased by the addition of short-chain inulin, as was the titratable acidity, indicating that the yogurt gel network was comparatively weaker than that of the other yogurts (Table 1).

The results of this study showed that yogurts supplemented with short- or long-chain inulins were characterized by lower values of consistency than those of yogurts lacking them. However, storage had a much more significant effect on the syneresis and consistency of the probiotic yogurt ($P < 0.01$). On the 28th day of storage, these values were significantly higher than before storage. The amount of syneresis was significantly lower during 14 days of storage compared with 28 days of storage (data not shown). This may be explained by the decrease in pH that occurs during storage (Kosikowski 1982), which may have caused the casein micelle matrix to contract, causing more whey-off. Kip *et al.* (2006) explained that inulin might become part of the protein structural network formed during fermentation by complexing with protein aggregates. These results are in agreement with those reported by Aryana *et al.* (2007) and Mazloomi *et al.* (2011), who found that yogurt containing long-chain inulin (HP) had less syneresis than a control yogurt because of the high water-holding capacity of inulin.

Paseephol *et al.* (2008) reported that the long polysaccharide chain of inulin might be dispersed among the casein micelles, interfering with the formation of the protein matrix and therefore responsible for the gel being softer. Studies have shown a reduction of syneresis and enhancement of the textural properties of yogurt from the addition of the inulin. Franck (2002) reported that inulin forms a particle gel network of insoluble sub-micron crystalline particles in the aqueous phase. Moreover, the utility of long-chain inulin as a fat substitute is thought to be related to its ability to form micro-crystals that interact with each other to form small aggregates. These aggregates encapsulate a great amount of water, increasing whey-off and creating a smooth and creamy texture (Bot *et al.* 2004). Ipsen *et al.* (2001) reported that use of an increasing amount of inulin in yogurt manufacture resulted in a gradual coarsening of the acid-induced protein network, increased syneresis and increased permeability. Bozanic *et al.* (2001, 2002) found that the firmness of fermented goat and cow milks and yogurts were improved by the addition of inulin. Ibrahim *et al.* (2004) reported that the viscosity and curd tension of set fermented milk were increased by supplementation with inulin and that syneresis during storage was decreased. Hassan *et al.* (1999) reported that the highest curd tension and the lowest amount of syneresis in yogurt occurred with the use of inulin and galactomannan in a 1:1 ratio.

Lactic acid and other organic acids in fermented dairy products play an important role as natural preservatives and contribute to the characteristic sensory properties of the product (Fernandez-Garcia *et al.* 1998; Adhikari *et al.* 2002). Table 1 shows the evolution of organic acids in yogurt made with inulins of different chain lengths. The mean concentration of organic acids, such as lactic, citric and formic acid, was increased by the addition of inulin to the probiotic yogurt with *L. rhamnosus*. The lactic acid contents of the yogurt samples on day 28 varied between 10.82 and 11.62 mg/g. Neither acetic (<0.042) and nor propionic acid (<0.024 mg/g) were detected in the yogurt samples. Lactic, citric, formic and butyric acids were steadily produced during fermentation. The results showed that the chain lengths of the inulins did not have much effect on the organic acid profile. Chen *et al.* (2004) found that the concentrations of organic acids in yogurt could be increased by increasing the activity and viability of the probiotics by adding prebiotics to the milk to be used for the manufacture of the yogurt. The chain length of prebiotics affected various attributes. Biomass and the production of lactate and acetate were greater when the substrate contained mostly shorter chain fructo-oligosaccharides instead of medium- or long-chain fructo-oligosaccharides because the shorter chain compounds were the first to be consumed by Bifidobacteria. The predominantly long-chain structure of inulins ensures long fermentation times in the colon. (Perrin *et al.* 2002; Vereyken *et al.* 2003). There is not much report available on the organic acid profile of yogurt with *L. rhamnosus*. More data are thus needed to explain the organic acid production of *L. rhamnosus* and probiotics in mixed cultures with yogurt strains.

Sensory Properties

Sensory tests of textural attributes that support the firmness, viscosity and creaminess properties of the yogurt samples were performed. The results of the sensory evaluation are summarized in Table 2. No significant effect of inulin on appearance was observed. However, significant differences were observed between the sensory evaluations regarding the structure and texture, odor, taste and overall

TABLE 2. SENSORY EVALUATION OF STORED YOGURT

	Appearance	Structure and texture	Odor	Taste	Overall acceptability
C	4.25 ± 0.08^{a}	4.15 ± 0.04^{ab}	4.70 ± 0.03^{a}	4.60 ± 0.04^{a}	4.10 ± 0.04^{b}
R	4.40 ± 0.05^{a}	4.50 ± 0.06^{a}	4.35 ± 0.04^{b}	4.60 ± 0.03^{a}	4.30 ± 0.05^{ab}
RSI	4.45 ± 0.04^{a}	4.55 ± 0.03^{a}	4.75 ± 0.03^{a}	4.55 ± 0.04^{a}	4.65 ± 0.03^{a}
RLI	4.40 ± 0.04^{a}	3.80 ± 0.07^{b}	4.25 ± 0.05^{b}	3.85 ± 0.08^{b}	4.15 ± 0.05^{b}
Significance	ns	**	**	**	**

Values are the mean values ± SD, and the statistical comparison of the mean values: mean values in the same column with different superscripted letters are significantly different $P < 0.01$(**); ns, non-significant.

C, control yogurt; ns, not significant; R, yogurt containing *L. rhamnosus*; RLI, yogurt containing *L. rhamnosus* and long-chain inulin; RSI, yogurt containing *L. rhamnosus* and short-chain inulin.

acceptability of the samples ($P < 0.01$). For overall acceptability, the sensory scores of the yogurt containing short-chain inulin and the yogurt containing *L. rhamnosus* were higher than those of the yogurt containing long-chain inulin and the control yogurt. In addition, the panelists indicated that RSI yogurt had "slight sweetness" and RLI yogurt had creamy and a fat-like mouthfeel. These results indicate that the addition of *L. rhamnosus* did not significantly inhibit the conversion of lactose to lactic acid via standard yogurt bacteria and thus did not have an effect on the flavor of the yogurt. Yogurt flavor is mainly influenced by the presence of lactic acid and other flavor compounds produced by lactic acid bacteria during the acid fermentation (Hekmat and Reid 2006).

Aryana *et al.* (2007) reported no significant effect of inulin chain length on the viscosity, color and product appearance of fat-free yogurt; but better sensory properties of yogurt containing medium- and long-chain inulin. Dello Staffolo *et al.* (2004) found that the yogurt containing inulin maintained a stable color during storage. Seydim *et al.* (2005) found that yogurts containing inulin had a good flavor and a smooth texture. Hassan *et al.* (1999) stated that the organoleptic properties of yogurt containing inulin were comparable with the organoleptic properties of yogurt prepared with commercial stabilizers, whereas Guven *et al.* (2005) found that the organoleptic quality of yogurt decreased with the increase in the inulin concentration.

CONCLUSION

The effects of inulins of different chain lengths on the physicochemical and sensory properties of yogurt were investigated. The results indicate that the addition of inulin as a prebiotic ingredient to the yogurt led to a remarkable increase in the viability of the probiotic strain *L. rhamnosus* during storage. Adding short-chain inulin resulted in improved probiotic viability and stability compared with adding long-chain inulin. Syneresis was increased by the addition of short-chain inulin, with higher titratable acidity but also higher flavor and taste scores compared with yogurt containing long-chain inulin. The increased DP of the inulin led to a decreased rate of consumption of inulin by *L. rhamnosus*. However, the chain lengths of the inulins did not affect the consistency or appearance of the yogurts. The presence of long-chain inulin did not enhance the viability *L. rhamnosus* bacteria during yogurt storage as much as did short-chain inulin Therefore, the use of long-chain inulin is not recommended to improve the viability of the probiotic strain or the sensory properties of yogurt. The presence of prebiotics in yogurt containing *L. rhamnosus* has a beneficial effect on its healthfulness. However, it is not known whether the chain lengths of the prebiotics affect the healthful characteristics of fat-free plain yogurts containing *L. rhamnosus.*

REFERENCES

ADHIKARI, K., GRUN, I.U., MUSTAPHA, A. and FERNANDO, L.D. 2002. Changes in the profile of organic acids in plain set and stirred yogurts during manufacture and refrigerated storage. J. Food Qual. *25*, 435–451.

AKALIN, A.S., GONC, S. and AKBAS, Y. 2002. Variation in organic acids content during ripening of pickled white cheese. J. Dairy Sci. *85*, 1670–1676.

AKIN, M.S. and KIRMACI, Z. 2007. Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. Food Chem. *104*, 93–99.

AOAC. 2000. *Official Methods of Analysis of AOAC International*, 17th Ed., AOAC International, Arlington, VA.

ARYANA, K.J. and MCGREW, P. 2007. Quality attributes of yogurt with *Lactobacillus casei* and various prebiotics. LWT-Food Sci. Technol. *40*, 1808–1814.

ARYANA, K.J., PLAUCHE, S., RAO, R.M., MCGREW, P. and SHAH, P. 2007. Fat-free plain yogurt manufactured with inulins of various chain lengths and *Lactobacillus acidophilus*. J. Food Sci. *72*, 79–84.

BEVILACQUA, A.E. and CALIFANO, A.N. 1989. Determination of organic acids in dairy products by high-performance liquid chromatography. J. Food Sci. *54*, 1076–1079.

BEYLOT, M. 2005. Effects of inulin-type fructans on lipid metabolism in man and in animal models. Br. J. Nutr. *93*, 163–168.

BHARTI, S.K., SHARMA, N.K., MURARI, K. and KUMAR, A. 2012. Functional aspects of dairy foods in human health: An overview. Int. J. Pharmacol. Therapeut. *2*, 29–35.

BOT, A., ERLE, U., VREEKER, R. and AGTEROF, W.G.M. 2004. Influence of crystallisation conditions on the large deformation rheology of inulin gels. Food Hydrocolloids. *18*, 547–556.

BOUSVAROS, A., GUANDALINI, S., BALDASSANO, R.N., BOTELHO, C., EVANS, J., FERRY, G.D., GOLDIN, B., HARTIGAN, L., KUGATHASAN, S., LEVY, J. *et al.* 2005. A randomized, double-blind trial of *Lactobacillus* GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. Inflamm. Bowel Dis. *11*, 833–839.

BOZANIC, R., ROGELJ, I. and TRATNIK, L.J. 2001. Fermented acidophilus goat's milk supplemented with inulin: Comparison with cow's milk. Milchwissenschaft. *56*, 618–622.

BOZANIC, R., ROGELJ, I. and TRATNIK, L.J. 2002. Fermentation and storage of probiotic yogurt from goat milk. Mljekarstvo. *52*, 93–111.

CANBULAT, Z. and OZCAN, T. 2007. The health impact of using *Lactobacillus rhamnosus* GG in infant formulas and children's dietary supplements. J Agric. Fac. Uludag Univ. *21*, 69–79 (in Turkish).

CAUSEY, J., FEIRTAG, J., GALLAGHER, D., TINGLAND, B. and SLAVIN, J. 2000. Effect of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. Nutrit Res. *20*, 191–201.

CAVALLINI, D.C.U. and ROSSI, E.A. 2009. Soy yogurt fortified with iron and calcium: Stability during the storage. Alim. Nutr. *20*, 7–13.

CHEN, M.J., CHEN, K.N., LIN, C.W. and MAO, H.M. 2004. Study on the optimal growth rates of probiotics in yogurt by genetic algorithms. Taiwan J. Chem. Food Sci. *42*, 306–314.

CHENG, H. 2010. Volatile flavor compounds in yogurt: A review. Criti. Rev. Food Sci. Nutr. *50*, 938–950.

DELLO STAFFOLO, M., BERTOLA, N., MARTINO, M. and BEVILACQUA, A. 2004. Influence of dietary fiber addition on sensory and rheological properties of yogurt. Int. Dairy J. *14*, 783–789.

FERNANDEZ-GARCIA, E., MCGREGOR, J.U. and TRAYLOR, S. 1998. The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yogurt. J. Dairy Sci. *81*, 655–663.

FLAMM, G., GLINSMANN, W., KRITCHEVSKY, D., PROSKY, L. and ROBERFROID, M. 2001. Inulin and oligofructose as dietary fiber: A review of the evidence. Crit. Rev. Food Sci. Nutr. *41*, 353–362.

FRANCK, A. 2002. Technological functionality of inulin and oligofructose. Bri. J. Nutr. *87*, 287–291.

GIBSON, G.R. and WANG, X. 1994. Bifidogenic properties of different types of fructo-oligosaccharides. Food Microbiol. *11*, 491–498.

GIONCHETTI, P., CALAFIORE, A., RISO, D., LIGUORI, G., CALABRESE, C., VITALI, G., LAURETI, S., POGGIOLI, G., CAMPIERI, M. and RIZZELLO, F. 2012. The role of antibiotics and probiotics in pouchitis. Ann. Gastroenterol. *25*, 100–105.

GUEIMONDE, M., DELGADO, S., MAYO, B., RUAS-MADIEDO, P., MARGOLLES, A. and LOS REYES-GAVILAN, C.G. 2004. Viability and diversity of probiotic *Lactobacillus* and *Bifidobacterium* populations included in commercial fermented milks. Food Res. Int. *37*, 839–850.

GUVEN, M., YASAR, K., KARACA, O.B. and HAYALOGLU, A.A. 2005. The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. Int. J. Dairy Tech. *58*, 180–184.

HARDI, J. and SLACANAC, V. 2000. Examination of coagulation kinetics and rheological properties of fermented milk products: Influence of starter culture, milk fat content and addition of inulin. Mljekarstvo *50*, 217–226.

HASSAN, F.A.M., HELMY, W.A. and ENAB, A.K. 1999. Utilization of some local polysaccharide in manufacturing of yoghurt. Egypt J. Dairy Sci. *27*, 281–289.

HEKMAT, S. and REID, G. 2006. Sensory properties of probiotic yogurt is comparable to standard yogurt. Nutr. Res. *26*, 163–166.

IBRAHIM, G.A., MEHANNA, N.S., EL-RAB, D.A.G., ABD EL-SALAM, M.H., KHOLIF, A.M., ABDOU, S.M. and EL-SHIBINY, S. 2004. Preparation and properties of set fermented milk containing inulin and different probiotics. In *9th Egyptian Conference for Dairy Science and Technology; 2004 Oct 9–11*, pp. 117–132, Egyptian Society of Dairy Science, Cairo, Egypt.

IPSEN, R., OTTE, J., LOZAHIC, G. and QVIST, K.B. 2001. Microstructure and viscosity of yoghurt with inulin added as a fat replacer. Annual Trans. Nordic Rheol. Soc. *8/9*, 59–62.

IZZO, M. and FRANCK, A. 1998. Nutritional and health benefits of inulin and oligofructose conference. Trends in Food Sci. Tech. *9*, 255–257.

KAUR, N. and GUPTA, A.K. 2002. Applications of inulin and oligofructose in health and nutrition. J. Biosci. *27*, 703–714.

KIP, P., MEYER, D. and JELLEMA, R.H. 2006. Inulins improve sensoric and textural properties of low-fat yoghurts. Int. Dairy J. *16*, 1098–1103.

KOSIKOWSKI, F.V. 1982. *Cheese and Fermented Milk Foods*, 2nd Ed., Cornell University, Ithaca, NY.

KUEHBACHER, T., OTT, S.J., HELWIG, U., MIMURA, T., RIZZELLO, F., KLEESSEN, B., GIONCHETTI, P., BLAUT, M.,

CAMPIERI, M., FOLSCH, U.R. *et al*. 2006. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. Gut *55*, 833–841.

LIN, D.C. 2003. Probiotics as functional foods. Nutr. Clin. Pract. *18*, 497–506.

LUCEY, J.A. and SINGH, H. 1998. Formation and physical properties of acid milk gels: A review. Food Res. Int. *30*, 529–542.

MARTEAU, P.R., DE VRESE, M., CELLIER, C.J. and SCHREZENMEIR, J. 2001. Protection from gastrointestinal diseases with the use of probiotics. Am. J. Clin. Nutr. *73*, 430–436.

MARTIN-DIANA, A.B., JANER, C., PELAAEZ, C. and REQUENA, T. 2003. Development of a fermented goat's milk containing probiotic bacteria. Int. Dairy J. *13*, 827–833.

MAZLOOMI, S.M., SHEKARFOROUSH, S.S., EBRAHIMNEJAD, H. and SAJEDIANFARD, J. 2011. Effect of adding inulin on microbial and physico- chemical properties of low fat probiotic yogurt. Iran J. Vet. Res. *12*, 93–98.

MOERMAN, F.T., VAN LEEUWEN, M.B. and DELCOUR, J.A. 2004. Enrichment of higher molecular fractions in inulin. J. Agric. Food Chem. *52*, 3780–3783.

OUWEHAND, A.C. and SALMINEN, S.J. 1998. The health effects of cultured milk products with viable and non-viable bacteria. Int. Dairy J. *8*, 749–758.

OUWEHAND, A.C., SALMINEN, S. and ISOLAURI, E. 2002. Probiotics: An overview of beneficial effects. A. Van Leeuw. *82*, 279–289.

OZCAN, T., LUCEY, J.A. and HORNE, D. 2008. Effect of tetrasodium pyrophosphate on the physicochemical properties of yogurt gels. J. Dairy Sci. *91*, 4492–4500.

OZCAN-YILSAY, T. and YILMAZ, L. 2007. Effect of probiotic yogurt culture on Hepatic Encephalopathy. Milk World *7*, 35–37 (in Turkish).

PASEEPHOL, T. 2008. *Characterisation of prebiotic compounds from plant sources and food industry wastes: Inulin from Jerusalem artichoke and lactulose from milk concentration permeate*. PhD Thesis, pp. 197, School of Applied Sciences, RMIT University.

PASEEPHOL, T., SMALL, D.M. and SHERKAT, F. 2008. Rheology and texture of set yogurt as affected by inulin addition. J. Texture Studies. *39*, 617–634.

PERRIN, S., FOUGNIES, C., GRILL, J.P., JACOBS, H. and SCHNEIDER, F. 2002. Fermentation of chicory fructo-oligosaccharides in mixtures of different degrees of polymerization by three strains of bifidobacteria. Can. J. Microbiol. *48*, 759–763.

RANADHEERA, R.D.C.S., BAINES, S.K. and ADAMS, M.C. 2010. Importance of food in probiotic efficacy. Food Res. Int. *43*, 1–7.

RASTALL, R.A., FULLER, R., GASKINS, H.R. and GIBSON, G.R. 2000. Colonic functional foods. In *Functional Foods* (G.R. Gibson and C.M. Williams, eds.) pp. 71–89, Woodhead Publishing, Cambridge, U.K.

REID, G., CHARBONNEAU, D., ERB, J., KOCHANOWSKI, B., BEUERMAN, D., POEHNER, R. and BRUCE, A.W. 2003. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: Randomized, placebo-controlled trial in 64 healthy women. FEMS Immunol. Med. Microbiol. *35*, 131–134.

ROBERFROID, M.B. 2000. Prebiotics and probiotics: Are they functional foods? Am. J. Clin. Nutr. *71*, 1682–1687.

SADEK, Z.I., EL-SHAFEI, K. and MURAD, H.A. 2004. Utilization of xanthan gum and inulin as prebioticsfor lactic acid bacteria. In *Egyptian Conference for Dairy Science and Technology, 9th,Cairo, Egypt, 9 (Research Papers 1); 2004 Oct 9–11*, pp. 269–283, Egyptian Society of Dairy Science, Cairo, Egypt.

SALMINEN, S., OUWEHAND, A., BENNO, Y. and LEE, Y.K. 1999. Probiotics: How should they be defined? Trends Food Sci. Tech. *10*, 107–110.

SAS. 2004. *SAS User's Guide: Statistics, Version*, 9.1 Ed., SAS Inst. Inc., Cary, NC.

SCHNEEMAN, B. 1999. Fiber, inulin and oligofructose: Similarities and differences. J. Nutr. *129*, 1424–1427.

SCHREZENMEIER, J. and DE VRESE, M. 2001. Probiotics, prebiotics and synbiotics – approaching a definition. Am. J. Clin. Nutr. *73*, 361–364.

SEYDIM, Z.B.G., SARIKUS, G. and OKUR, O.D. 2005. Effect of inulin and Dairy-Lo as fat replacers on the quality of set type yogurt. Milchwissenschaft. *60*, 51–55.

SIP, A. and GRAJEK, W. 2010. Probiotics and Prebiotics. In *Functional Food Product Development* (J. Smith, E. Charter and I. Wiley, eds.) pp. 146–177, Wiley-Blackwell, Ltd., Publication, Oxford, U.K.

SZAJEWSKA, H., KOTOWSKA, M., MRUKOWICZ, J.Z., ARMANSKA, M. and MIKOLAJCZYK, W. 2001. Efficacy of *Lactobacillus* GG in prevention of nosocomial diarrhea in infants. J. Pediatr. *138*, 361–365.

SZYMANSKI, H., PEJCZ, J., JAWIEN, M., CHMIELARCZYK, A., STRUS, M. and HECZKO, P.B. 2006. Treatment of acute infectious diarrhoea in infants and children with a mixture of three *Lactobacillus rhamnosus* strains – a randomized, doubleblind, placebo-controlled trial. Aliment Pharmacol. Therapeut. *23*, 247–253.

THARMARAJ, N. and SHAH, N.P. 2003. Selective enumeration of *L. delbrueckii* ssp *bulgaricus*, *Streptococcus thermophillus*, *Lactococcus acidophillus*, *Bifidobacteria*, *Lactobacillus casei*, *Lactobacillus rhamnosus* and *Propioniobacteria*. J. Dairy Sci. *86*, 2288–2296.

VEREYKEN, I.I., CHUPIN, V., HOEKSTRA, F.A., SMEEKENS, S.C.M. and DE KRUIJFF, B. 2003. The effect of fructan on membrane lipid organization and dynamics in the dry state. Biophys. J. *84*, 3759–3766.

VINDEROLA, C., COSTA, G.A., REGENHARDT, S. and REINHEIMER, J.A. 2002. Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. Int. Dairy J. *12*, 579–589.

VINDEROLA, C.G. and REINHEIMER, J.A. 2003. Lactic acid bacteria: A comparative "*in vitro*" study of probiotic characteristics and biological barrier resistance. Food Res. Int. *36*, 895–904.

VINDEROLA, C.G., PROSELLO, W., GHIBERTO, T.D. and REINHEIMER, J.A. 2000. Viability of probiotic (*Bifidobacterium*, *Lactobacillus acidophilus and Lactobacillus casei*) and non probiotic microflora in Argentinean fresco cheese. J. Dairy Sci. *83*, 1905–1911.

WACHER-RODARTE, C., GALVIN, M.V., FARRES, A., GALLARDO, F., MARSHALL, V.M.E. and GARCIA-GARIBAY, M. 1993. Yogurt production from reconstituted skim milk using different polymer and non-polymer forming starter cultures. J. Dairy Res. *60*, 247–254.

WADA, T., SUGATANI, J., TERADA, E., OHGUCHI, M. and MIWA, M. 2005. Physicochemical characterization and biological effects of inulin enzymatically synthesized from sucrose. J. Agr. Food Chem. *53*, 1246–1253.

WILLIAMS, C.M. 1999. Effects of inulin on lipid parameters in humans. J. Nutr. *129*, 1471–1473.

WULLT, M., HAGSLATT, M.L. and ODENHOLT, I. 2003. *Lactobacillus plantarum* 299v for the treatment of recurrent *Clostridium difficile*-associated diarrhea: A double-bind, placebo-controlled trial. Scand J. Infect Dis. *35*, 365–367.

ZIMERI, J.E. and KOKINI, J.L. 2003. Rheological properties of inulin-waxy maize starch systems. Carbohydr. Polymer. *52*, 67–85.