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Production of Probiotic Traditional *Lighvan* Cheese

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ABSTRACT

The aim of this research was to produce probiotic *Lighvan* cheese based on enriched *Bifidobacterium lactis*. The survival of *B. lactis*, some chemical composition and organoleptical characteristics of probiotic *Lighvan* cheese were studied during 60 day of storage. *B. lactis* cells survived in cheese samples at concentrations up to 6.84 log10 cfu/g for at least 60 days of storage time. The addition of *B. lactis* had a significant ($P<0.05$) effect on the chemical composition but it did not affect the sensory characteristics of the traditional cheese.

Keywords: *Lighvan*, Cheese, Survival, Composition

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INTRODUCTION

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 [1].

Cheese is fresh or matured product obtained by draining of whey after coagulation of milk, cream, skimmed or partly skimmed milk, buttermilk or a combination of some or all of these products [2]. The three basic steps of cheese production, acidification, coagulation and dehydration or syneresis are common in all cheese varieties [3]. 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 matrix and high fat content of cheese may protect the organisms during passage through the gastrointestinal tract [4, 5, 6]. 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 made to produce *Lighvan* in cheese 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. Incorporating bifidobacteria in cheese through the cheese milk is not difficult, as cheese offers the necessary anaerobic conditions and a suitable pH. In one study, the microbiological and chemical characteristics of traditional *Lighvan* cheese were investigated [7].

In another study, effect of type of milk on sensory properties of *Lighvan* cheese was studied. There were not any significant differences ($P > 0.05$) between cheese made from either raw goat's milk, raw cow's milk or mixed of them [8]. Recently the physicochemical and biochemical changes of traditional Iranian cheese *Lighvan* were studied over 90 days of ripening in brine. Dry matter and fat values decreased during ripening. Lipolysis level, RI, TCA-SN values and salt content increased continuously until the end of the ripening period, but total nitrogen decreased throughout a 90-day storage period. The ripening stage was the main factor affecting the cheese's sensory properties [9]. In this work, traditional cheese supplemented with B. *lactis* was produced and the survival of probiotic bacteria and eventual changes of physico-chemical characteristics of the traditional cheese was studied.

MATERIALS AND METHODS

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

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MRS broth (Merck, Germany), under anarobiosis at 37°C for 24 h. Cells as seed cultures were harvested by centrifugation (SANYO, MISTRAL, Germany) at 10,000 ×g for 10 min, washed twice with sterile skim milk and resuspended in cheese-milk at a concentration of 9.0 log10 cfu/ml for *B.* lactis.

Cheese manufacture

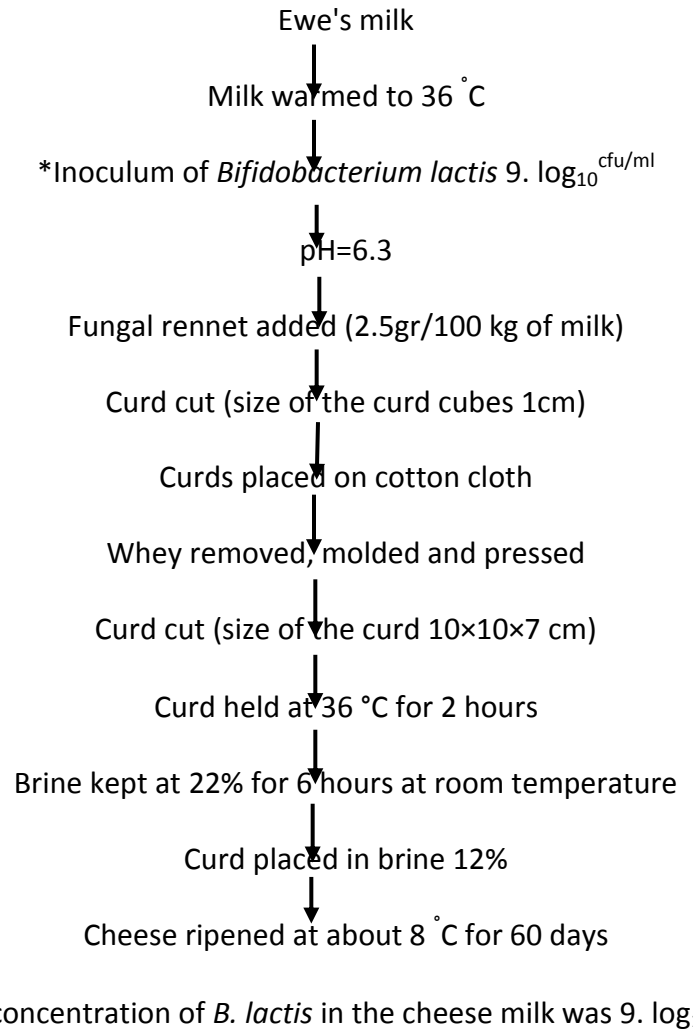

Figure-1: Protocol for the production of Probiotic *Lighvan* cheese

The different steps of cheese manufacture are summarized in 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 earlier mentioned protocol. The compositions of cheese milk were as follows: dry matter 17.86% (w/ w), protein5.6% (w/w), fat 6.5% (w/w), lactose 4.7% (w/w), ash 0.88% (w/w), and conversion coefficient of milk to cheese was 4.86 kg of milk to 1 kg of cheese. The raw milk was warmed to 36 °C and *B. lactis* was

inoculated with an initial concentration of 10^9 cfu/ml of milk and then milk was coagulated with fungal rennet for 45 min; after curdling, the curd was cut into small cubes, approximately 1 cm3, and left to rest (15 min). The pieces of curds after cutting were 10 × 10 × 7 cm. These pieces were immersed into brine with 22% concentration for 6 h 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 at 8 °C.

Microbiological analysis

10 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 min. 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 [10]. Cultivation was carried out using the pour-plate technique, and the plates were incubated, under anaerobiosis, for 72 h at 37°C for *B. lactis*. The *B. lactis* in the cheese samples was enumerated after 5, 25, 45 and 60 days. The characterizations of probiotic *Lighvan* cheese were determined during the ripening period. Each experiment was done in triplicate.

Chemical analysis

Samples of cheese were analyzed for pH using Metrohm, Model 632 pH-meter, Swiss, and also percentages of titratable acidity, total solids, total fat and salt were analyzed according to the AOAC method [11].

Organoleptic assessment

Organoleptic assessment of the cheese after 5, 25, 45 and 60 days of ripening was carried out by a ten-member panel selected on the basis of interest in sensory evaluation of traditional Lighvan cheese. The samples were presented to panelists in randomized order after storage for 2 h at room temperature, and were graded between 1 and 10 (1 being very bad and 10 being very good) for appearance, body and texture, colour, flavour and acceptability [12]. 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.

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 [13]. Means comparison was performed with LSD's test at the $P<0.05$ level of significance.

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RESULT AND DISCUSSION

Enumeration of *B. lactis*

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
25	8.09
45	7.02
60	6.84

†Means of each parameter in the same column without a superscript differ significantly (P<0.05).

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 [14]. 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 at the highest level. According to statistical analysis, significance differences (P<0.05) were observed in the enumeration of *B. lactis* during 60 days of ripening. After 25 days, the cheese contained ca. 8.0 log10 cfu/g of *bifidobacterium*, and after 60 days of ripening, the survival of B. *lactis* was 6.84 log10 cfu/g. 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*. Mupirocin susceptibility showed that bifidobacteria 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 log10 cfu/g during the 60 days (Figure 2). The minimum concentration of probiotic microorganisms that must be 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 log10 cells. This concentration should ensure the therapeutic minimum dose of 5.0 log10 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 and 7.0 log10 cfu/g or ml [15]. 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<0.05) 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[16], also Corbo *et al*. reported that after 56 days of ripening of Canestrato Pugliese Hard cheese supplemented with bifidobacteria, the survival of bifidobacteria was 6.0 log10 cfu/g [1].The use of bifidobacteria as a starter adjunct to produce probiotic cheeses was recently done. Not all the strains exhibited the same stability during

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ripening and storage of the dairy products [4, 5, 17, 18, 19, and 20], suggesting that strain survival should be evaluated individually prior to commercial use. Indeed, in cottage cheese, *Bifidobacterium infantis* reached levels of approximately 7.0 log10 cfu/g of cheese after 1 day of storage, but large viability losses were observed after 15 days at 4°C [17]. Other reports showed that bifidobacteria added to Cheddar [4] or Cheddar-like cheese [21] survived up to 24 weeks at approximately 7.3 log10 cfu/g, or remained above 6.5 log10 cfu/g.

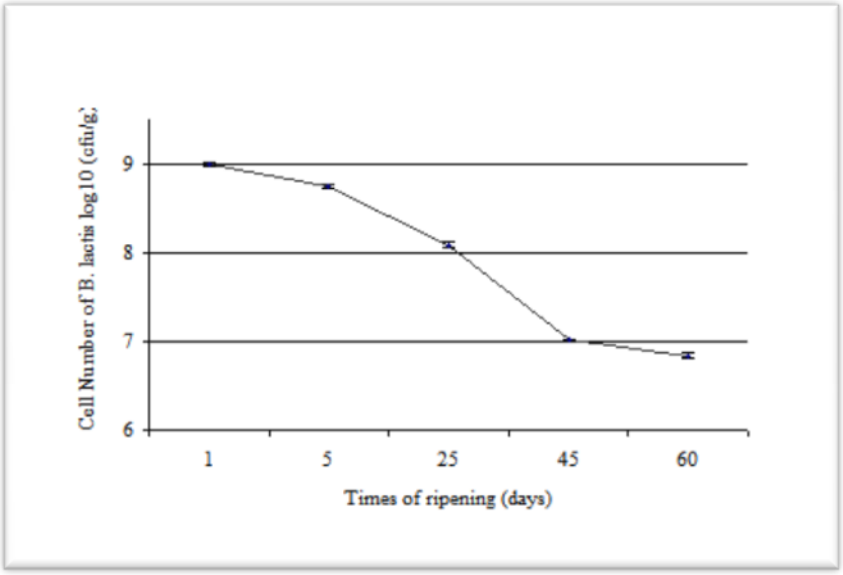

Figure- 2: Survival of *B.lactis* during 60 days of storage

Chemical Composition of Cheese

Table -2: Chemical composition of probiotic *Lighvan* cheese[†‡]

Ripening period (days)	Total solid (g/100g)	Fat (g/100g)	Salt (g/100g)	Acidity (g/100 g lactic acid)	pH
5	48.6^{a}±0.02	20.1^{a}±0.1	3.20^{a}±0.01	2.21^{a}±0.01	5.16^{a}±0.01
25	48.3^{b}±0.01	19.9^{b}±0.02	3.41^{b}±0.01	2.22^{a}±0.001	5.06^{b}±0.01
45	46.5^{c}±0.01	17.3^{c}±0.01	3.46^{c}±0.00	2.23^{a}±0.001	4.89^{c}±0.01
60	46.1^{d}±0.01	17.2^{c}±0.003	3.50^{d}±0.01	2.27^{a}±0.01	4.78^{d}±0.00

[†]Means of each parameter in the same column with a superscript differ significantly (P<0.05).
[‡]Mean values ± standard deviation of three trials.

Table 2, presents the chemical composition of probiotic *Lighvan* cheese at different ripening times. According to statistical analysis, except for titratable acidity, significant (P<0.05) difference was observed in the main chemical composition (pH, total solids, total fat and salt) of probiotic *Lighvan* cheese during 60 days of ripening. Titratable acidity of the samples increased until the 60th day of ripening, while pH value decreased. Titratable acidity (g/100g lactic acid) of probiotic *Lighvan* cheese was 2.21(g/100g lactic acid) at the day 5 of storage, and

2.27(g/100g lactic acid) at the day 60 of storage. 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 [22, 23]. 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*. reported that lactose is converted into lactic acid during cheese-making by the starter culture[24]. Therefore, lactic acid is the most abundant organic acid in all types of cheese [25]. Similar to our results, Tarakci and Kucukoner reported that titratable acidity increased during ripening; these results also agree with those reported by Sameen *et al*. [26, 27]. The insignificant increase in titratable acidity of probiotic *Lighvan* cheese was attributed to the low proteolytic activity of the probiotic organism. The pH of probiotic *Lighvan* cheese was 5.16 at the day 5th of storage, and 4.78 at the day 60 of storage. The total solids (%) of probiotic *Lighvan* cheese was 48.58(g/100g) at the day 5 of storage, and 46.12(g/100g) at the day 60 of storage. Significance (P>0.05) difference was observed during the ripening of probiotic *Lighvan* cheese. Decreases in total solid content of probiotic *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*. reported that the total solid content of Urfa cheese decreased throughout storage as a result of extended proteolysis. Subsequent to the breaking of peptide bonds and the release of new ionic groups[28, 29], 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. Total fat decreased gradually during ripening. Significant (P>0.05) difference was observed during the ripening of probiotic *Lighvan* cheese. The total fat content (g ⁄100 g) of the Lighvan cheese at the start of ripening was 20.1g ⁄100 g; this decreased to 17.2 g 100 g during ripening. Changes in fat content during storage could be due to a decrease in total solids and lipolysis. The salt (g ⁄100 g) in probiotic *Lighvan* cheese was 3.2 g ⁄100 g at the day 5 of storage, and 3.5 g ⁄100 g at the day 60 of storage. The effect of storage time of cheese on salt content was significant (P<0.05). The salt (NaCl) penetration into the cheese was much faster during the early stage of storage than during ripening. Salt is driven into cheese by the concentration gradient between the cheese blocks and brine; this gradient is much larger at the beginning of ripening [30]. Increase in salt content during ripening could be attributed to higher water content, as salt penetrates the cheese matrix in water.

Sensory Evaluation

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 *Lighvan* cheese was 7.6, at the day 5 of storage, and 8.6 at the day 60 of storage. The appearance and colour of the experimental cheese was considered good and did not have a

significant difference (P>0.05) throughout the ripening period. The mean value of body and texture for probitic *Lighvan* cheese was 7.01, at the day 5 of storage, and 8.0 at the day 60 of storage. The body and texture scores of the probiotic *Lighvan* cheese did not differ significantly (P>0.05). The mean value of flavour for probitic *Lighvan* cheese was 7.2, at the day 5 of storage, and 8.5 at the day 60 of storage. The flavour scores of probiotic *Lighvan* cheese did not differ significantly (P>0.05). The mean value of acceptability for probitic *Lighvan* cheese was 7.1, at the day 5 of storage, and 8.5 at the day 60 of storage. The acceptability scores of probiotic *Lighvan* cheese did not differ significantly (P>0.05). Appearance and colour, body and texture, flavour and acceptability scores generally increased during the ripening period. As regards acceptability scores, panel members' preferred ripen probiotic *Lighvan* cheeses over unripened. Identifying its taste, texture, colour and appearance was better.

Table- 3: Sensorial scores of probiotic *Lighvan* cheese

Ripening period (days)	Appearance and colour	Body and texture	Flavour	Acceptability
5	7.6±0.1	7.0±0.01	7.2±0.2	7.1±0.1
25	7.9±0.1	7.4±0.4	7.5±0.0	7.3±0.3
45	8.2±0.2	7.6±0.1	7.9±0.1	7.8±0.1
60	8.6±0.0	8.0±0.2	8.5±0.4	8.5±0.1

† Means of each parameter in the same row without a superscript did not differ significantly (P>0.05).
‡Mean values ± standard deviation of three trials.

CONCLUSIONS

The production of functional cheese products was recently proposed as a suitable and promising alternative to fermented milks [6], 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 characterize it as unreceptive 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 log10 cfu/g for at least 60 days of ripening. In contrary to sensory evaluation, *B. lactis* affect the chemical composition 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 have 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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