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Chemical, proteolysis and sensory attributes, and probiotic microorganisms viability of Iranian ultrafiltered-Feta cheese as a function of inulin concentration and storage temperature

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RESEARCH ARTICLE

Abstract

The aim of the present work was to study the effects of inulin addition (0, 1.5 and 3% w/w) and the storage temperature (8 and 12 °C) on the viability of probiotic organisms (*Lactobacillus acidophilus* la-5 and *Bifidobacterium lactis* BB-12), chemical composition, proteolysis and sensory characteristics of ultrafiltered (UF)-Feta cheeses over 60 days storage time. Storage time was the most effective factor on dependent variables. Population of both strains remained above 10^6 cfu/g at the end of storage time and their viability was neither significantly affected by the inulin concentration, nor by the storage temperatures. No significant (P>0.05) differences were observed in sensory scores, syneresis, water soluble nitrogen, trichloroacetic acid soluble nitrogen and other physicochemical properties (except for dry matter) among different cheese samples at different temperatures. The levels of flavour, odour and overall acceptability were significantly increased as storage period progressed. The results suggest UF-Feta cheese is a suitable carrier for *L. acidophilus* la-5 and *B. lactis* BB-12 with increased acceptability during storage.

Keywords: probiotic bacteria, probiotic viability, quality characteristics, sensory evaluation, UF Feta cheese

1. Introduction

There is a long history of the usage of probiotics in human diet. Probiotics are defined by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) as 'live microorganisms that, when administered in adequate amounts, confer health benefits on the host' (FAO/WHO, 2001). Probiotic foods including dairy products, have been classically defined as 'foods containing live microorganisms believed actively to enhance human health by the improving balance of micro-flora in the gut' (Tamime *et al.*, 2005).

Some of the main beneficial health related effects of probiotics consumption are: enhanced immune response, alleviation of symptoms of lactose intolerance, diarrhea treatment, reduction of serum cholesterol, vitamin synthesis and anti-carcinogenic and antimicrobial activities (Boylston *et al.*, 2004; Shah, 2007).

Along with the growing consumer awareness about health benefits of probiotics, there is a greater interest in developing of new probiotic foods other than yoghurts and fermented milks. In general, cheese is potentially appropriate alternative substrate for probiotic bacteria. Cheese has higher levels of pH, solid consistency, fat content and buffering capacity than yoghurt, thus would offer more protection to probiotic organisms during storage and gastrointestinal tract transit time (Ong and Shah, 2009).

Ultrafiltered (UF)-Feta cheese is the most produced cheese (approximately >150,000 tonne/year) in Iran and this fresh cheese has been well-accepted by Iranian and Middle East people (Karami *et al.*, 2009). Due to its manufacturing

process, fresh cheese appears to be ideally suited to serve as a carrier for probiotic bacteria. One reason is that fresh cheese is an un-ripened cheese; thus, storage occurs at refrigeration temperature, shelf life is rather limited, and no prolonged periods of ripening are necessary (Heller *et al.*, 2003). Other advantages of UF-Feta cheeses are their production from concentrated retentate of milk and it is possible to add probiotic to retentate that have two advantages: (1) the number of probiotic bacteria in the product can be controlled; and (2) there is not any loss of probiotic from coagulant during drainage of whey that can be observed in other types of cheese (Ong and Shah, 2009).

Starter culture and probiotic bacteria by means of carbohydrate metabolism, proteolysis and, to a lesser extent with lipolysis contribute to develop aroma and flavour of cheese during storage period (Ergönül, 2012). Storage temperature may influence on the concentration and balance of flavour components through its effect on the activity of microorganisms and various involved enzymes. Temperature is also a critical factor influencing probiotic survival during this storage period. In practical terms, the lower temperature led to a more viability of the probiotics in the food products. Therefore, storage appropriate temperature is required to achieve an optimum quality and probiotic viability in cheeses (Heller *et al.*, 2003).

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 that can improve the host health (Gibson and Roberfroid, 1995). Few studies have indicated the positive effect of inulin on the survival of probiotic bacteria in ice cream and yogurt (Akalin and Erişir, 2008; Magariños *et al.*, 2007). Prebiotics may exert a protective effect on selected probiotic bacteria by improving their survival and activity rates during storage of the product as well as on their passage through the upper parts of the gastrointestinal tract (Akalin and Erişir, 2008; Magariños *et al.*, 2007).

The many researchers investigated a number of cheese varieties as carriers of probiotic microorganisms, including cottage cheese (Blanchette *et al.*, 1996), Gouda (Gomes *et al.*, 1998), Cheddar (Gardiner *et al.*, 1999; Mc Brearty *et al.*, 2001; Ong *et al.*, 2006; Phillips *et al.*, 2006), Crescenza (Gobbetti *et al.*, 1997), Fresco (Vinderola *et al.*, 2000), cream cheese (Buriti *et al.*, 2007a), Minas fresh cheese (Buriti *et al.*, 2005) and goat cheese (Gomes and Malcata, 1998). However, potential efficacy of Iranian UF-Feta cheese as a carrier for probiotics have not been extensively studied, especially in relation to the effects of storage temperature and inulin addition to retentate. Therefore, the present work was designed to study the influence of inulin inclusion into cheese and storage temperature on the viability of probiotics, physicochemical properties, proteolysis as well as sensory characteristics of Iranian UF-Feta cheese during the storage period.

2. Materials and methods

Materials

Probiotic cultures used in this study were *Lactobacillus acidophilus* la-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 (Chr. Hansen, Hørsholm, Denmark). A cheese starter culture (R-704, Chr. Hansen) composed of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* was obtained from the same company. The cultures were provided in freeze-dried form and added directly to retentate at 10 mg/100 ml. Microbial rennet (Fromase® 2200 TL, 2200 IMCU/g) from *Rhizomucor miehei* was provided by DSM Food Specialities (Seclin, France) and Inulin (Raftiline HP) was obtained from Orafti (Oreye, Belgium).

UF-Feta cheese manufacture

UF-Feta cheese was manufactured using ultra filtered and pasteurised bovine milk following the procedure employed by Iranian dairies. Cheese milk was first standardised to a fat content of 3% w/w. Then, it was pasteurised at 72 °C for 16s and ultrafiltered at 50 °C to a total solids content of 35% w/w (corresponding to a concentration factor of 5:1). Based on Table 1, the retentate was mixed with inulin at three levels of 0% w/w (Treatment 1 (T1) and 4 (T4)), 1.5% w/w (T2, T5) and 3% w/w (T3, T6), and then homogenised at 50 bar at 65 °C, pasteurised at 80 °C for 60 s and cooled to 30 °C before the inoculation. The probiotic cultures were added along with the starter culture at sufficient levels to obtain approximately 10^6 cfu/g of probiotic bacteria in the retentate. Then, rennet at a concentration of 2 g/100 kg was added to the inoculated retentate and the mixture was dispensed into polystyrene cups. After the coagulation, dry salt (3% w/w) was added on the parchment paper and then the cups were sealed by using aluminium foil and transferred to an incubator at 30 °C and held to reach pH around 4.8 after the cooling period. After that, all types of cheese were divided into two equal portions and stored at 8 and 12 °C for 60 days. Each treatment (denoted T1-T6) of cheese was manufactured in triplicates (Table 1).

Physicochemical properties

The moisture content by oven drying at 102±2 °C (IDF, 1982), salt by Volhard method (ISIRI, 1976), protein by Kjeldhal method (IDF, 1993) and fat by the Gerber method (BSI, 1995) were analysed. The pH values of cheese samples were determined by direct insertion of electrode of pH meter (Micro-pH 2000, Crison Instruments, Barcelona, Spain).

Table 1. Treatments of probiotic ultrafiltered-Feta cheese.

Treatments	Temperature (°C)	Inulin (% w/w)
T1	8	0
T2	8	1.5
T3	8	3
T4	12	0
T5	12	1.5
T6	12	3

The procedure by Karami *et al.* (2009) was followed for the extraction of water soluble nitrogen (WSN) in 20 g cheese sample. Trichloroacetic acid soluble nitrogen (TCASN) was determined in the same cheese extract according to Katsiari *et al.* (1997) by mixing 10 ml of above mentioned extract with 10 ml of 24% aqueous solution of TCA, holding the mixture at room temperature for 1 h and then filtering it through Whatman no. 40 filter paper (Whatman, Göttingen, Germany). The nitrogen content in each fraction was determined by the Kjeldahl method (IDF, 1993).

The syneresis amount was calculated as the weight of whey (g) released from each cheese in its package divided by the weight of cheese in the same package (g) and multiplied by 100 (Souza and Saad, 2009). All physicochemical properties were measured on the days of 1 and 60 of storage time (except for WSN, TCASN and pH, which were measured on the days of 1, 15, 30, 45 and 60).

Survival of probiotic bacteria in cheeses

Enumeration of probiotic bacteria was carried out during storage period of 60 days at 8 and 12 °C. For this purpose, cheese samples (10 g) were blended with 90 ml of 0.1% w/v peptone water (Oxoid, Basingstoke, UK) in a stomacher (Lab-Blender 400; Seward Medical, London, UK) and then serial dilutions were prepared. *L. acidophilus* la-5 was enumerated on MRS agar (Merck, Darmstadt, Germany) containing 0.15 g/100 ml of bile after aerobic incubation at 37 °C for 72 h. Enumeration of *B. lactis* BB-12 was carried out on MRS agar modified with the addition of 0.2 g/100 ml of lithium chloride and 0.3 g/100 ml sodium propionate after anaerobic incubation (GasPak system; Oxoid) at 37 °C for 72 h (Vinderola and Reinheimer, 2000). All plate counts were carried out in duplicates at five sampling points (days 1, 15, 30, 45 and 60).

Sensory evaluation of cheese samples

Sensory evaluation of the cheese samples was carried out at day 1 and at the end of storage at 8 and 12 °C by a group of 15-member trained panel familiar with Iranian UF-Feta cheese. The cheese samples were tempered at room temperature for 1 h and then presented to the panellists randomly on plates labelled with randomised three number codes. Mineral water was provided to the panellists for mouth washing between cheese samples. The panel was asked to evaluate cheese attributes such as flavour, odour, texture, colour and appearance and overall acceptability by using a 7-point hedonic scale, with 1 = least desirable and 7 = most desirable.

Statistical analysis

Each experiment was triplicated using the prepared samples and the values reported are means of these replicates. The obtained results were subjected to analysis of variance (ANOVA) using SPSS 13 (SPSS Inc., Chicago, IL, USA) software. Mean values were compared using Tukey's test (P=0.05).

3. Results and discussion

Physicochemical properties during storage

The composition of UF-Feta cheese samples over 60 days of storage period at 8 and 12 °C is given in Table 2. The composition of the cheese sample met the Iranian UF-Feta cheese standard for the first quality cheese indicating that addition of probiotics and inulin did not adversely affect UF-Feta cheese composition. There were no significant (P>0.05) differences in the main compositional variables (except for dry matter) among different treatments. Due to addition of inulin, the cheese samples with higher amounts of inulin contained higher amounts of dry matter, compared to cheese samples with lower amount of inulin. The main compositional variables of the different cheese samples (dry matter, fat in dry matter, salt, salt in dry matter and protein) remained relatively constant (P>0.05) during storage period at 8 and 12 °C. However, an insignificant (P>0.05) increase in dry matter and a slight decrease in fat in dry matter was observed for all studied cheese samples. At the end of storage period, composition of all various cheese samples were similar and no significant (P>0.05) difference were observed.

The pH values of the cheese samples on day 1 were very similar and no significant differences (P>0.05) were observed among different treatments for pH (Table 3). However, by the end of the storage period, pH reduced significantly (P<0.05) at the both storage temperatures as the result of acid production by starter and probiotic bacteria.

The results showed that inulin addition to cheese did not affect (P>0.05) the pH but storage temperature had a significant (P<0.05) effect on this parameter, so that the pH of the cheese samples which were stored at 12 °C were significantly (P<0.05) lower than the samples stored at

Table 2. Changes in chemical composition (% w/w) and syneresis (% w/w) of ultrafiltered-Feta cheeses in the different treatments on storage (8 and 12 °C) for 60 days.[1]

Composition/ syneresis[2]	Storage period (day)	Treatment[3]					
		T1	T2	T3	T4	T5	T6
DM	1 day	37.4 ± 0.9^{cA}	37.5 ± 0.5^{bcA}	38.3 ± 0.9^{abA}	37.4 ± 0.6^{bcA}	37.6 ± 0.9^{bcA}	38.8 ± 1.1^{aA}
	60 days	38.1 ± 1.1^{abA}	38.0 ± 0.4^{bA}	38.6 ± 1.3^{abA}	38.0 ± 0.3^{bA}	38.2 ± 0.5^{abcA}	38.9 ± 0.4^{aA}
Salt	1 day	3.1 ± 0.1^{aA}	3.1 ± 0.0^{aA}	3.2 ± 0.0^{aA}	3.1 ± 0^{aA}	3.1 ± 0.1^{aA}	3.2 ± 0.0^{aA}
	60 days	3.1 ± 0.0^{aA}	3.2 ± 0.0^{aA}	3.2 ± 0.0^{aA}	3.1 ± 0.1^{aA}	3.1 ± 0.1^{aA}	3.1 ± 0.1^{aA}
SDM	1 day	8.3 ± 0.4^{aA}	8.3 ± 0.2^{aA}	8.3 ± 0.2^{aA}	8.4 ± 0.1^{aA}	8.1 ± 0.4^{aA}	8.1 ± 0.1^{aA}
	60 days	8.1 ± 0.1^{aA}	8.3 ± 0.1^{aA}	8.3 ± 0.2^{aA}	8.2 ± 0.2^{aA}	8.1 ± 0.1^{aA}	8.0 ± 0.2^{aA}
FDM	1 day	41.5 ± 1.0^{aA}	40.9 ± 2.1^{aA}	40.0 ± 1.7^{aA}	40.6 ± 1.4^{aA}	40.4 ± 1.5^{aA}	38.7 ± 1.1^{aA}
	60 days	39.9 ± 1.5^{aA}	39.5 ± 0.9^{aA}	39.7 ± 1.3^{aA}	40.8 ± 0.3^{aA}	39.7 ± 1.3^{aA}	39.4 ± 0.4^{aA}
Protein	1 day	13.7 ± 0.1^{aA}	13.7 ± 0.1^{aA}	13.6 ± 0.3^{aA}	13.8 ± 0.2^{aA}	13.9 ± 0.3^{aA}	13.8 ± 0.2^{aA}
	60 days	14.1 ± 0.2^{aA}	14.0 ± 0.2^{aA}	13.7 ± 0.2^{aA}	13.9 ± 0.3^{aA}	13.9 ± 0.2^{aA}	13.7 ± 0.1^{aA}
Syneresis	1 day	10.1 ± 2.8^{aA}	9.5 ± 2.9^{aA}	8.8 ± 1.1^{aA}	10.2 ± 2.4^{aA}	9.4 ± 1.4^{aA}	8.8 ± 1.5^{aA}
	60 days	11.8 ± 1.9^{aA}	10.7 ± 1.5^{aA}	9.9 ± 1.9^{aA}	12.1 ± 1.4^{aA}	10.5 ± 1.8^{aA}	10.1 ± 1.8^{aA}

[1] Mean±standard deviation with different capital letters in a column and small letters in a row differ significantly (P<0.05) for each parameter (n≥6).
[2] DM = dry matter; FDM = fat in dry matter; SDM = salt in dry matter.
[3] Refer to Table 1 for T1-T6.

8 °C (P<0.05) at day 60. At higher storage temperature, probiotic microorganisms and starter lactococci had higher metabolic activity, which probably contributed to the increased acidification and more intense pH reduction (Ong and Shah, 2009).

During storage time, the syneresis as the result of the drop in pH of cheese samples insignificantly increased slightly (Table 2). It has been reported that with the continuous increase in acidification of the product, the repulsive forces among casein micelles decrease, promoting their aggregation and, consequently, whey expulsion, thus characterizing the appearance of syneresis (Fox *et al.*, 2000; Fritzen-Freire *et al.*, 2010). This fact may explain the results obtained for the reduction in moisture content or increasing the dry matter content in our experimental cheese samples. Increase in inulin concentration from 0 to 1.5 and 3% w/w reduced the percentage of syneresis. However, the syneresis percentages among cheese samples with 0, 1.5 and 3% inulin were not significantly different (P>0.05). The syneresis was also not affected by levels of storage temperature used in this study.

Proteolysis in the treatments throughout the 60 days storage period is summarised in Table 3. The amounts of WSN and TCASN in all cheese samples progressively increased (P<0.05) during the storage. During 60 days storage period, no significant (P>0.05) differences were observed in the level of WSN among cheese samples with different concentrations of inulin. This result was expected, since primary proteolysis is mainly governed by the activity of chymosin, of plasmin, or of both (Visser 1993). Also, inulin concentration did not show any significant (P>0.05) influence on the TCASN which indicated inulin was not an effective factor on proteolytic activity of probiotic strains used in this study.

There were no significant (P>0.05) differences in the level of WSN and TCA-SN among cheese samples stored at different levels of temperature, indicating that storage temperature levels used in the present study had not significant influence (P>0.05) on the proteolysis indices. However, increase of ripening temperature from 8 to 12 °C had a positive effect on the proteolysis causing a greater increase in proteolysis indices which were not significant (P>0.05). The increase in the amount of soluble nitrogen by increasing ripening temperature was also reported by Jin and Park (1995). However, the used temperature levels in that study was higher than that used in the present study.

Survival of probiotic bacteria during storage

The changes in the viability of *L. acidophilus* la-5 and *B. lactis* BB-12 in the experimental cheese samples are presented in Table 4. The viable cell numbers of *L. acidophilus* la-5 (7.96-8.15 log cfu/g, at day 1) and *B. lactis* BB-12 (8.11-8.2 log cfu/g, at day 1) significantly decreased during 60 days storage period (P<0.05) and both strains showed a similar viable loss trend during this period with the biggest fall (0.89-1.41 log cfu/g) in the period of 15-30

Table 3. Changes in the values of pH, WSN/TN and TCASN/TN of ultrafiltered-Feta cheeses in the different treatments during storage (8 and 12 °C) for 60 days.[1]

pH/proteolytic index[2]	Storage period	Treatment[3]					
		T1	T2	T3	T4	T5	T6
pH	1 day	4.81±0.02^{aA}	4.80±0.02^{aA}	4.78±0.02^{aA}	4.81±0.04^{aA}	4.79±0.05^{aA}	4.79±0.05^{aA}
	15 days	4.73±0.01^{abB}	4.74±0.03^{aB}	4.71±0.04^{abB}	4.71±0.04^{abB}	4.70±0.04^{abB}	4.70±0.05^{abB}
	30 days	4.69±0.02^{abC}	4.71±0.02^{aBC}	4.68±0.02^{abcB}	4.66±0.03^{bcC}	4.65±0.02^{cdC}	4.62±0.03^{dC}
	45 days	4.65±0.03^{abD}	4.68±0.03^{aCD}	4.64±0.00^{bcC}	4.57±0.03^{eD}	4.61±0.02^{cdD}	4.59±0.03^{deCD}
	60 days	4.63±0.03^{aD}	4.65±0.02^{aD}	4.65±0.01^{aC}	4.58±0.05^{bD}	4.55±0.03^{bE}	4.56±0.02^{bD}
Proteolytic index (%)							
WSN/TN	1 day	7.12±0.33^{aE}	6.94±0.35^{aE}	7.15±0.39^{aE}	6.86±0.31^{aE}	6.87±0.17^{aE}	7.13±0.26^{aE}
	15 days	7.67±0.12^{aD}	7.62±0.29^{aD}	7.60±0.24^{aD}	7.54±0.22^{aD}	7.59±0.31^{aD}	7.61±0.41^{aD}
	30 days	8.23±0.16^{aC}	7.80±0.26^{aC}	7.85±0.12^{aC}	8.23±0.09^{aC}	8.05±0.29^{aC}	8.06±0.35^{aC}
	45 days	8.68±0.28^{aB}	8.36±0.34^{aB}	8.53±0.15^{aB}	8.82±0.27^{aB}	8.43±0.09^{aB}	8.76±0.42^{aB}
	60 days	9.13±0.33^{aA}	9.38±0.25^{aA}	8.98±0.23^{aA}	9.53±0.27^{aA}	9.23±0.13^{aA}	9.28±0.24^{aA}
TCASN/TN	1 day	4.55±0.22^{aE}	4.56±0.40^{aE}	4.48±0.35^{aE}	4.61±0.31^{aD}	4.55±0.34^{aE}	4.56±0.14^{aE}
	15 days	4.78±0.21^{aD}	5.06±0.19^{aD}	4.89±0.35^{aD}	5.23±0.39^{aC}	5.04±0.15^{aD}	4.92±0.06^{aD}
	30 days	5.43±0.32^{aC}	5.46±0.17^{aC}	5.56±0.53^{aC}	5.40±0.35^{aC}	5.42±0.15^{aC}	5.65±0.15^{aC}
	45 days	5.99±0.31^{aB}	5.72±0.16^{aB}	5.94±0.38^{aB}	6.11±0.34^{aB}	5.70±0.21^{aB}	6.12±0.41^{aB}
	60 days	6.88±0.40^{aA}	7.08±0.22^{aA}	6.78±0.04^{aA}	7.13±0.35^{aA}	7.06±0.35^{aA}	6.99±0.13^{aA}

[1] Mean±standard deviation with different capital letters in a column and small letters in a row differ significantly (P<0.05) for each parameter (n≥6).
[2] TN = total nitrogen; TCASN = trichloroacetic acid soluble nitrogen; WSN = water soluble nitrogen.
[3] Refer to Table 1 for T1-T6.

Table 4. Changes in viable count (log cfu/g) of probiotic bacteria of ultrafiltered-Feta cheeses in the different treatments during storage (8 and 12 °C) for 60 days.[1]

Probiotic organism	Storage period	Treatment[2]					
		T1	T2	T3	T4	T5	T6
Lactobacillus acidophilus la-5	1 day	8.01±0.32^{aA}	7.96±0.34^{aA}	8.10±0.27^{aA}	8.12±0.35^{aA}	8.05±0.23^{aA}	8.15±0.24^{aA}
	15 days	7.81±0.31^{aA}	8.03±0.46^{aA}	7.81±0.29^{aA}	7.80±0.29^{aB}	7.92±0.27^{aA}	7.85±0.30^{aB}
	30 days	6.70±0.27^{aB}	6.62±0.24^{aB}	6.57±0.34^{aB}	6.62±0.33^{aC}	6.60±0.36^{aB}	6.71±0.38^{aC}
	45 days	6.45±0.24^{aBC}	6.33±0.26^{aBC}	6.51±0.22^{aB}	6.20±0.09^{aD}	6.25±0.16^{aC}	6.41±0.22^{aD}
	60 days	6.37±0.20^{aC}	6.31±0.25^{aC}	6.46±0.27^{aB}	6.22±0.15^{aD}	6.15±0.15^{aC}	6.20±0.17^{aD}
Bifidobacterium lactis BB-12	1 day	8.11±0.19^{aA}	8.15±0.23^{aA}	8.12±0.28^{aA}	8.20±0.13^{aA}	8.17±0.23^{aA}	8.19±0.23^{aA}
	15 days	7.70±0.28^{aB}	7.81±0.35^{aB}	7.80±0.23^{aB}	7.60±0.16^{aB}	7.62±0.18^{aB}	7.58±0.27^{aB}
	30 days	6.50±0.32^{aC}	6.65±0.26^{aC}	6.65±0.25^{aC}	6.40±0.27^{aC}	6.50±0.23^{aC}	6.69±0.40^{aC}
	45 days	6.40±0.15^{aC}	6.36±0.28^{aCD}	6.48±0.15^{aC}	6.20±0.09^{aC}	6.31±0.15^{aCD}	6.29±0.13^{aD}
	60 days	6.30±0.14^{aC}	6.28±0.22^{aD}	6.39±0.09^{aC}	6.11±0.09^{aC}	6.17±0.19^{aD}	6.29±0.12^{aD}

[1] Mean±standard deviation with different capital letters in a column and small letters in a row differ significantly (P<0.05) for each parameter (n≥6).
[2] Refer to Table 1 for T1-T6.

days. After 30 days probiotic counts of cheese samples remained almost stable up to the 60th days with an amount of 6.11 to 6.46 log cfu/g which was the end of storage period. At the day 60, viable counts of probiotic bacteria among all treatments did not differ significantly (P>0.05). As can be seen in Table 4, viable counts of both strains throughout storage period were always above recommended threshold level (>6 log cfu/g) for probiotic products (Shah, 2000).

Poor and good survival of probiotics in various cheese samples are reported by several authors. For example, Mc Brearty *et al.* (2001) in an attempt to develop probiotic Cheddar cheese, found that *B. lactis* BB-12 survived at high numbers (8 log cfu/g), while numbers of *Bifidobacterium longum* BB536 were reduced to 5 log cfu/g cheese, following six months of ripening.

In the current study, no significant (P>0.05) differences were observed between the probiotic counts in the different cheese samples made by different inulin concentration. Therefore, inulin addition had no significant (P>0.05) influence on the viability of the probiotic strains. The present results are in accordance with the data made by Buriti *et al.* (2007b) who reported that the presence of inulin in fresh cream cheese had no implications on the growth and viability of *Lactobacillus paracasei* during storage period. Similarly, Cardarelli *et al.* (2008) reported, that the presence of prebiotics in symbiotic petit-suisse cheese lead to a slight or absent reduction in loss of viable counts of *B. animalis* subsp. *lactis* and *L. acidophilus*, compared to the control batches during 28 days storage.

Contrary to these results, some studies indicated that inulin-type prebiotics may have positive effect on the survival of probiotic bacteria in yoghurt and milk supplemented by these compounds during the storage (Buriti *et al.*, 2007b; Cardarelli *et al.*, 2008). Özer *et al.* (2005) compared yogurt samples with added inulin at the levels of 0.5 and 1.0% with control and found that inulin had no effect on the growth of yogurt starter culture, but stimulated the growth of *B. bifidum* BB-02 to a great extent. Similar conclusions were obtained by Capela *et al.* (2006) and Desai *et al.* (2004) that the addition of prebiotics improved viability of probiotic bacteria. There is some evidence that the addition of prebiotics can improve survival of probiotics during the storage of probiotic-containing foods, specially fermented milk products, but the mechanism of effect are unclear. Hypothesis can be made that obtained beneficial results may be due to a partial fermentation of the carbohydrate during storage time. Results may depend upon the choice of combination of pro-and pre-biotics, their dose, as well as the food matrix into which they are incorporated.

The increase of ripening temperature from 8 to 12 °C did not effect on the viability of the probiotic strains studied. However, the probiotic populations in cheese samples stored at 12 °C were always slightly lower than that stored at 8 °C. Our finding is in agreement with the results obtained by Ong and Shah (2009) who reported that viability of six probitic strains in Cheddar cheese was not affected by the increasing the ripening temperature (from 4 to 8 °C). The present results show that UF-Feta cheese (with or without the addition of inulin) can be a good vehicle for the delivery of *L. acidophilus* la-5 and *B. lactis* BB-12.

Sensory analysis

The effect of storage time, storage temperature and inulin concentration on flavour, odour, texture, colour and appearance and overall acceptability of the cheese samples are shown in Table 5. Storage time had a significant (P<0.05) effect on the sensory scores of the cheese samples, so that the level of flavour, odour and overall acceptability increased significantly (P<0.05) as storage progressed. This increase could be due to the development of flavour components as a result of ripening process. Our finding is in agreement with the result obtained by McBreaty *et al.* (2001) who observed more extensive proteolysis and improved flavour in *B. lactis* BB-12 cheese during the storage.

Keeping at 12 °C as compared to 8 °C also slightly increased (not significantly; P>0.05) the level of flavour, odour and overall acceptability of the cheese samples. This result was expected as in higher temperature the reactions involved in cheese maturation are accelerated and more flavour and taste components will be produced as storage in higher temperature is considered as a tool for faster maturation of cheese.

Addition of inulin did not affect the flavour intensity, odour and overall acceptability, texture and appearance of the cheese samples. There were no significant (P>0.05) differences in the colour and appearance of cheese at the end of storage period as inulin in solution format is a clear liquid with no odour, flavour and colour. Additionally, inclusion of inulin into cheese had no effect on the activities of the responsible strains for flavour and taste. The present results indicate that incorporation of such probiotics into UF cheese could be resulted in a good quality cheese with the increased acceptability during storage time.

4. Conclusions

L. acidophilus la-5 and *B. lactis* BB-12 could be successfully used to manufacture of probiotic UF-Feta cheese as these probiotic bacteria survived well (>10^6 cfu/g) by the end of storage period and did not impair the composition of UF-Feta cheese. Generally, storage time was the most effective factor on the viability, pH, WSN, TCASN, flavour, odour and overall acceptability. It can be concluded that the viability of both probiotic strains was not significantly (P>0.05) affected by the inulin concentration and storage

Table 5. Changes in the sensory attributes of ultrafiltered-Feta cheeses in the different treatments during storage (8 and 12 °C) for 60 days.[1]

Sensory attributes	Storage period	Treatment[2,3]					
		T1	T2	T3	T4	T5	T6
Flavour	1 day	4.90±0.52^{aB}	4.80±0.26^{aB}	4.70±0.54^{aB}	4.70±0.54^{aB}	5.00±0.47^{aB}	4.80±0.59^{aB}
	60 days	5.70±0.48^{aA}	5.60±0.52^{aA}	5.80±0.42^{aA}	5.80±0.59^{aA}	5.60±0.52^{aA}	5.97±0.46^{aA}
Texture	1 day	5.30±0.54^{aA}	5.30±0.35^{aA}	4.90±0.46^{aA}	5.20±0.42^{aA}	5.10±0.51^{aA}	5.50±0.33^{aA}
	60 days	5.10±0.52^{aA}	5.00±0.33^{aA}	5.30±0.42^{aA}	5.20±0.48^{aA}	5.40±0.46^{aA}	5.30±0.59^{aA}
Odour	1 day	4.80±0.63^{aB}	4.70±0.63^{aB}	4.70±0.42^{aB}	4.60±0.32^{aB}	4.80±0.54^{aB}	4.60±0.46^{aB}
	60 days	5.57±0.67^{aA}	5.67±0.52^{aA}	5.67±0.62^{aA}	5.87±0.67^{aA}	6.00±0.62^{aA}	5.70±0.48^{aA}
Colour/appearance	1 day	6.00±0.53^{aA}	6.20±0.48^{aA}	6.40±0.46^{aA}	6.20±0.26^{aA}	6.20±0.35^{aA}	6.10±0.33^{aA}
	60 days	6.10±0.46^{aA}	6.10±0.39^{aA}	6.20±0.43^{aA}	6.30±0.35^{aA}	6.00±0.33^{aA}	6.20±0.26^{aA}
Overall	1 day	4.70±0.48^{aB}	4.50±0.41^{aB}	4.90±0.39^{aB}	4.80±0.42^{aB}	5.00±0.41^{aB}	4.50±0.41^{aB}
	60 days	5.70±0.48^{aA}	5.60±0.65^{aA}	5.80±0.54^{aA}	5.90±0.39^{aA}	6.00±0.47^{aA}	5.80±0.42^{aA}

[1] Mean±standard deviation with different capital letters in a column and small letters in a row differ significantly (P<0.05) for each parameter (n≥6).
[2] Scores: 1 = least desirable and 7 = most desirable.
[3] Refer to Table 1 for T1-T6.

temperature. Since the probiotic UF-Feta cheese, without any alternation can be manufactured by using industrial protocol, the obtained probiotic cheese is attractive for commercial purposes as cheese with higher numeration of probiotic bacteria. The present study confirms that Iranian UF-Feta cheese can be a good carrier for *L. acidophilus* la-5 and *B. lactis* BB-12. Future direction will focus on production of different probiotic cheeses using other bacterial strains. Investigation of the profile of aromatic components of probiotic cheese during the storage time to find optimum condition of the production is necessary.

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