https://doi.org/10.17221/453/2016-CJFS

Biochemical Changes of Iranian Probiotic Lighvan Cheese

*Alireza SHAHAB LAVASANI**

Innovative Technologies in Functional Food Production Research Center, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

**Corresponding author: shahablavasani@iauvaramin.ac.ir*

Abstract

Shahab Lavasani A. (2018): Biochemical changes of Iranian probiotic Lighvan cheese. Czech J. Food Sci., 36: 181–186.

Lighvan cheese from ewe and goat milk was produced according to a traditional protocol, and with the addition of 9 $\log_{10}$ CFU/g fresh cells of *Bifidobacterium lactis* subsp. *animalis*. Probiotic Lighvan cheese was studied to determine the survival of *B. lactis* subsp. *animalis* and biochemical changes during 60 days of ripening of probiotic Lighvan cheese. Lipolysis level and organoleptic assessments were analysed. *B. lactis* subsp. *animalis* cells survived in cheese samples at concentrations up to 6.84 $\log_{10}$ CFU/g for at least 60 days of storage time. The lipolysis level increased continuously until the end of the ripening period.The ripening stage was the main factor affecting the cheese sensory properties.

Keywords: Lighvan cheese; lipolysis; organoleptic; probiotic; ripening

Ewe and goat milk represents 4.25% of the world milk production (Kalantzopoulos *et al.* 2002). The largest quantities are produced in Asia. The main use of sheep and goat milk is for the production of traditional cheeses at a farm level and in small local dairies, or in regional cheese industries. Additionally, quite large amounts of goat milk are consumed and sold directly (Kalantzopoulos *et al.* 2002). Cheese is valued as an excellent food because of its high nutritional value (particularly because of its relatively high amounts of protein, calcium, phosphorus and vitamins including A and D), high bioavailability and palatability. Because of their chemical composition and high assimilation, grade milk and dairy products are significant as an accessible source of protein of animal origin in the human diet. Among the varieties of cheeses manufactured in Iran, brined cheeses are the most widespread and popular with customers (Uliescu *et al.* 2007). Lighvan cheese is a traditional semi-hard Iranian cheese type produced mainly in northwestern Iran from raw ovine milk or appropriate mixtures of ovine and caprine milk, without a starter culture. Because the lactation period of sheep and goats is very short in Iran, approximately 6 months, it is not always possible to safely extend Lighvan cheese production throughout the entire year; this is the driving force behind the common use of pasteurised milk in cheese-making (Pandey *et al.* 2003). Despite the safety concern, there is still a large demand in Iran and elsewhere for cheeses made from raw milk because they possess strong and unique flavours. In Iran, brined cheese is a major item in the diet: the yearly per-capita consumption is about 4.6 kg, generally higher than in other countries in the region. At the industrial level, the ripening period is 45–90 days, but the cheeses made from raw milk by small, rural producers may be ripened for 6–8 months (Azarnia *et al.* 1997). Cheese ripening involves a very complex series of interrelated events, resulting in the development of the flavour and texture characteristic of the cheese variety. The triglycerides in all cheese varieties undergo hydrolysis by the action of indigenous or endogenous lipases, which result in the liberation of fatty acids in cheese during maturation (Sausa *et al.* 2001). The degree of lipolysis in cheese depends on the variety and ranges from slight to very extensive. Maintaining the viability of bifidobacteria in dairy products over a long time at refrigeration temperatures is still a problem with most fermented products. Antagonistic effects of antimicrobial substances such as bacteriocins, organic acids and protease produced by starter cultures decrease the viability of probiotic

https://doi.org/10.17221/453/2016-CJFS

strains. 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 provides 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 (Dinakar & Mistry 1994; Gomes *et al.* 1995; Stanton *et al.* 1998). Incorporating bifidobacteria into cheese through the cheese milk is not difficult, as cheese offers the necessary anaerobic conditions and a suitable pH. As mentioned above, the aim of this study was to produce probiotic Lighvan cheese and explore its biochemical changes during ripening periods.

MATERIAL AND METHODS

Bifidobacterial cultures. A lyophilised culture of *Bifidobacterium lactis* subsp. *animalis* was supplied by CAMINOX (Spain) with lot number D 540. The enumeration of *B. lactis* subsp. *animalis* was 125 × 10^9 CFU/g and 0.5 g of *B. lactis* subsp. *animalis* was cultured in MRS broth (Merck, Germany), under anaerobiosis at 37°C for 24 hours. Cells as seed cultures were harvested by centrifugation (Mistral 6000; Sanyo, Germany) at 10 000 *g* for 10 min, washed twice with sterile skim milk and resuspended in cheese milk at a concentration of 9 $\log_{10}$ CFU/g for *Bifidobacterium lactis* subsp. *animalis*.

Cheese manufacture. The different steps of cheese manufacture are summarised in Figure 1. Ewe milk was supplied from a farm 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 raw milk was coagulated with fungal rennet for 60 min; after curdling, the curd was cut into small cubes, approximately 1 cm^3, and left to rest (15 min). The pieces of curds after cutting were 10 × 10 × 7 cm^3. 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.

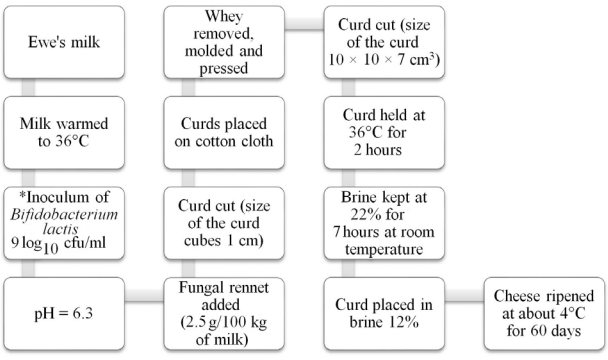

Figure 1. Protocol for the production of probiotic Lighvan cheese (*the concentration of *B. lactis* in the cheese milk was 9 $\log_{10}$ CFU/ml)

Microbiological analysis. Ten grams of cheese was first diluted in 90 ml of 2% sodium citrate solution and homogenized in a Stomacher 400 Lab Blender (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 and Sharpe (MRS) agar (Merck, Germany) was modified with L-cysteine HCl (0.05%) for reducing the redox 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 h at 37°C for *Bifidobacterium lactis* subsp. *animalis*. The *Bifidobacterium lactis* subsp. *animalis* 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 cheese (without *Bifidobacterium lactis* subsp. *animalis*) as a blank. Each experiment was done in triplicate.

Lipolysis. The level of lipolysis was assessed in the cheese samples 5, 25, 45, and 60 days old by measuring the contents of free fatty acids (FFA).

Sample preparation. Extraction of cheese lipids and isolation of the FFA were executed by GC as described by De Jong and Badings (1990).

Organoleptic assessment. Cheese after 5, 25, 45, and 60 days of ripening was assessed by a five-member panel selected on the basis of interest in sensory evaluation of traditional Lighvan cheese. The samples were presented to panellists 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 (Larmond 1978). 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 analysed using a completely randomized design (CRD)

https://doi.org/10.17221/453/2016-CJFS

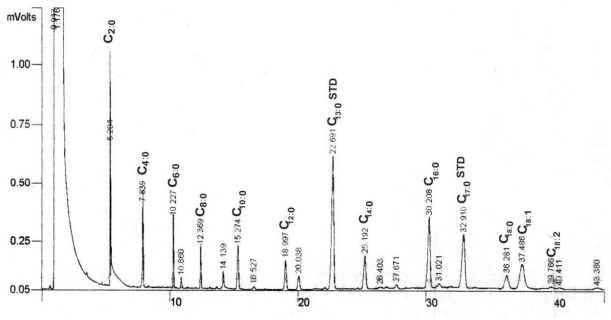

Figure 2. Gas chromatogram of FFA extracted from a probiotic *Lighvan* cheese (60 days old) spiked with internal FFA standards $C_{13:0}$ and $C_{17:0}$

with three replications. Data were subjected to analysis of variance using the SAS statistical software package (1988). Mean comparison was performed by the LSD test at the $P < 0.01$ level of significance.

RESULTS AND DISCUSSION

***Enumeration of Bifidobacterium lactis* subsp. *animalis*.** Table 1 shows the number of cells of *Bifidobacterium lactis* subsp. *animalis* during the 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 microorganisms. MRS agar was used to obtain the highest survival of the bacteria and inhibition effect of this medium to preserve the microbial population at the highest level. According to statistical analysis, significant differences ($P < 0.01$) were observed in the enumeration of *B. lactis* subsp. *animalis* during 60 days of ripening. After 25 days, the cheese contained ca. 8 $\log_{10}$ CFU/g of *Bifidobacterium*,

Table 1. Survival of *Bifidobacterium lactis* during 60 days of the ripening period of Lighvan cheese

Day	*Bifidobacterium lactis* ($\log_{10}$ CFU/ml)
1	9.00
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 ($P < 0.01$)

and after 60 days of ripening, the survival of *Bifidobacterium lactis* subsp. *animalis* was 6.84 $\log_{10}$ CFU/g.

After comparison of several selective media for isolation and enumeration of *B. lactis* subsp. *animalis* under the conditions in this study, MRS agar modified with L-cysteine HCl (0.05%) and lithium mupirocin was the best for the cell count of *B. lactis* subsp. *animalis*. Mupirocin susceptibility showed that bifidobacteria were consistently resistant to mupirocin, whereas all lactobacilli were susceptible. *B. lactis* bacteria decreased slightly throughout cheese ripening: a fall of only ca. 2.00 $\log_{10}$ CFU/g during the 60 days. 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 millilitre of product defined as a probiotic food should be at least 7 $\log_{10}$ cells. This concentration should ensure the therapeutic minimum dose of 5 $\log_{10}$ 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 and 7 $\log_{10}$ CFU/g or ml (Gardiner *et al.* 1999). 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* subsp. *animalis* showed a significant ($P < 0.01$) decrease during 60 days of ripening.

Similar to our results, 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 $\log_{10}$ CFU/g. The use of bifidobacteria as a starter adjunct to produce probiotic cheeses was recently done. Not all the strains exhibited the same stability during ripening and storage of the dairy products (Dinakar & Mistry 1994; Gomes *et al.* 1995; Blanchette *et al.* 1996; Ghoddusi & Robinson 1996; Roy *et al.* 1997; Gobbetti *et al.* 1998), suggesting that strain survival should be evaluated individually prior to commercial use. Indeed, in cottage cheese, *Bifidobacterium infantis* reached levels of approximately 7 $\log_{10}$ CFU/g of cheese after 1 day of storage, but large viability losses were observed after 15 days at 4°C (Blanchette *et al.* 1996). Other reports showed that bifidobacteria added to Cheddar as published by Dinakar and Mistry (1994) or

https://doi.org/10.17221/453/2016-CJFS

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.

Lipolysis of cheese-free fatty acids profile. Chromatographic separation of underivatised FFA (Figure 2) allowed the quantification of all major fatty acids in one run. The concentration of acetic acid and total C4:0-C18:2 increased throughout ripening, showing the significant effect ($P < 0.05$) of the ripening stage on cheese lipolysis (Table 2). The mean concentration of acetic acid and individual FFAs of probiotic Lighvan cheese increased throughout the ripening process. The content of acetic acid was 28.91 mg/100 g of cheese at the fifth day of storage and 29.85 mg/100 g of cheese at the 60th day of storage. Acetic acid made up 25% of total FFA present in probiotic Lighvan cheese. The mean concentration of acetic acid and individual FFAs of probiotic Lighvan cheese increased throughout the ripening process. Acetic acid contributes greatly to the final flavour of traditional Lighvan cheese and is the major volatile acid extracted with FFAs. It is not produced from lipolysis by lipase but from several biochemical pathways. It is formed during the early stages of ripening and is probably a product of citrate or lactate fermentation or of amino acid catabolism by bacteria (Abd El-Salam *et al.* 1993; McSweeney & Sousa 2000). The increase of acetic acid up to 60 days may be due to lactate fermentation, since lactose can be present even in mature cheese (Abd El-Salam *et al.* 1993). Acetic acid made up 25% of total FFA present in probiotic Lighvan cheese. Butyric acid was the main FFA in the SCFFA experimental cheese samples, ranging from 6.01 mg/100 g cheese at 5th day of storage time to 6.2 mg/100 g cheese at 60th day of storage time. Butyric acid is also an important component of Lighvan cheese, which contributes greatly to its flavour and piquant taste. The predominant FFA was myristic acid in MCFFA and its value ranged from 8.85 mg/100 g cheese at 5th day of storage time to 8.97 mg/100 g cheese at 60th day of storage time, and palmitic acid was dominant FFA among LCFFA in probiotic Lighvan cheese, and its value ranged from 23 mg/100 g cheese at 5th day of storage time to 23.2 mg/100 g cheese at 60th day of storage time. The relatively higher increase was observed in the concentration of SCFFA (C4:0 to C8:0), which has a more significant impact on the development of the cheese characteristic aroma during ripening than medium-chain free fatty acids (MCFFA) (C10:0 to C14:0) and long-chain free fatty acids (LCFFA) (C16:0 to C18:2) (Table 3). This could mainly be due to the specificity of milk lipoprotein lipase towards FFA located at the positions sn-1 and sn-3 of the triglyceride. Despite the quantitative importance of medium- and long-chain FFA, they are not the main contributors to cheese flavour (Rahmat & Richter 1996; Freitas & Malcata 1998). The predominant FFA was myristic acid in MCFFA and palmitic acid was a dominant FFA among LCFFA in probiotic Lighvan cheese; researchers have demonstrated that palmitic and oleic acids, which do not

Table 2. Free fatty acids (mg/100 g) of probiotic Lighvan cheese[a,b]

Fatty acid	Ripening (days)			
	5	25	45	60
$C_{2:0}$	28.91^{a} ± 0.01	28.97^{b} ± 0.01	29.00^{c} ± 0.01	29.85^{d} ± 0.01
$C_{4:0}$	6.01^{a} ± 0.01	6.12^{b} ± 0.01	6.18^{c} ± 0.01	6.20^{d} ± 0.01
$C_{6:0}$	3.90^{a} ± 0.02	3.95^{b} ± 0.01	3.98^{c} ± 0.01	4.01^{d} ± 0.01
$C_{8:0}$	3.02^{a} ± 0.01	3.17^{b} ± 0.01	3.22^{c} ± 0.01	3.25^{d} ± 0.01
$C_{10:0}$	7.21^{a} ± 0.01	7.29^{b} ± 0.01	7.34^{c} ± 0.01	7.37^{d} ± 0.01
$C_{12:0}$	6.78^{a} ± 0.01	6.85^{b} ± 0.01	6.90^{c} ± 0.01	6.92^{c} ± 0.01
$C_{14:0}$	8.85^{a} ± 0.01	8.90^{b} ± 0.01	8.93^{c} ± 0.01	8.97^{d} ± 0.02
$C_{16:0}$	23.00^{a} ± 0.02	23.10^{b} ± 0.02	23.15^{c} ± 0.01	23.20^{d} ± 0.01
$C_{18:0}$	9.01^{a} ± 0.01	9.10^{b} ± 0.02	9.14^{c} ± 0.01	9.18^{d} ± 0.01
$C_{18:1}$	19.85^{a} ± 0.01	19.90^{b} ± 0.01	19.95^{c} ± 0.01	19.98^{d} ± 0.01
$C_{18:2}$	1.01^{a} ± 0.02	1.20^{b} ± 0.01	1.24^{c} ± 0.01	1.28^{d} ± 0.01
Total	117.55^{a} ± 0.02	118.55^{b} ± 0.01	119.03^{c} ± 0.02	120.21^{d} ± 0.02

[a]means of each parameter in the same column with a superscript differ significantly ($P < 0.05$); [b]mean values ± standard deviations of three trials

https://doi.org/10.17221/453/2016-CJFS

Table 3. Sensory scores of probiotic and traditional Lighvan cheese

Cheese type	Ripening period (days)	Appearances and colour	Body and texture	Flavour	Acceptability
RPC	5	7.60 ± 0.89	7.01 ± 1.30	7.20 ± 1.00	7.10 ± 0.45
RC		7.30 ± 0.55	7.00 ± 0.67	7.00 ± 0.85	7.00 ± 0.68
RPC	25	7.90 ± 1.23	7.40 ± 0.90	7.50 ± 0.84	7.30 ± 0.89
RC		7.70 ± 1.25	7.20 ± 1.10	7.30 ± 1.29	7.10 ± 0.60
RPC	45	8.20 ± 1.34	7.60 ± 0.97	7.90 ± 0.88	7.80 ± 0.91
RC		8.00 ± 1.30	7.50 ± 0.87	7.70 ± 1.52	7.60 ± 0.74
RPC	60	8.60 ± 1.64	8.00 ± 1.79	8.50 ± 0.45	8.50 ± 0.71
RC		8.40 ± 1.56	7.80 ± 0.55	8.30 ± 1.32	8.30 ± 0.82

RPC – raw probiotic cheese; RC – raw cheese; means of each parameter in the same row without a superscript do not differ significantly ($P > 0.01$); values are means ± standard deviations of three trials

intrinsically contribute to cheese flavour quite as much as short-chain FFAs, since they have higher perception thresholds, dominated among the saturated and unsaturated long-chain FFAs of halloumi cheese, which is processed with high thermal kneading of the curd after processing, and kept in brine like traditional Lighvan cheese (Kaminarides *et al*. 2007).

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 were considered good and did not show a significant difference ($P > 0.01$) throughout the ripening period. The mean value of body and texture for probiotic and traditional Lighvan cheeses was 7.01 and 7, respectively, at the fifth day of storage, and 8 and 7.8 at the 60th day of storage. The body and texture scores of the probiotic and traditional Lighvan cheeses did not differ significantly ($P > 0.01$). The mean value of flavour for probiotic and traditional Lighvan cheeses was 7.2 and 7, 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 probiotic and traditional Lighvan cheeses was 7.1 and 7, 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 ripening. As regards acceptability scores, the panel members preferred ripened probiotic Lighvan cheeses over unripened ones. Identifying their taste, texture, colour and appearance was better.

CONCLUSIONS

The production of functional cheeses 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 characterise this ecosystem as hostile for microorganisms. However, the results of this study demonstrated that Lighvan cheese made with added *B. lactis* subsp. *animalis* seemed to be an effective way to produce a semi-hard ewe's cheese with a considerable number of viable bifidobacterium cells. In particular, *B. lactis* subsp. *animalis* cells survived in cheese at concentrations up to 6.84 $\log_{10}$ CFU/g for at least 60 days of ripening. *B. lactis* subsp. *animalis* affected proteolysis and lipolysis, characteristics of the traditional Lighvan cheese but it did not have any effect on sensory properties of probiotic 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 but we can conclude that in cheeses ripened in brine, a significant part of ripening products is transferred into brine and their effects on sensory properties of the final product are limited.

https://doi.org/10.17221/453/2016-CJFS

References

Abd El-Salam M.H., Alichanidis E., Zerfiridis G.K. (1993): Domiati and feta-type cheeses. In: Fox P.F. (ed.): Cheese: Chemistry, Physics and Microbiology. London, Chapman and Hall: 301–335.

Azarnia S., Ehsani M.R., Mirhadi S.A. (1997): Evaluation of the physicochemical characteristics of the curd during the ripening of Iranian brine cheese. International Dairy Journal, 7: 473–478.

Blanchette L., Roy D., Belanger G., Gauthier S.F. (1996): Production of cottage cheese using dressing fermented by bifidobacteria. Journal of Dairy Science, 79: 8–15.

Corbo M.R., Albenzio M., De Angelis M., Sevi A., Gobbetti M. (2001): Microbiological and biochemical properties of canestrato pugliese hard cheese supplemented with bifidobacteria. Journal of Dairy Science, 84: 551–561.

Daigle A., Roy D., Vuillemand J.C. (1999): Production of probiotic cheese (Cheddar-like cheese) using enriched cream fermented by *Bifidobacterium infantis*. Journal of Dairy Science, 82: 1081–1091.

De Jong C., Badings H.T. (1990): Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up and capillary gas chromatographic analysis. Journal of High Resolution Chromatography, 13: 94–98.

Dinakar P., Mistry V.V. (1994): Growth and viability of *Bifidobacterium bifidum* in Cheddar cheese. Journal of Dairy Science, 77: 2854–2864.

Freitas A.C., Malcata F.X. (1998): Lipolysis in Picante cheese: influence of milk type and ripening time on free fatty acid profile. Lait, 78: 251–258.

Gardiner G., Ross R.P., Collins J.K., Fitzgerald G., Stanton C. (1999): Development of a probiotic Cheddar cheese containing humanderived *Lactobacillus paracasei* strains. Journal of Applied Environmental Microbiology, 6: 2192–2199.

Ghoddusi H.B., Robinson R.K. (1996): Enumeration of starter cultures in fermented milks. Journal of Dairy Research, 63: 151–181.

Gobbetti M., Corsetti A., Rossi J. (1998): The sourdough microflora. Interaction between lactic acid bacteria and yeasts: metabolism of carbohydrates. Journal of Applied Microbiology Biotechnology, 41: 456–460.

Gomes A.M.P., Malcata F.X., Klaver F.A.M., Grande H.J. (1995): Incorporation and survival of *Bifidobacterium* sp. Strain Bo and *Lactobacillus acidophilus* strain Ki in a cheese product. Netherland Milk Dairy Journal, 49: 71–95.

Heidarpour M., Mokhtari F., Mirdamadi S., Gharashi A. (2008): Isolation and Identification of *Bifidobacterium* sp. in Iranian Traditional Dairy Products. Research Journal of Biological Science, 3: 979–983.

Kalantzopoulos G., Dubeuf J.P., Vallerand F., Pirisi A., Casalta E., Lauret A. Trujillo T. (2002): Characteristics of sheep and goat milks: quality and hygienic stakes for the sheep and goat dairy sectors. IDF SC on Microbiological Hygiene, Agenda item 4.8.

Kaminarides S., Stamou P., Massouras T. (2007): Changes of organic acids, volatile aroma compounds and sensory characteristics of Halloumi cheese kept in brine. Journal of Food Chemistry, 100: 219–225.

Ketney O., Tita M., Tita O., Bretan L., Boltea F. (2008): Researches regarding viability of probiotic level of some lactic bacterium and *Bifidobacterium* colonies in the feta cheese. Journal of Agronomy Proceeding Technology, 14: 446–454.

Larmond E. (1987): Laboratory Methods for Sensory Evaluation of Food. Ottawa, Canadian Government Publishing Center: 64–67.

McSweeney P.L.H., Sousa M.J. (2000): Biochemical pathways for the production of flavour compounds in cheese during ripening. Lait, 80: 293–324.

Pandey P.K., Ramaswamy H.S., St-Gelais D. (2003): Evaluation of pH change kinetics during various stages of Cheddar cheese-making from raw, pasteurized, microfiltered and high-pressure-treated milk. Lebensmittel-Wissenchaft and -Technologie, 36: 497–506.

Payne J.F., Morris A.E.J., Beers P. (1999): Note: Evaluation of selective media for the enumeration of *Bifidobacteria* sp. in milk. Journal of Applied Microbiology, 86: 353–358.

Rahmat A., Richter R. (1996): Formation of volatile free fatty acids during ripening of Cheddar-like goat cheese. Journal of Dairy Science, 79: 717–724.

Roy D., Pitre M., Blanchette Savoie L., Belanger G., Ward P., Maubois J.L. (1997): Monitoring proteolysis and cheese juice composition during ripening of Cheddar cheese made from microfiltered milk. Lait, 77: 521–541.

Sausa M.J., Ardo Y., McSweeney P.L.H. (2001): Advances in the study of proteolysis in cheese during ripening. International Dairy Journal, 11: 327–345.

Stanton C., Gardiner G., Lynch P.B., Collins J.K., Fitzgerald G., Ross R.P. (1998): Probiotic cheese. International Dairy Journal, 8: 491–496.

Uliescu M., Rotaru G., Mocanu D., Stanciu V. (2007): Study of the probiotic telemea cheese maturation. In: 8th International Symposium EuroAliment, Sept 7–8, 2017, Galati, Romania: 92–99.

Received: 2016–12–02
Accepted after corrections: 2018–01–24
Published online: 2018–04–14