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USE OF SYNBIOTICS FOR PRODUCTION OF FUNCTIONAL LOW FAT LABNEH

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In the present work, the compared effect of 1% inulin and fat replacers (1% Dairy-Lo and 0.1% Dariloid) on chemical properties, growth and stability of probiotic bacteria and sensory attributes in Labneh cheese during storage (30 days) at 5°C was studied. Three strains of probiotic bacteria were used, *Lactobacillus reuteri* B-14171, *Lactobacillus johnsonii* B-2178 and *L. salivarius* B-1950, with a yoghurt starter cultures.

Addition of inulin, Dairy-Lo and Dariloid increased the soluble nitrogen, total volatile fatty acids, acetaldehyde and diacetyl. The effect of added prebiotics on soluble tyrosine and tryptophan was more pronounced. All strains showed good growth and survival in the presence of prebiotics. Initial counts of probiotic bacteria and their subsequent survival were better in the products supplemented with inulin. The most acceptable cheeses were those supplemented with 1% inulin.

INTRODUCTION

In recent years, there has been a growing interest in using probiotic microorganisms as dietary adjuncts in the dairy industry. However, there are several problems associated with incorporating probiotic bacteria into the starter used to make cultured dairy products. One problem is slow growth of probiotic bacteria, probably due to their low level of proteolytic activity [Oliveira *et al.*, 2002]. Another problem is possible growth suppression of probiotic bacteria, by the traditional yoghurt cultures [McComas & Gilliland, 2003]. For probiotic bacteria to deliver their health benefits, sufficient numbers must be present at the time of consumption. Thus, producing a product that contains an adequate number of probiotic bacteria, at the point of consumption in dairy products, is very important. Several countries have established standards for numbers of probiotic bacteria in dairy products [Sanders *et al.*, 1996; Shin *et al.*, 2000]. Swiss Food Regulation requires that such products contain at least 1×10^6 probiotic organisms per milliliter or gram [Shin *et al.*, 2000].

A possible method of ensuring adequate numbers of probiotic bacteria in cultured dairy products is to supplement milk to be fermented with substances stimulatory toward the growth of probiotic bacteria. In recent years, there has been more focus on synbiotics, a combination of pre- and probiotics in a single product [Helland *et al.*, 2004]. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon [Desal *et al.,* 2004]. There has been a considerable interest in the use of prebiotics to enhance the survival and colonization of probiotic bacteria that are added in food products [Ziemer & Gibson, 1998]. Inulin non-digestible carbohydrate containing naturally occurring fructo-oligosaccharides, possesses characteristics of dietary fiber, and as such is of particular interest for its metabolic properties. Alongside its health benefits, inulin is also considered to have functional properties such as the ability to act as a fat or sugar replacer without adversely affecting flavour [Tungland, 2000]. The fat-substituting property of inulin is based on the product ability to stabilize the structure of the aqueous phase, which creates an improved creaminess mouth feel [Ibrahim *et al.*, 2004].

Over the past decade, there has been substantial interest in the development of a new range of dairy products, which are similar to the existing products but in which the fat content is substantially reduced to avoid health problems associated with high dietary fat [Akoh, 1998]. The use of fat replacers in cheese while keeping the same functional and organoleptic properties as full fat cheeses has attracted great attention in the past few years [Drake *et al.*, 1996; Kebary *et al.*, 2002]. Many commercial fat replacers are available for use in foods and they are classified as fat-based fat replacers, protein-based fat replacers (Dairy-Lo) and carbohydrate fat replacers (Dariloid-100) [Huyghebaert *et al.*, 1996].

Labneh is the product obtained from ordinary yoghurt after removal of a part of its water, lactose and salts. It should be soft, smooth and spreadable with a consistency resembling cultured cream, acidic clean flavour and milky white colour. It is usually consumed with bread as a part of a main meal in the Middle East [El-Samragy, 1997]. The variation in quality of Labneh (yoghurt cheese) in different countries is due to the variation of starter cultures used [Sharaf *et al.*, 1996]. Lactobacilli have been used widely in dairy products because of their health-promoting effects [Shah, 2000, 2001]. Therefore, the objective of this study

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was to investigate the influence of commercially available inulin, Dairy-Lo and Dariloid on viability of probiotic lactobacilli and to verify the effect of different prebiotics on the physical and sensory characteristics of Labneh cheese.

MATERIALS AND METHODS

MATERIALS

Bacterial strains. The microorganisms used in this study were as follows: *Lactobacillus johnsonii* B-2178, *Lactobacillus reuteri* B-14171 and *Lactobacillus salivarius* B-1950. These strains were provided by Northern Regional Research Laboratory, Illinois, USA [NRRL]. All strains had previously been shown to possess properties required of probiotic microorganisms including bile salt tolerance, tolerance to low-pH values and antagonistic activity [Amin *et al.*, 2002]. In addition, *Streptococcus thermophilus* and *Lactobacillus delbreuckii* ssp. *bulgaricus* were obtained from Dairy Microbiology Lab., National Research Centre, Dokki, Cairo, Egypt.

Additives. The following additives were used as prebiotics in the study: (1) Dairy-Lo (a protein-based fat replacer), Pfizer Inc., New York, USA; (2) Inulin, Sensus Food Ingredients, Netherlands, and (3) Darioloid 100 (Guar gum, locvst bean gum, xanthan gum), Kelco, Division of Merck and Co. Inc. Rahway, New Jersey, USA).

Milk. Fresh whole buffalos' milk was obtained from the herd of the Faculty of Agriculture, Cairo Univ., Egypt.

METHODS

Procedure for Labneh manufacturing. Labneh was manufactured according to El-Sayed *et al.* [1993] from standardized fresh buffalos' milk (1.5% fat). Milk was heated to 85°C for 15 min, cooled to 42°C and divided into 4 main portions. The first portion was applied as a control, without addition of prebiotics. The other three portions were supplemented with 1% (w/v) inulin, 1% (w/v) Dairy-Lo and 0.1% (w/v) Dariloid, respectively. Then each portion was divided into three equal portions. Starters were added as follows: (1) *S. thermophilus, L. delbreuckii* ssp. *bulgaricus* and *L. johnsonii* B-2178; (2) *S. thermophilus, L. delbreuckii* ssp. *bulgaricus* and *L. reuteri* B-14171; and (3) *S. thermophilus, L. delbreuckii* ssp. *bulgaricus* and *L. salivarius* B-1950.

All starters were added at the level of 3% and incubated at 42°C until pH 4.8. Fermented milk of all treatments was left overnight, mixed and put into cloth bags which were hung for 24 h in a refrigerator to allow for whey drainage. The resulting Labneh cheese was mixed with 1% salt (NaCl), submerged in corn oil in glass containers and stored in a refrigerator (5±1°C) for 30 days. The experiments were carried out in triplicate.

Analytical procedures. Labneh cheeses were sampled when fresh and after 10, 20 and 30 days of storage.

Chemical analysis. Protein, fat, lactose and ash contents of Labneh were determined according to AOAC [1995]. pH and titratable acidity (expressed as grams of lactic acid/100 g of Labneh) were determined as outlined by Marshall [1993]. Total solids and soluble nitrogen were measured according to IDF [1982]. Acetaldehyde and diacetyl content were determined as described by Lees & Jago [1969] and Pack *et al.* [1964], respectively. Total volatile free fatty acids (TVFA) were measured according to Kosikowski [1982]. Soluble tyrosine and tryptophan were measured according to Vakaleris & Price [1959]. Carbonyl compounds were determined according to Berry & Mckerrigan [1959]. Total energy was calculated based on conversion factors as follows: protein 4 kcal/g, carbohydrate 4 kcal/g and fat 9 kcal/g.

Bacteriological analysis. *Enumeration of Labneh cheese starters.* Labneh cheese samples were diluted in sterile tryptone diluent (0.1% w/v) and subsequently plated in duplicate onto selective media. *S. thermophilus* and *L. delbreuckii* ssp. *bulgaricus* were enumerated on M_{17} media (Oxoid) and MRS, respectively, at pH 5.4. Viability of *L. reuteri, L. johnsonii* and *L. salivarius* was determined on MRS-arabinose agar [Effat, 2000], MRS-raffinose agar [Abd El-Khalek *et al.*, 2004], and MRS-mannitol agar, respectively. The plate's incubation was done at 37°C for 72 h, in an anaerobic environment (BBL Gas Pak, Becton Dickinson, Cockeysville MA, USA) for all lactobacilli.

Percent viabilities of cultures in the presence of prebiotics were calculated as follows: % viability = (CFU at 30 days of storage/initial CFU)×100 [Desal *et al.,* 2004].

Sensory evaluation. All Labneh cheese samples were organoleptically evaluated when fresh and during refrigerated storage every 10 days according to Amer *et al.* [1997] for flavour (60 points), consistency (30 points), and appearance (10 points) by 20 panelists of the experienced staff members of the Dairy Science Department, National Research Centre.

RESULTS AND DISCUSSION

Chemical composition of Labneh cheese

The gross chemical composition of all Labneh cheese treatments are summarized in Table 1. It is clear that probiotic control Labneh cheese (C_3) with *L. reuteri* had highest content of total solids than all treatments followed by Labneh cheese (I_3) manufactured with 1% inulin and *L. reuteri* and Labneh cheese (Da_3) made with 1% Dairy-Lo and *L. reuteri*, respectively. On the other hand, Labneh cheese (D_1) made with 0.1% Dariloid and *L. johnsonii* had the lowest content of total solids than all treatments. This may be due to the effect of starter.

Fat is the same in all treatments except the control had a slight increase than all treatments (Table 1). From the same table fat/d.m. is highest in Labneh cheese (control) than all treatments. Labneh cheese made from 1% inulin and *S. thermophilus*, *L. delbreuckii* ssp. *bulgaricus* and *L. reuteri* (I_3) had lowest fat/d.m.% than all treatments.

As for total protein (TP) (Table 1) Labneh cheese manufactured with *L.reuteri* and 1% Dairy-Lo (Da_3) had the highest total protein than all treatments. This effect may be due to the effect of starter.

Also from Table 1, it is clear that Labneh cheese manufactured with 0.1% Dariloid with *L. johnsonii* (D_1) had a highest TP/d.m. than all treatments whereas Labneh cheese manufactured with *L. reuteri* (C_3) had the lowest TP/d.m. than all treatments.

Table 1 showed that Labneh cheese manufactured with 1% inulin and *L. reuteri* (I_3) had the highest content of lactose (22.71%) than all treatments whereas Labneh cheese manufactured with 0.1% Dariloid and *L. johnsonii* (D_1) had the lowest lactose content than all treatments. This may be due to the effect of starter.

Both Labneh cheese manufactured with 1% Dairy-Lo, and 0.1% Dariloid and *L. reuteri* (Da_3 and D_3) had the highest content of ash, whereas Labneh cheese manufactured with *L. johnsonii* (C_1) had lowest content of ash than all treatments. On the other hand, ash/DM was a highest (7.28%) in Labneh cheese manufactured with 0.1% Dariloid with *L. salivarius* (D_2). While it was a lowest in Labneh cheese manufactured with *L. reuteri* (C_3). In general, addition of fat replacers caused an increase in ash content. These results could be attributed to the ash content of Dairy-Lo and Dariloid [Kebary *et al.*, 1996].

From the same Table 1, we noticed that Labneh cheese manufactured with *L. reuteri* (C_3) had the highest caloric value than all treatments. On the other hand Labneh cheese made with *L. johnsonii* (D_1) had a lowest caloric value than all treatments.

Changes of some chemical properties during storage

Data presented in Table 2 show that the T.A. varied widely between the three types of probiotic bacteria used in fresh and stored Labneh, probably due to the nature of the individual probiotic bacteria. Highest T.A. was obtained in fresh Labneh made with *S. thermophilus, L. delbreuckii* ssp. *bulgaricus* and *L. johnsonii* and supplemented with 0.1% Dariloid (D_1) followed by that made with yoghurt starter and *L. johnsonii* and supplemented with 1% Dairy-Lo. On the other hand, Labneh made with yoghurt starter and *L. reuteri* had the lowest T.A. During storage, the T.A. of all Labneh cheese samples increased as the storage period progressed, while the pH values (Table 2) of all treatments had an opposite trend. In general, our findings are partly in agreement with those reported for fermented milk by Ibrahim *et al.* [2004].

Data in Table 2 show a noticeable increase in SN content in all Labneh cheeses with added prebiotics as compared to the control especially D_1 and D_3 made with the use of mixed cultures of *S. thermophilus, L. delbreuckii* ssp. *bulgaricus* and *L. johnsonii* and *S. thermophilus, L. delbreuckii* ssp. *bulgaricus* and *L. reuteri*. During storage, gradual increase in SN was observed in cheese from all treatments till the end of storage. Similar trends were reported for Domiati and Tallaga cheeses by Kebary *et al.* [1996] and Badawi & Kebary [1998].

Data presented in Table 3 show that the volatile fatty acids in Labneh cheeses made with prebiotics as of fresh cheeses had higher values especially Labneh manufactured with *S. thermophilus, L. delbreuckii* ssp. *bulgaricus* and *L. salivarius* supplemented with 1% inulin as a prebiotic (I_2). The T.V.F.A. gradually increased in Labneh cheese from different treatments with extended storage. These findings were in agreement with those reported by Taha *et al.* [1997].

Labneh cheese made with adding Dairy-Lo or Dariloid fat replacers had higher T.V.F.A. than corresponding cheeses without fat replacers (control) at the end of storage (Table 3). The increase in T.V.F.A. could be explained on the basis that the increase in the cheese proteolysis as amino acids can serve as precursors for the development of certain volatile fatty acids [Nakae & Elliott, 1965]. Similar results were

TABLE 1. Chemical composition of fresh Labneh as affected by probiotics and prebiotics.

Cheese treatment	TS (%)	Fat (%)	Fat/d.m. (%)	Total protein (%)	Total protein/d.m.	Lactose (%)	Lactose/ d.m. (%)	Ash (%)	Ash/d.m. (%)	Caloric value (kcal/100 g)
C_1	17.72	3.0	16.93	10.10	57.00	3.40	19.19	1.20	6.77	81.42
C_2	17.74	3.0	16.91	10.09	56.88	3.42	19.28	1.21	6.82	81.04
C_3	18.36	3.0	16.34	10.12	55.12	4.00	21.79	1.22	6.65	83.48
I_1	17.62	2.7	15.32	10.23	58.06	3.46	19.64	1.22	6.92	79.06
I_2	17.67	2.7	15.28	10.24	57.95	3.48	19.69	1.23	6.96	79.18
I_3	18.32	2.7	14.74	10.29	55.73	4.16	22.71	1.24	6.77	81.73
Da_1	17.58	2.7	15.36	10.28	58.48	3.34	19.00	1.26	7.17	78.78
Da_2	17.62	2.7	15.32	10.30	58.46	3.34	18.96	1.28	7.26	78.86
Da_3	18.28	2.7	14.77	10.38	56.78	3.86	21.12	1.32	7.22	81.26
D_1	17.56	2.7	15.38	10.28	58.54	3.32	18.91	1.24	7.06	78.70
D_2	17.58	2.7	15.36	10.26	58.36	3.34	19.00	1.28	7.28	78.70
D_3	18.20	2.7	14.84	10.34	56.81	3.84	21.10	1.32	7.25	81.02

C_1 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii*
C_2 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius*
C_3 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri*
I_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 1% inulin
I_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 1% inulin
I_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 1% inulin
Da_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 1% Dairy-Lo
Da_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 1% Dairy-Lo
Da_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 1% Dairy-Lo
D_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 0.1% Dariloid
D_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 0.1% Dariloid
D_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 0.1% Dariloid

TABLE 2. Influence of probiotics and prebiotics on acidity, pH and soluble nitrogen of Labneh during storage at 5±1°C.

Cheese treatment	Acidity (% lactic acid)				pH				Soluble nitrogen (SN), %			
	Storage period (days)											
	0	10	20	30	0	10	20	30	0	10	20	30
C_1	1.30	1.35	1.42	1.46	4.62	4.60	4.54	4.50	0.24	0.28	0.34	0.42
C_2	1.28	1.32	1.38	1.42	4.61	4.58	4.56	4.52	0.26	0.36	0.42	0.46
C_3	0.90	1.12	1.20	1.28	4.64	4.60	4.58	4.54	0.28	0.38	0.44	0.50
I_1	1.29	1.32	1.38	1.42	4.60	4.56	4.52	4.50	0.30	0.34	0.46	0.52
I_2	1.26	1.34	1.40	1.44	4.58	4.54	4.48	4.44	0.32	0.38	0.44	0.56
I_3	0.80	0.96	1.01	1.05	4.68	4.64	4.60	4.58	0.36	0.40	0.46	0.50
Da_1	1.31	1.36	1.38	1.42	4.61	4.54	4.50	4.46	0.36	0.42	0.48	0.52
Da_2	1.30	1.34	1.37	1.41	4.60	4.56	4.52	4.48	0.34	0.40	0.52	0.60
Da_3	0.95	1.02	1.04	1.08	4.67	4.62	4.60	4.57	0.40	0.44	0.50	0.58
D_1	1.32	1.36	1.40	1.44	4.61	4.58	4.50	4.44	0.42	0.48	0.54	0.62
D_2	1.28	1.32	1.38	1.40	4.60	4.56	4.49	4.46	0.40	0.46	0.50	0.60
D_3	0.58	1.04	1.12	1.18	4.66	4.62	4.60	4.56	0.41	0.50	0.56	0.64

C_1 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii*
C_2 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius*
C_3 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri*
I_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 1% inulin
I_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 1% inulin
I_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 1% inulin
Da_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 1% Dairy-Lo
Da_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 1% Dairy-Lo
Da_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 1% Dairy-Lo
D_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 0.1% Dariloid
D_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 0.1% Dariloid
D_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 0.1% Dariloid

reported for Edam cheese [Kebary *et al.,* 2002] and for Tallaga cheese by Badawi & Kebary [1998].

Soluble tyrosine and soluble tryptophan contents followed similar trends of variations in all cheeses (Table 3). As for tyrosine content, Labneh made with yoghurt culture and *L. reuteri* and supplemented with 0.1% Dariloid (D_3) had the highest values whether fresh or during storage. During storage, the tyrosine content increased in all samples indicating a continuous breakdown of the proteins. As for tryptophan content, it is clear that its contents ran parallel to tyrosine content being the highest in fresh Labneh made with Dariloid addition followed by Labneh made with the addition of Dairy-Lo. During storage, tryptophan content of all samples decreased with a higher rate in the case of Labneh made with fat replacers. Similar trends were reported for Labneh cheese [Amer *et al.*, 1997] and for Edam cheese [Kebary *et al.*, 2002]. Addition of Dairy-Lo or Dariloid caused a pronounced increase in the values of soluble tyrosine and soluble tryptophan contents. These results might be due to the increase in moisture content that enhances proteolysis [Banwart, 1981].

Data presented in Table 4 show that Labneh produced by yoghurt culture and *L. reuteri* containing inulin had the highest acetaldehyde and diacetyl contents whether when fresh or stored followed by that made with yoghurt culture and *L. salivarius* containing inulin. Both acetaldehyde and diacetyl contents increased to reach maximum values after 10 days and then decreased until the end of storage in all treatments. The concentration of acetaldehyde and diacetyl can differ to a great extent, depending on the medium composition, growth conditions and the specific activity of the bacteria and their enzymes. In yoghurt, the pathway leading to acetaldehyde production is generally considered to be via lactose degradation [Gonzalez *et al.*, 1994]. Some of the end products of citrate and pyruvate metabolism, such as diacetyl and acetaldehyde, have distinct aroma properties and contribute significantly to the quality of fermented foods [Helland *et al.,* 2004].

Concerning total carbonyl compounds, it is obvious that Labneh cheese manufactured with yoghurt culture and *L. salivarius* supplemented with Dairy-Lo (Da_2) had higher content than all treatments throughout storage (Table 4). During storage, total carbonyl compounds in all Labneh cheese samples increased as the storage period progressed. Similar results were reported by Hassan *et al.* [2001].

Influence of prebiotics on traditional yoghurt cultures

The changes in the viable counts of *S. thermophilus* and *L. delbreuckii* ssp. *bulgaricus* during storage of Labneh cheese are presented in Figures 1a, 2a and 3a. As shown, counts of *S. thermophilus* predominated in all Labneh cheeses at 0 day of storage period, as Sharaf *et al.* [1996] reported for Labneh cheese and Effat [2000] for Domiati cheese. As no differences of *S. thermophilus* counts were detected for the different Labneh cheeses, means of all cheeses at each time of storage were considered. In average, *S. thermophilus* counts decreased slightly from 8.9 $\log_{10}$ cfu/g to 5.90 $\log_{10}$ cfu/g during storage (Figures 1a, 2a and 3a).

The counts of *L. delbreuckii* ssp. *bulgaricus* exhibited approximately the same behavior of *S. thermophilus* in all Labneh cheeses throughout the storage period (Figure 1b, 2b and 3b). Sharaf *et al.* [1996] reported that the count of *L. delbreuckii* ssp. *bulgaricus* decreased during storage of Lab-

TABLE 3. Influence of probiotics and prebiotics on total volatile fatty acids, soluble tyrosine and soluble tryptophan in Labneh during storage at 5±1°C.

Cheese treatment	Total volatile fatty acids (mL 0.1 N NaOH/100 g cheese)				Soluble tyrosine (mg/100 g cheese)				Soluble tryptophan (mg/100 g cheese)			
	Storage period (days)											
	0	10	20	30	0	10	20	30	0	10	20	30
C_1	16	18	22	24	16.7	29.1	35.2	38.1	92.6	80.3	72.3	50.4
C_2	14	16	20	22	16.6	25.2	33.9	36.2	98.1	92.2	80.4	62.2
C_3	16	18	21	24	15.5	24.8	30.6	34.8	95.3	82.6	78.1	60.0
I_1	25	28	30	32	14.8	22.4	26.8	31.0	90.4	80.4	70.8	60.8
I_2	28	30	34	36	16.0	24.8	28.2	34.6	91.0	81.2	72.0	65.0
I_3	26	29	32	34	15.0	24.0	28.0	34.0	88.6	78.6	68.2	60.2
Da_1	20	22	28	30	25.10	30.0	36.2	41.6	101.2	90.4	70.8	60.2
Da_2	22	24	26	38	26.3	30.8	34.8	40.0	106.0	92.3	72.0	61.8
Da_3	20	23	28	32	24.2	29.4	33.4	38.6	108.0	94.0	74.3	65.6
D_1	26	30	34	38	28.4	34.6	39.6	42.0	118.2	106.0	92.8	71.2
D_2	25	29	36	40	29.3	36.0	40.2	44.3	116.1	101.3	90.4	70.6
D_3	24	28	32	39	30.8	38.4	42.1	46.8	119.0	108.2	95.6	68.0

C_1 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii*
C_2 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius*
C_3 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri*
I_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 1% inulin
I_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 1% inulin
I_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 1% inulin
Da_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 1% Dairy-Lo
Da_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 1% Dairy-Lo
Da_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 1% Dairy-Lo
D_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 0.1% Dariloid
D_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 0.1% Dariloid
D_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 0.1% Dariloid

TABLE 4. Influence of probiotics and prebiotics on acetaldehyde, diacetyl and total carbonyl compounds in Labneh during storage at 5±1°C.

Cheese treatment	Acetaldehyde content (μm/100 gm)				Diacetyl content (μm/100 gm)				Total carbonyl compounds (μmol/100 kg cheese)			
	Storage period (days)											
	0	10	20	30	0	10	20	30	0	10	20	30
C_1	320	342	320	225	104	125	115	95	68.21	165.23	262.00	352.96
C_2	325	345	322	228	105	126	114	94	65.49	158.00	250.82	348.22
C_3	330	348	330	230	108	132	120	105	70.00	168.14	268.00	356.08
I_1	330	408	318	208	106	130	118	96	70.22	168.80	269.08	377.08
I_2	335	412	332	226	108	134	122	106	84.36	182.00	280.14	382.00
I_3	340	410	321	212	110	132	120	104	78.07	174.00	278.92	380.76
Da_1	285	300	260	225	100	122	92	80	80.46	188.90	272.62	380.41
Da_2	290	318	268	230	101	120	90	86	85.73	195.11	304.11	401.32
Da_3	295	325	270	235	104	126	116	92	78.24	180.18	285.01	395.11
D_1	310	408	382	215	102	124	114	93	76.38	168.40	254.70	362.18
D_2	322	424	392	218	104	125	115	95	76.64	170.18	261.11	368.80
D_3	318	416	390	220	103	122	92	79	80.04	175.42	268.00	372.01

C_1 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii*
C_2 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius*
C_3 : Control with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri*
I_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 1% inulin
I_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 1% inulin
I_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 1% inulin
Da_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 1% Dairy-Lo
Da_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 1% Dairy-Lo
Da_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 1% Dairy-Lo
D_1 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. johnsonii* containing 0.1% Dariloid
D_2 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. salivarius* containing 0.1% Dariloid
D_3 : Labneh cheese with *S. thermophilus* + *L. delbreuckii* ssp. *bulgaricus* + *L. reuteri* containing 0.1% Dariloid

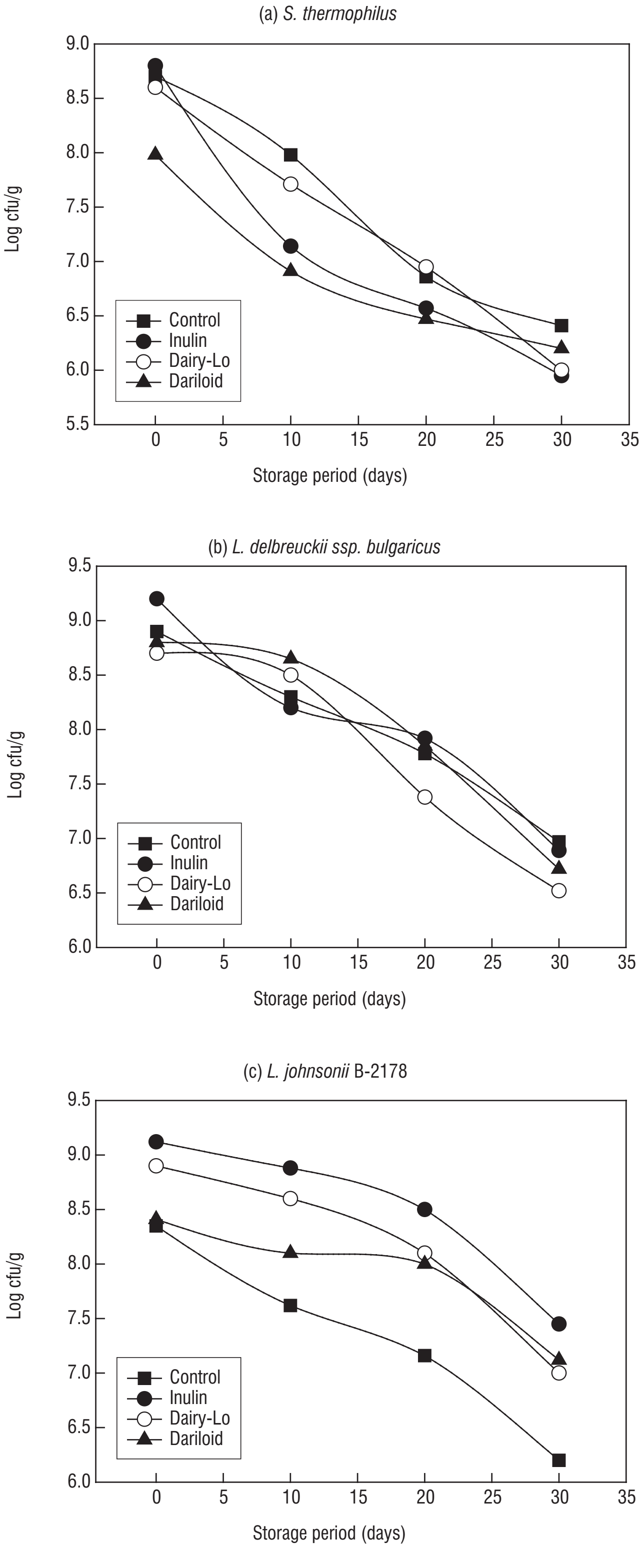

FIGURE 1. Viability of *S. thermophilus* (a) *L. delbreuckii* ssp. *bulgaricus* (b) and *L. johnsonii* B-2178 (c) in Labneh cheese containing prebiotics during storage in a refrigerator.

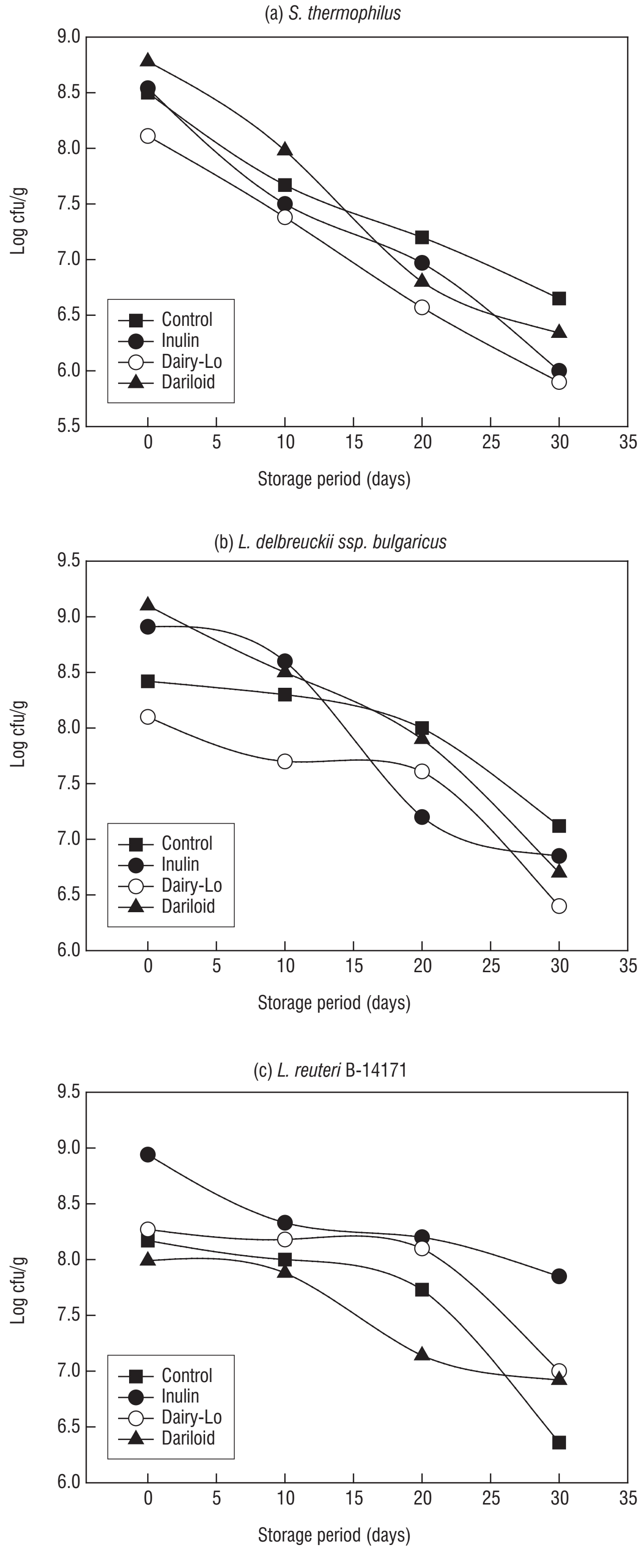

FIGURE 2. Viability of *S. thermophilus* (a) *L. delbreuckii* ssp. *bulgaricus* (b) and *L. reuteri* B-14171 (c) in Labneh cheese containing prebiotics during storage in a refrigerator.

neh cheese. The decreased numbers was due to the sensitivity of microorganisms to the produced acidity. Similarly, Hamann & Marth [1984] reported that the population of viable yoghurt bacteria increased immediately after manufacturing the yoghurt and decreased during refrigerated storage of the product. In general, these results coincide with those obtained by Sharaf *et al.* [1996].

From the foregoing results, it is obvious that prebiotics had no influence on the growth pattern of both *S. thermophilus* and *L. delbreuckii* ssp. *bulgaricus* during cold storage of Labneh cheeses. Generally, our results confirm the findings of Akin [2005] who recorded that the addition of inulin had no effect on numbers of *S. thermophilus* and *L. delbreuckii* ssp. *bulgaricus* in probiotic fermented ice cream. Moreover,

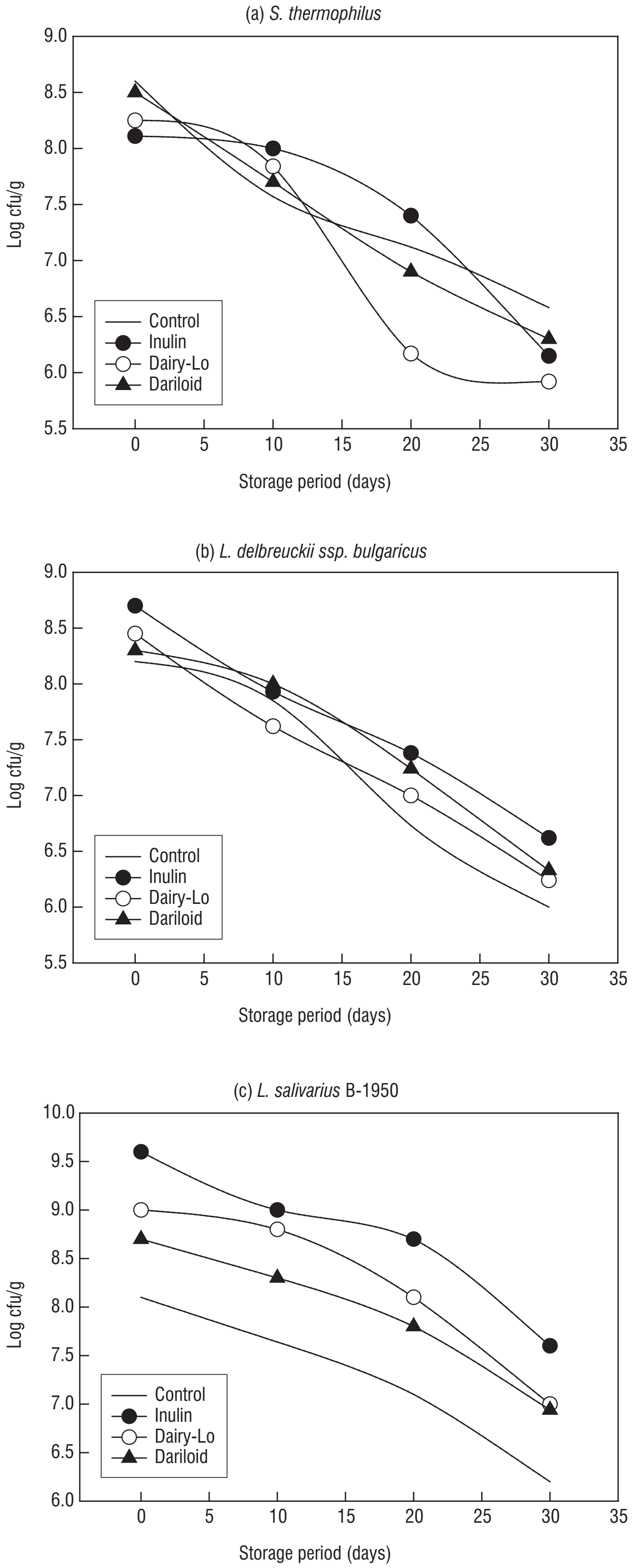

FIGURE 3. Viability of *S. thermophilus* (a) *L. delbreuckii* ssp. *bulgaricus* (b) and *L. salivarius* B-1950 (c) in Labneh cheese containing prebiotics during storage in refrigerator.

the foregoing results are in accordance with other reports which stated that the addition of non-heated xanthan gum showed no marked effect on growth level of lactic acid bacteria [El-Sayed *et al.,* 2002; Sadek *et al.*, 2004].

Influence of prebiotics on the shelf stability of probiotic cultures in Labneh cheese

The viability of the 3 strains *L. reuteri*, *L. johnsonii* and *L. salivarius* in Labneh cheese containing prebiotics stored at a refrigerator (~5°C) for 30 days are shown in Figures 1c, 2c and 3c. In general, the viability of all 3 strains decreased during storage. However, the viability was in many cases higher than that of the control, without prebiotics. On an average, best viability was observed with inulin. The highest viability of 87.8%, 81.68% and 79.16% was recorded for *L. reuteri, L. johnsonii* and *L. salivarius* with inulin, respectively. Overall, Dairy-Lo was the least effective in maintaining viability, with average viabilities of 84%, 78.6%, and 79.77%. The lowest viability was recorded by *L. johnsonii* with an average viability of 78.6%. The control samples containing no prebiotics had average survival rates of 77.8%, 74% and 76% for *L. reuteri, L. johnsonii* and *L. salivarius,* respectively. Out of 3 strains studied, 2 strains (*L. reuteri* and *L. johnsonii*) showed a strong correlation between viability and pH.

Viability of lactobacilli is affected because of several factors including acid produced during fermentation, oxygen content in the product and oxygen permeation through the packaging material and antimicrobial substances produced by lactic acid bacteria [Desal *et al.,* 2004]. Although viability of probiotic bacteria decreased during storage, it was observed that the Labneh cheeses contained 6.92 $\log_{10}$ cfu/g of probiotics, in average, after 30 days of storage. These counts are close to 10^7 cfu/g, which is the level of suggested by some authors to have a health promoting effect [Oliveira *et al.,* 2002; Akin, 2005]. Generally, our results confirm the observation of Desal *et al.* [2004], Ibrahim *et al.* [2004] and Akin [2005] who reported that the viability of lactobacilli after storage was greatest with inulin.

Sensory evaluation

The Labneh cheeses were evaluated for flavour, consistency and appearance. Data pertaining to the overall evaluation and preference of Labneh during storage at 5°C are depicted in Table 5. It appears that Labneh cheese manufactured with yoghurt culture and *L. reuteri* containing 1% inulin (I_1) gained higher scores either when fresh or during storage. Its consistency and appearance were better than other treatments. This Labneh was preferred over the Labneh cheeses of other treatments allover storage period. The resultant Labneh, fresh as well as stored, was characterised by desirable pungent flavour with smoother consistency. This could be due to that the main characteristics of *S. thermophilus* are its abilities to produce aroma [Mohamed *et al.,* 1999]. Moreover, the presence of *L. reuteri* which is acid tolerant [Toit *et al.*, 1998] and possesses flavor qualities and also represents nutritional benefits [Effat, 2000] was observed till the end of storage period. On the other hand, Labneh made with yoghurt culture and *L. johnsonii* without addition any prebiotics (C_1) gained the least scores. As storage progressed the organoleptic scores decreased in all treatments. These results are in agreement with Taha *et al.* [1997].

TABLE 5. Organoleptic properties of probiotic Labneh cheeses containing prebiotics throughout storage course on refrigerator.

Properties	Storage period (days)	Labneh cheese treatments											
		C_1	C_2	C_3	I_1	I_2	I_3	Da_1	Da_2	Da_3	D_1	D_2	D_3
Flavor (60)	0	50	51	54	55	52	54	54	54	48	50	52	54
	10	48	49	50	52	50	52	50	52	46	47	49	50
	20	44	45	47	48	48	48	48	48	42	44	46	46
	30	35	36	37	38	40	40	41	38	36	38	38	36
Consistency (30)	0	25	26	26	30	30	30	30	24	23	28	28	30
	10	22	24	24	28	28	28	27	22	22	26	26	28
	20	18	19	18	26	25	26	24	20	21	24	24	26
	30	16	16	14	24	22	22	23	18	20	22	20	24
Appearance (10)	0	8	8	8	9	9	9	9	8	7	9	8	8
	10	6	7	6	7	8	8	8	7	6	8	7	7
	20	5	5	5	6	7	6	6	6	5	7	6	6
	30	4	4	4	5	6	5	5	4	4	6	5	5
Total (100)	0	82	85	88	93	91	94	93	88	84	87	88	92
	10	76	80	80	87	86	88	85	81	79	81	82	85
	20	67	69	70	80	80	80	78	74	70	75	76	78
	30	55	56	55	67	68	67	69	60	61	66	63	65

C_1, C_2, C_3, I_1, I_2, I_3, Da_1, Da_2, Da_3, D_1, D_2, and D_3: Please refer to previous table.

CONCLUSIONS

The growth, activity and viability of *L. reuteri, L. johnsonii* and *L. salivarius* strains in Labneh were dependent on prebiotics as well as strain. Addition of prebiotics improves the growth rates. There was also slight improvement in survival rate with prebiotics, thus incorporating prebiotics will improve the efficiency of the product. The survival probiotic bacteria was better in the samples supplemented with inulin. This could be due to prebiotic effects of this oligofructose. These results suggested that the addition of inulin stimulated the growth of *L. reuteri, L. johnsonii* and *L. salivarius*, which resulted in improved viability of these organisms.

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