African Journal of Biotechnology Vol. ~~x~~10(~~xx~~69), pp. ~~xxx~~15600-~~xxx~~15606, ~~xx~~7 ~~xxxxx~~November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1826
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

~~Accepted 5 September, 2011~~

~~Effect of B.*lactis* on Some Physico-Chemical and Organoleptical Properties of Lighvan Cheese~~

Effect of *Bifidobacterium lactis* on some physico-chemical and organoleptical properties of Lighvan cheese

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Accepted 5 September, 2011

~~ASummary~~BSTRACT

The aim of this study was to determine survival of Bifidobacterium~~B.~~ lactis and its effect on some physico-chemical properties during the ripening of Lighvan cheese. Lighvan cheese from ewe's and goat's milk was produced according to a traditional protocol, and with the addition of 9.0 log_{10} cfu of fresh cells per gram of *~~Bifidobacterium~~* *B. lactis*. The effect of time on survival of *B. lactis* during the ripening of probiotic Lighvan cheese was significant (*p<0.01*). *B. lactis* cells survived in cheese samples at concentrations up to 6.84 log_{10} cfu/g for at least 60 days of storage time. ~~In~~ Parallel to the variation in pH values, titratable acidity values increased until the 60th day of ripening. Salt content increased continuously until the end of the ripening period. The addition of *B. lactis* did not have a significant (*p>0.01*) effect on the chemical composition and sensory characteristics of the traditional Lighvan cheese.

Key words: Probiotic, Lighvan~~,~~ cheese, Bifidobacterium lactis.

1.

INTRODUCTION

Maintaining the viability of bifidobacteria in dairy products over a long time at refrigeration temperatures is still a problem with most fermented products. Antagonistic effect of antimicrobial substances such as bacteriocines, organic acids, and protease produced by starter cultures decrease viability of probiotic strains. onOn the other hands, no report was found on effect of salt concentration on viability of *Bifidobacterium lactis*. The sensitivity of bifidobacteria to the antagonistic growth of starter lactic-acid bacteria and, especially, to low pH, limits their viability (Daigle et al., 1999). Cheese can offer certain advantages in delivering probiotics to the gastrointestinal tract, the target organ. Since cheese generally possesses a higher pH than fermented milk products and therefore provide a more stable environment, it can result in a long term survival of probiotic bacteria. Furthermore, the terms survival of probiotic bacteria. Furthermore, the matrix and high fat content of cheese may protect the organisms during passage through the gastrointestinal tract (Dinakar and Mistry, 1994; Gomes et al., 1995; Stanton et al., 1998).

The incorporation of cultures with beneficial effects into a functional food is successful when the cultures maintain viability until being consumed, and if the added cultures do not adversely affect the product's composition, texture or sensory features. Results from cheeses used as functional foods are limited, and those reported are contradictory (Corbo et al., 2001).

Traditional Lighvan, a semi hard cheese with a large market demand, is one of the most popular traditional cheese in Iran. It is mostly produced from ewe's or goat's milk, or a mixture of the two. Traditional Lighvan cheese which ripened in brine is a major component especially in the diet of consumers in north-west of the country. Some efforts have been done made to produce Lighvan in cheese plant using pasteurized milk. The ripening period of this

plant using pasteurized milk. The ripening period of this type of product is about 90 days but the cheeses made from raw milk in small, rural production units may be ripened for six to eight months. Cheese ripening is a complex process which its main characteristic is proteolysis involving different enzymes such as chymosin from rennet, plasmin and the microbial enzymes. This population is affected mainly by brine concentration and pH, (Alais, 1984; Desnouveaux et al., 1985; Furtado and Partidge, 1988; Bhowmik and Marth, 1990; Law et al., 1992). Incorporating bifidobacteria in cheese through the cheese milk is not difficult, as cheese offers the necessary anaerobic conditions and a suitable pH. In this work, It was tried to produce traditional cheese supplemented with B. *lactis* was produced and study the survival of probiotic bacteria and eventual changes of physico-chemical characteristics of the traditional cheese was studied.

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2.

MATERIALS AND METHODS

2.1.

Bifidobacterial cultures

A lyophilised culture of *B. lactis* was supplied by CAMINOX, Spain. The manufacturer claims in its technical literature that this strain has probiotic effects. B. *lactis* was cultured in MRS broth (Merck, Germany), under anarobiosis at 37 °°C for 24 hours. Cells as seed cultures were harvested by centrifugation (SANYO, MISTRAL, Germany) at 10,000 × g for 10 minutes, washed twice with sterile skim milk, and resuspended in cheese-milk at a concentration of 9.0 $\log_{10}$cfu/ml for *B. lactis*.

Ewe's milk

Milk warmed to 36 °C

†Inoculum of lyophilised starters (*Mesophilic and Thermophilic starters 4 to 3 proportionally,1% of milk weight)

‡Inoculum of *Bifidobacterium lactis* 9.0 $\log_{10}$ cfu/ml

pH=6.3

Fungal rennet added (2.5gr/100 kg of milk)

Curd cut (size of the curd cubes 1cm^3)

Curds placed on cotton cloth

Whey removed, molded and pressed

Curd cut (size of the curd 10×10×7 cm)

Curd held at 36 °C for 2 hours

Brine kept at 22% for 6 hours at room temperature

Curd placed in brine 12%

Cheese ripened at about 8 °C for 60 days

†So the ratio of the lyophilized starter to the cheese milk was 1 % w/w.

‡The concentration of *B. lactis* in the cheese milk was always 9.0 $\log_{10}$ cfu/ml

*Mesophilic culture, supplied by Chr. Hansen, contained *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. lactis*, *Leuconostoc mesenteroides subsp. cremoris* and *Lactococcus lactis subsp. lactis biovar diacetylactis*. The thermophilic starter contained *Streptococcus thermophillus* and *Lactobacillus bulgaricus*.

Figure 1. Protocol for the Production of Probiotic Lighvan Cheese

2.2. Cheese manufacture

The different steps of cheese manufacture are summarized in the Figure 1. Ewe's milk was supplied from an animal husbandry in

Varamin from the Zandy breed. Experimental cheese samples were made in three replications at the Tehran Pegah dairy plant (Tehran, Iran). Lighvan cheese was produced using raw milk according to the above earlier mentioned protocol. The raw milk was coagulated with fungal rennet for 60 minutes; after curdling, the curd was cut into small cubes, approximately 1 cm^3, and left to rest (15 minutes). The pieces of curds after cutting were 10 × 10 × 7 cm. These pieces were immersed into brine with 22% concentration for 6 hours at room temperature and then, placed into tin-plate containers with brine at about 12% concentration. The containers were sealed and stored for 60 days.

2.3.

Microbiological analysis

10Ten grams10 g of cheese was first diluted in 90 ml of 2% sodium citrate solution and homogenized in a Stomacher Lab-Blender 400 (Seward, England) for 1 minute. Subsequent serial dilutions were made in Ringer's solution and plated on specific media for viable counts. DE MAN, ROGOSA, SHARPE (MRS agar) (Merck, Germany) was modified with L-cysteine. HCl (0.05%) for reducing the Red/ox potential and 60 mg of lithium mupirocin (Li-MUP) (Sigma-Aldrich, USA) for its inhibitory effect (Heidarpour et al., (2008). Cultivation was carried out using the pour-plate technique, and the plates were incubated, under anaerobiosis, for 72 hours at 37 °C for *B. lactis*. The *B. lactis* in the cheese samples was enumerated after 5, 15, 25, 35, 45 and 60 days. The characterizations of probiotic Lighvan cheese were compared with traditional Lighvan

Lavasani et al. 15601 cheese (without B. *lactis*) as a blank. Each experiment was done in

cheese (without B. *lactis*) as a blank. Each experiment was done in

cheese (without B. *lactis*) as a blank. Each experiment was done in triplicate.

2.4.

Chemical analysis

Samples of cheese were analyzed for pH using (Metrohm, Model 632 pH-meter, Swiss), and also titratable acidity, total solids, total fat and salt were analyzed according to the AOAC method (1990).

2.5.

Organoleptic assessment

Organoleptic assessment of the cheese after 5, 25, 45 and 60 days of ripening was carried out by a five-member panel selected on the basis of interest in sensory evaluation of traditional Lighvan cheese. The samples were presented to panelists in randomisedrandomized order after storage for 2 hours at room temperature, and were graded between 1 and 10 (1 being very bad and 10 being very

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good) for appearance, and body, and texture, colour, flavour and acceptability (Larmond, 1987). (Larmond, 1987). Panel members were also instructed to report any defects in appearance, body and texture, flavour and acceptability. Water was provided for mouth-washing between evaluations of samples.

2.6.

Statistical analysis

The data were statistically analyzed using a completely randomized design (CRD) with three replications. Data were subjected to analysis of variance using the SAS statistical software package (SAS, 1995). Means comparison was performed with LSD's test at the p<0.01 level of significance.

3.

RESULTS

3.1

Enumeration of *Bifidobacterium* B. *lactis*

Table 1 shows the number of cells of B. *lactis* during ripening of probiotic Lighvan cheese. Different culture media have been reported for the selective enumeration of bifidobacteria in dairy products (Payne et al., 1999). The traditional technology was modified slightly to favour the survival of probiotic microorganism. mMRS agar was used to obtain the highest survival of the bacteria and inhibition effect of this media to preserve the microbial population is at the highest level. According to statistical analysis, significance differences (p<0.01) were observed in the enumeration of *B. lactis* during 60 days of ripening. After 25 days, the cheese contained ca. 8.0 log_{10} cfu/g of bifidobacterium, and after 60 days of ripening, the survival of B. *lactis* was 6.84 log_{10} cfu/g.

3.2. Compositional and physicochemical properties

Table 2 presents the chemical composition of probiotic and traditional Lighvan cheeses at different ripening times. According to statistical analysis, no significant (p>0.01) differences were was observed in the main chemical composition (pH and percent titratable acidity, total solids, total fat and salt) between probiotic and traditional traditional Lighvan cheese. Titratable acidity of the samples increased until the 60th day of ripening, while pH values

Ewe's milk

↓

Milk warmed to 36°C

↓

[†]Inoculum of lyophilised starters ([•]Mesophilic and Thermophilic starters 4 to 3 proportionally, 1% of milk weight)

‡Inoculum of *Bifidobacterium lactis* 9.0 $\log_{10}$ cfu/ml

↓

pH = 6.3

↓

Fungal rennet added (2.5 g/100 kg of milk)

↓

Curd cut (size of the curd cubes 1cm^3)

↓

Curds placed on cotton cloth

↓

Whey removed, molded and pressed

↓

Curd cut (size of the curd 10 × 10 × 7 cm)

↓

Curd held at 36°C for 2 h

↓

Brine kept at 22% for 6 h at room temperature

↓

Curd placed in brine 12%

↓

Cheese ripened at about 8°C for 60 days

Figure 1. Protocol for the production of probiotic Lighvan cheese. [†]The ratio of the lyophilized starter to the cheese milk was 1% (w/w); [‡]the concentration of *B. lactis* in the cheese milk was 9.0 $\log_{10}$ cfu/ml; [•]mesophilic culture, supplied by Chr. Hansen, contained Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Leuconostoc mesenteroides subsp. cremoris and Lactococcus lactis subsp. lactis biovar diacetylactis. The thermophilic starter contained Streptococcus thermophillus and Lactobacillus bulgaricus.

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Table 1. Survival of *B. lactis* in Lighvan Cheese[†] during storage.

Frequency of sampling days for counting (day)	The number of *B. lactis* (10^9/cfu/ml)
1	9
5	8.75
15	8.69
25	8.09
35	7.64
45	7.02
60	6.84

[†]Means of each parameter in the same column without a superscript differ significantly (p<0.01).

Table 2. Physicochemical composition of probiotic and traditional Lighvan cheese[†‡].

Cheese type[a,b]	Ripening period (days)	Total solid (%)	Fat (%)	Salt (%)	Acidity (%)	pH
RPC	5	48.58±2.5^{c}	20.1±0.7^{c}	3.2±0.1^{c}	2.21±0.05^{c}	5.16±0.12^{c}
RC		48.66±2.7^{c}	20.19±0.9^{c}	3.24±0.1^{c}	2.16±0.04^{c}	5.3±0.14^{c}
RPC	25	48.29±2.2^{d}	19.92±0.8^{d}	3.41±1.0^{d}	2.218±0.04^{d}	5.06±0.03^{d}
RC		48.35±2.4^{d}	20.02±0.8^{d}	3.58±1.1^{d}	2.19±0.04^{d}	5.12±0.03^{d}
RPC	45	46.52±2.0^{e}	17.25±1.5^{e}	3.46±1.2^{e}	2.227±0.06^{e}	4.89±0.02^{e}
RC		46.61±2.1^{e}	18.52±1.6^{e}	3.62±1.2^{e}	2.21±0.05^{e}	5.03±0.03^{e}
RPC	60	46.12±1.3^{f}	17.21±1.6^{f}	3.5±0.4^{f}	2.27±0.02^{f}	4.78±0.03^{f}
RC		46.25±1.1^{f}	17.27±1.7^{f}	3.68±0.6^{f}	2.268±0.01^{f}	4.87±0.03^{f}

[†]Means of each parameter in the same column with a superscript differ significantly (p<0.01); [‡]mean values ± standard deviation of three trials; [a] RPC, raw probiotic cheese (probiotic Lighvan cheese); [b]RC, raw cheese (traditional Lighvan cheese).

increased until the 60th day of ripening, while pH values Lighvan cheese. Titratable acidity of the samples increased until the 60th day of ripening, while pH values decreased. The acidities (%) of probiotic and traditional Lighvan cheeses were 2.21% and 2.16%, respectively at the day 5 of storage, and 2.27% and 2.268% at the day 60 of storage. The pH of probiotic and traditional Lighvan cheeses was 5.16 and 5.3, respectively, at the day 5th of storage, and 4.78 and 4.87 at the day 60 of storage.

The total solids (%) of probiotic and traditional Lighvan cheeses was were 48.58% and 48.66%, respectively, at the fifth day of storage, and 46.12% and 46.25% at the 60th day of storage. There were no significant (p>0.01) differences between probiotic and traditional Lighvan cheeses. The total fat (%) of probiotic and traditional Lighvan cheeses was 20.1% and 20.19%, respectively, at the fifth day of storage, and 17.21% and 17.27% at the 60th day of storage. Total fat decreased gradually during ripening, with no significant (p>0.01) differences between probiotic and traditional Lighvan cheeses. The salt (%) in probiotic and traditional Lighvan cheeses was 3.2% and 3.24%, respectively, at the fifth day of storage, and 3.5% and 3.68% at the 60th day of storage. A marked increase was observed in salt content after 60 days of ripening. There were no significant (p<0.01) differences between probiotic and traditional Lighvan cheeses.

3.3. Organoleptic assessment

Table 3 shows the results of the sensory panel's assessment of cheese quality after ripening for 5, 25, 45 and 60 days. The mean value of appearance and colour for probiotic and traditional Lighvan cheeses was 7.6 and 7.3, respectively, at the fifth day of storage, and 8.6 and 8.4 at the 60th day of storage. The appearance and colour of the experimental cheese was considered good and did not have a significant difference (p>0.01) throughout the ripening period. The mean value of body and texture for probitic and traditional Lighvan cheeses was 7.01 and 7.00, respectively, at the fifth day of storage, and 8.0 and 7.8 at the 60th day of storage. The body and texture scores of the probiotic and traditional

Table 3. Sensorial scores of probiotic and traditional Lighvan Cheese[†‡].

Cheese type[a,b]	Ripening period (days)	Appearance and colour	Body and texture	Flavour	Acceptability
RPC	5	7.6±0.89	7.01±1.30	7.2±1.00	7.1±0.45
RC		7.3±0.55	7±0.67	7.0±0.85	7±0.68
RPC	25	7.9±1.23	7.4±0.90	7.5±0.84	7.3±0.89
RC		7.7±1.25	7.2±1.1	7.3±1.29	7.1±0.6
RPC	45	8.2±1.34	7.6±0.97	7.9±0.88	7.8±0.91
RC		8±1.30	7.5±0.87	7.7±1.52	7.6±0.74
RPC	60	8.6±1.64	8±1.79	8.5±0.45	8.5±0.71
RC		8.4±1.56	7.8±0.55	8.3±1.32	8.3±0.82

[†] Means of each parameter in the same row without a superscript do not differ significantly (p>0.01); [‡]mean values ± standard deviation of three trials; [a]RPC, raw probiotic cheese (probiotic Lighvan cheese); [b]RC, raw cheese (traditional Lighvan cheese).

body and texture scores of the probiotic and traditional Lighvan cheeses did not differ significantly (p>0.01). The mean value of flavour for probitic and traditional Lighvan cheeses was 7.2 and 7.0, respectively, at the fifth day of storage, and 8.5 and 8.3 at the 60th day of storage. The flavour scores of probiotic and traditional Lighvan cheeses did not differ significantly (p>0.01). The mean value of acceptability for probitic and traditional Lighvan cheeses was 7.1 and 7.0, respectively, at the fifth day of storage, and 8.5 and 8.3 at the 60th day of storage. The acceptability scores of probiotic and traditional Lighvan cheeses did not differ significantly (p>0.01). Appearance and colour, body and texture, flavour and acceptability scores generally increased during the ripening period.

~~4.~~

DISCUSSION

~~4.1.~~

Microbiological characteristics

After comparison of several selective media for isolation and enumeration of *B. lactis*, under the conditions in this study, mMRS agar modified with L-cysteine HCl (0.05%) and lithium mupirocin was the best for cell count of *B. lactis*. ~~Because~~ Mupirocin susceptibility showed that bifidobacteria ~~were~~ was consistently resistant to mupirocin, whereas all Lactobacilli were susceptible. *B. lactis* bacteria decreased slightly throughout cheese ripening: a fall of only ca. 2.0 $\log_{10}$ cfu/g during the 60 days (Fig~~.~~ure 2). The minimum concentration of probiotic microorganisms that must be ~~contained~~ present in a food product to exert a beneficial effect is unclear. The Fermented Milks and Lactic Acid Bacteria Beverages Association in Japan introduced a standard that stipulates that the minimum concentration of viable bifidobacteria per gram or milliliter of product defined as a probiotic food should be at least 7.0 $\log_{10}$ cells. This concentration should ensure the therapeutic minimum dose of 5.0 $\log_{10}$ viable cells/g or ml of product. Other international food associations and results from several studies have proposed that the concentration should range between 6.0 ~~to~~ and 7.0 $\log_{10}$ cfu/g or ml (Gardiner et al. ~~1999~~1998). Intrinsic characteristics of the Lighvan cheese matrix (low a_w and pH, high concentration of NaCl) could have caused severe cellular stress that reduced cell recovery. When added individually, *B. lactis* showed a significant (p<0.01) decrease during 60 days of ripening. Similar to our result, Ketney et al. (2008) reported that bifidobacteria had a satisfactory viability in the Feta cheese during 60 days of refrigerated storage, also Corbo et al. (2001) reported that after 56 days of ripening of Canestrato Pugliese Hard cheese supplemented with bifidobacteria, the survival of bifidobacteria was 6.0 $\log_{10}$ cfu/g .The use of bifidobacteria as a starter adjunct to produce probiotic cheeses was recently ~~applied~~done. Not all the strains exhibited the same stability during ripening and storage of the dairy products (Blanchette et al. 1996; Dinakar and Mistry, 1994; Ghoddusi and Robinson, 1996; Gobbetti et al. 1998; Gomes et al. 1995; Roy et al. 1997), suggesting that strain survival should be evaluated individually prior to commercial use. Indeed, in cottage cheese, *Bifidobacterium* ~~B.~~ *infantis* reached levels of approximately 7.0 $\log_{10}$ cfu/g of cheese after 1 day of storage, but large viability losses were observed after 15 days ~~at~~ at 4 °C (Blanchette et al. 1996). Other reports ~~showed that bifidobacteria added in to Cheddar (Dinakar~~

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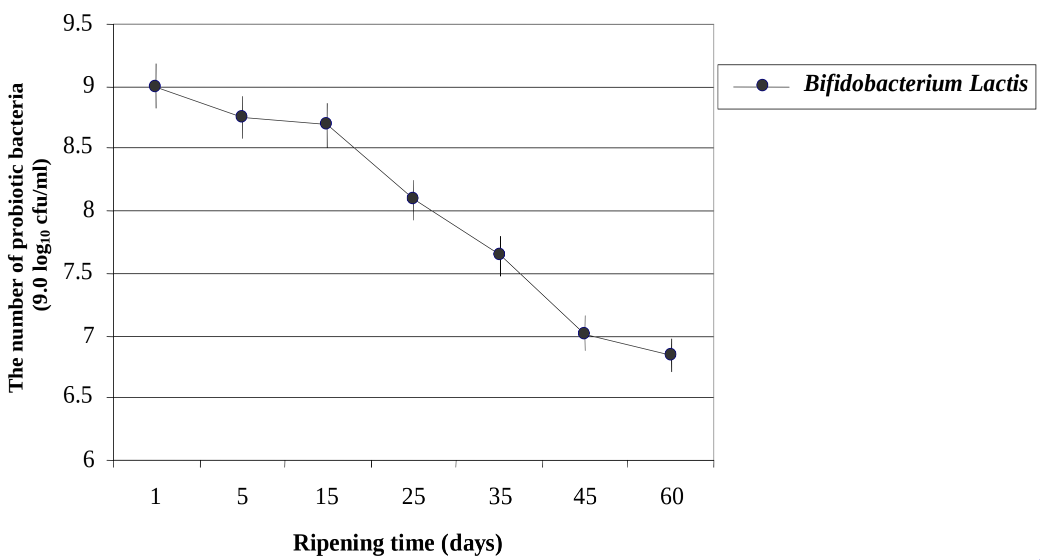

Figure 2. Survival of *B. lactis* colonies in Cheese during storage.

showed that bifidobacteria added to Cheddar (Dinakar and Mistry, 1994) or Cheddar-like cheese (Daigle et al., 1999) survived up to 24 weeks at approximately 7.3 log_{10} cfu/g, or remained above 6.5 log_{10} cfu/g.

~~4.2.~~

Compositional and physicochemical properties

The cheese acidity at a certain moment of the technological process is determined by the starting level of milk acidity and the lactic acid generated by the presence of the starter culture. The cheese acidity level has great importance, influencing the growth of microorganisms and enzymatic activity throughout the maturation process, as well as affecting rheological properties and flavour (Watkinson et al., 2001; Pappa et al., 2007). The increase in titratable acidity during the 60 days of ripening in brine was due mainly to the near completion of lactose fermentation and the liberation of amino and free fatty acids following proteolysis and lipolysis. Similar to our results, Azarnia et al. (2006) reported that lactose is converted into lactic acid during cheese-making by the starter culture. Therefore, lactic acid is the most abundant organic acid in all types of cheese (Izco et al., 2002). Similar to our results, Tarakci and Kucukoner (2006) reported that titratable acidity increased during ripening; these results also agree with those reported by Sameen et al. (2010). The titratable acidity of probiotic Lighvan cheese was slightly greater than traditional Lighvan cheese. The insignificant increase in titratable acidity of probiotic Lighvan cheese was attributed to the low proteolytic activity of the probiotic organism.

Decreases in total solid content of ~~probiotic and~~

~~Decreases in total solid content of probiotic and traditional~~ probiotic and tradi-tional Lighvan cheeses throughout ripening, generally originate from water-soluble proteins and peptides passing from the cheese matrix to the brine; this decrease may be due mainly to the breaking of peptide bonds and the release of new ionic groups. Creamer and Olson (1982) and Atasoy et al. (2003) reported that the total solid content of Urfa cheese decreased throughout storage as a result of extended proteolysis. In this research, the total solid content of the probiotic Lighvan cheese was slightly less than traditional Lighvan cheese. This may be related to differences between proteolytic activity of probiotic and traditional Lighvan cheese. Subsequent to the breaking of peptide bonds and the release of new ionic groups, probiotic Lighvan cheeses contain, in addition to chymosin, plasmin and proteinases contamination bacteria, and proteolytic enzymes related to probiotic microorganism; this leads to the breaking of peptide bonds.

Changes in fat content during storage could be due to a decrease in total solids and lipolysis. The fat content

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decreased in probiotic and traditional Lighvan cheese during storage but we did not observed any significant changes between probiotic and traditional Lighvan cheese. The salt (NaCl) penetration into the cheese was much faster during the early stage of storage than it had been during ripening. Salt is driven into cheese by the concentration gradient between the cheese blocks and and brine; this

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gradient is much larger at the beginning of ripening gradient is much larger at the beginning of ripening (Azarnia et al., 1997). Increase in salt content during ripening could be attributed to higher water content, as salt penetrates the cheese matrix in water.

4.3.

Organoleptic assessment

Appearance and colour, body and texture, flavour and acceptability scores generally increased during ripening. As regards to acceptability scores, panel members' members' pre-ferred ripen probiotic Lighvan cheeses over unripened. Perceiving Identifying its taste, texture, colour and appearance was better.

5. Conclusions

The production of functional cheese products was recently proposed as a suitable and promising alternative to fermented milks (Stanton et al., 1998), because cheese could offer certain advantages as a carrier of probiotic microorganisms. Semi hard Lighvan cheese has intrinsic features (pH, moisture and a_w) that may characterisecharacterize it as hostileunreceptive for microorganisms. However, the results of this study demonstrated that traditional Lighvan cheese proved to be an appropriate probiotic delivery vehicle for *B. lactis*. In particular, *B. lactis* cells survived in cheese at concentrations up to 6.84 log_{10} cfu/g for at least 60 days of ripening. *B. lactis* did not affect the chemical composition and sensory characteristics of the traditional Lighvan cheese. Besides meeting precise consumer demand, the production of functional or probiotic cheeses may be useful for differentiating and increasing the market popularity of various Iranian cheeses such as traditional Lighvan, which still has a strict regional tradition. If eaten daily, probiotic Lighvan cheese can be considered as a probiotic vector or as an additional variety supporting other probiotic foods that are eaten daily.

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Table 1. Survival of *B. lactis* in Lighvan Cheese[‡] during storage.

Frequency of sampling days for counting (day)	**The number of *B. lactis* (10^9/cfu/ml)**
1	9
5	8.75
15	8.69
25	8.09
35	7.64
45	7.02
60	6.84

[‡]Means of each parameter in the same column without a superscript differ significantly (p<0.01).

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Table 2. Physicochemical composition of probiotic and traditional Lighvan cheese[†‡]

Cheese type[a,b]	Ripening period (days)	Total solids (%)	Fat (%)	Salt (%)	Acidity (%)	pH
RPC		48.58±2.5[c]	20.1±0.7[c]	3.2±0.1[c]	2.21±0.05[c]	5.16±0.12[c]
RC	5	48.66±2.7[c]	20.19±0.9[c]	3.24±0.1[c]	2.16±0.04[c]	5.3±0.14[c]
RC		48.66±2.7[c]	20.19±0.9[c]	3.24±0.1[c]	2.16±0.04[c]	5.3±0.14[c]
RPC		48.29±2.2[d]	19.92±0.8[d]	3.41±1.0[d]	2.218±0.04[d]	5.06±0.03[d]
RC	25	48.35±2.4[d]	20.02±0.8[d]	3.58±1.1[d]	2.19±0.04[d]	5.12±0.03[d]
RC		48.35±2.4[d]	20.02±0.8[d]	3.58±1.1[d]	2.19±0.04[d]	5.12±0.03[d]
RPC		46.52±2.0[e]	17.25±1.5[e]	3.46±1.2[e]	2.227±0.06[e]	4.89±0.02[e]
RC	45	46.61±2.1[e]	18.52±1.6[e]	3.62±1.2[e]	2.21±0.05[e]	5.03±0.03[e]
RC		46.61±2.1[e]	18.52±1.6[e]	3.62±1.2[e]	2.21±0.05[e]	5.03±0.03[e]
RPC		46.12±1.3[f]	17.21±1.6[f]	3.5±0.4[f]	2.27±0.02[f]	4.78±0.03[f]
RC	60	46.25±1.1[f]	17.27±1.7[f]	3.68±0.6[f]	2.268±0.01[f]	4.87±0.03[f]
RC		46.25±1.1[f]	17.27±1.7[f]	3.68±0.6[f]	2.268±0.01[f]	4.87±0.03[f]

[†]Means of each parameter in the same column with a superscript differ significantly (p<0.01).);

[‡]mean values ± standard deviation of three trials.;

[a] RPC,: raw probiotic cheese (probiotic Lighvan cheese).);

[b]RC,: raw cheese (traditional Lighvan cheese).

Table 3. Sensorial scores of probiotic and traditional Lighvan Cheese[†‡]

Cheese type[a,b]	Ripening period (days)	Appearance and colour	Body and texture	Flavour	Acceptability
RPC		7.6±0.89	7.01±1.30	7.2±1.00	7.1±0.45
RC	5	7.3±0.55	7±0.67	7.0±0.85	7±0.68
RC		7.3±0.55	7±0.67	7.0±0.85	7±0.68
RPC		7.9±1.23	7.4±0.90	7.5±0.84	7.3±0.89
RC	25	7.7±1.25	7.2±1.1	7.3±1.29	7.1±0.6
RC		7.7±1.25	7.2±1.1	7.3±1.29	7.1±0.6
RPC		8.2±1.34	7.6±0.97	7.9±0.88	7.8±0.91
RC	45	8±1.30	7.5±0.87	7.7±1.52	7.6±0.74
RC		8±1.30	7.5±0.87	7.7±1.52	7.6±0.74
RPC		8.6±1.64	8±1.79	8.5±0.45	8.5±0.71
RC	60	8.4±1.56	7.8±0.55	8.3±1.32	8.3±0.82
RC		8.4±1.56	7.8±0.55	8.3±1.32	8.3±0.82

[†] Means of each parameter in the same row without a superscript did do not differ significantly (p>0.01).);

[‡] mean values ± standard deviation of three trials.;

[a] RPC,: raw probiotic cheese (probiotic Lighvan cheese).);

[b] RC,: raw cheese (traditional Lighvan cheese).

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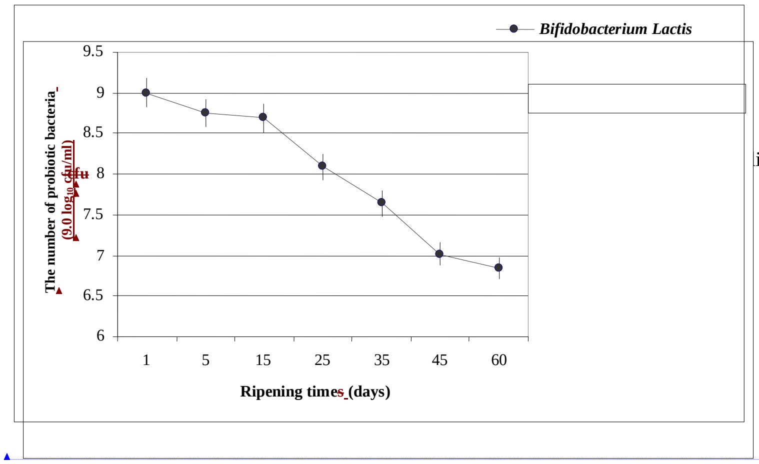
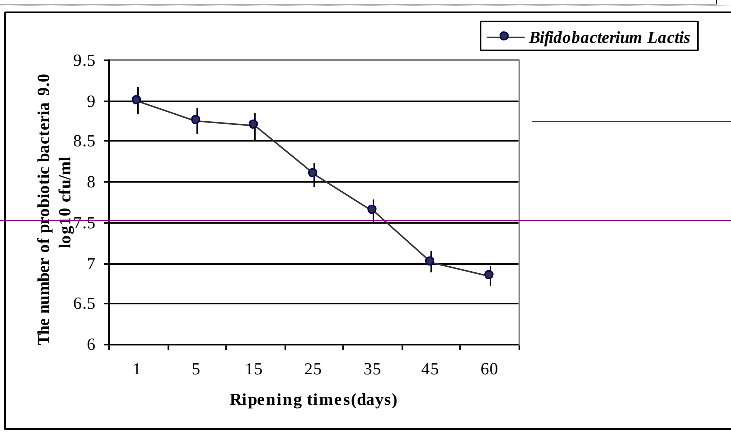
Figure 2. Survival of *Bifidobacterium lactis* Colonies in Cheese during Storage





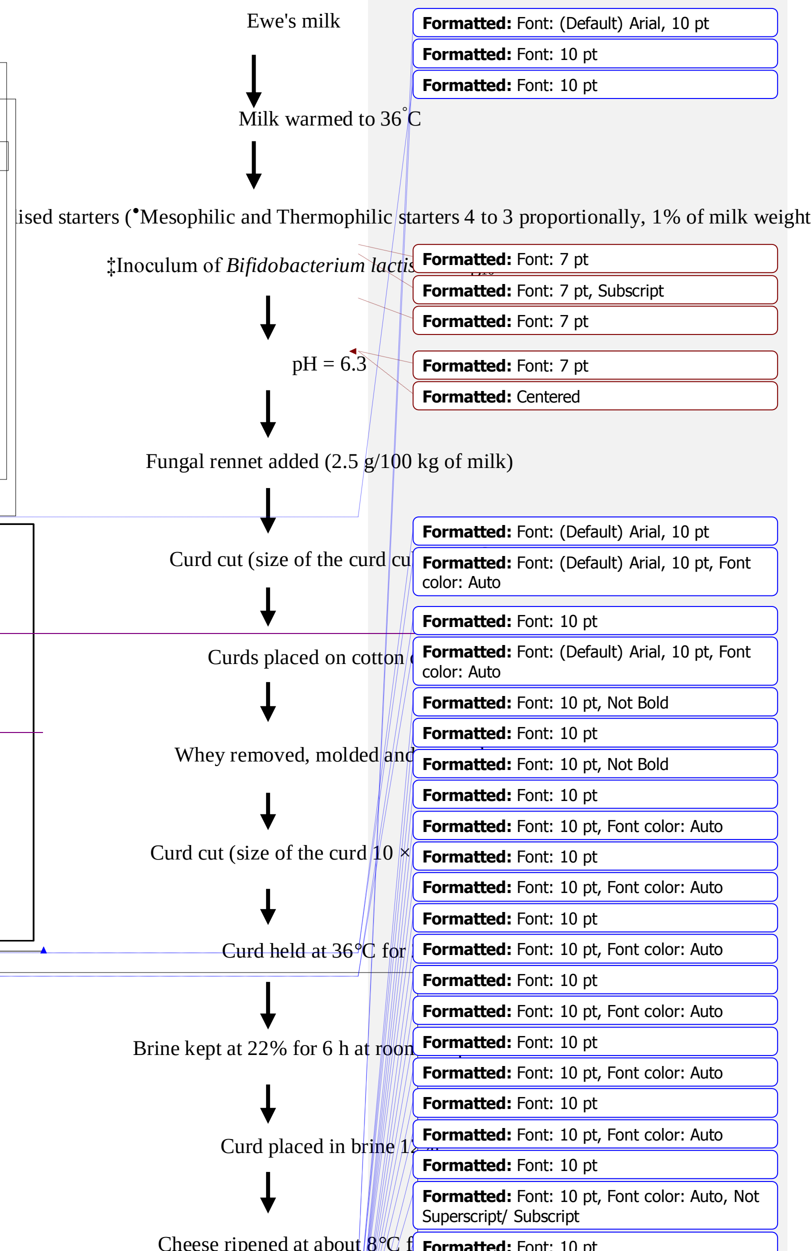


Figure 1. Protocol for the production of probiotic Lighvan cheese. †The ratio of the lyophilized starter to the cheese milk was 1% (w/w); ‡the concentration of *B. lactis* in the cheese milk was 9.0 log_{10} cfu/ml; •mesophilic culture, supplied by Chr. Hansen, contained *Lactococcus lactis* subsp. *cremoris*.

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Lactococcus lactis subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis biovar diacetylactis*. The thermophilic starter contained *Streptococcus thermophillus* and *Lactobacillus bulgaricus*.

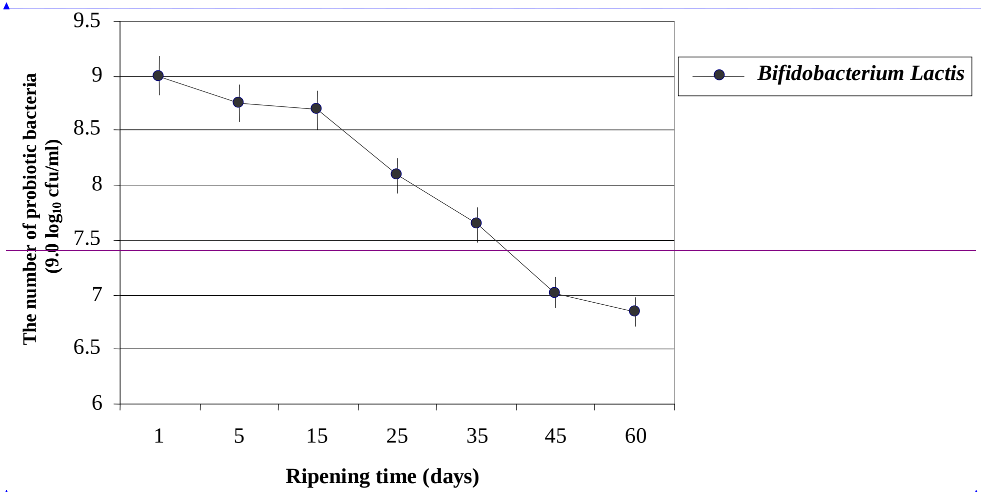

Figure 2. Survival of *B. lactis* colonies in Cheese during storage.

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