The effect of ovine and bovine milk on the textural properties of Lighvan cheese during ripening

MEHRNAZ AMINIFAR, 1 MANOUCHEHR HAMEDI, 2 ZAHRA EMAM-DJOMEH 2* and ALI MEHDINIA 3

1Faculty of Food Industry and Agriculture, Department of Food Science & Technology, Standard Research Institute (SRI), PO Box 31745-139, Karaj, Iran, 2Transfer Phenomena Laboratory, Department of Food Science, Technology and Engineering, Faculty of Agricultural Engineering and Technology, Agricultural Campus, University of Tehran, Karaj, 31587-11167, Iran, and 3Department of Marine Living Resources, Iranian National Institute for Oceanography, Tehran, 14155-4781, Iran

The effect of milk origin on the physicochemical characteristics, microstructure and texture of Lighvan cheese was investigated over a 90-day ripening period. Besides fat, other physicochemical properties of Lighvan cheese were affected by milk type. The moisture content of Lighvan cheese decreased when half or all the ovine milk was substituted with bovine milk. The Lighvan cheese’s microstructural properties and porosity were affected by type of milk and ripening time. Compaction of cheeses manufactured from ovine and mixed ovine and bovine milk is similar, and more than that of bovine Lighvan cheese. Ovine Lighvan cheese is harder and less brittle than bovine and mixed bovine and ovine.

Keywords Lighvan cheese, Cheese microstructure, Hardness, Brittleness, Milk origin.

INTRODUCTION

Lighvan cheese, one of the most popular Iranian traditional cheeses, is a starter-free cheese from the Azerbaijan region, in the north-west of Iran, and manufactured from raw ewe’s milk. It ripens in 10 to 12% salt brine for 3 or 4 months at an average temperature of 10 ± 2 °C. In spite of the increasing popularity of Lighvan cheese, there are few studies on its chemical composition and microbial communities (Abdi et al. 2006; Kafli et al. 2009; Aminifar et al. 2010).

Lighvan cheese is traditionally produced from ovine milk. Recently, Iranian researchers have endeavoured to make it from bovine milk due to the limitations of the seasonal production of ovine milk and short lactation period of small ruminants in Iran. Currently, some cheese traditionally produced from ovine milk is industrially manufactured using bovine milk; examples include Urfa in Turkey (Atasoy and Turkoğlu 2008) and Belir Sir U Kriskama in the countries of former Yugoslavia (Anifantakis and Moatsou 2006).

Texture is one of the most important cheese characteristics for consumer acceptability. It is greatly affected by factors such as milk type, processing technology, operation temperature and ripening time (Pappas et al. 1996; Tsigkros et al. 2003). The texture of cheese made with bovine milk is significantly different from that of ewe’s and goat’s milk cheese. Differences have been noted between industrial Halloumi cheese (which has bovine milk as the major constituent) and the traditional product made with mixture of sheep’s and goat’s milk (Papademas and Robinson 2000). Cheese ripening is a complex process, consisting of a series of biochemical reactions (Singh et al. 2003) that convert rubbery bland curd to a mature cheese with a desirable texture (Fox et al. 1990). The texture of traditional Lighvan cheese changes significantly during ripening (Aminifar et al. 2010).

The texture of cheese can be evaluated by studying its mechanical properties using uniaxial force compression (Hort et al. 1997; Awad 2006). Moreover, studying the microscopic structure of cheese provides beneficial information about its texture (Mckenna 2003). In the
present study, physicochemical, textural and microstructural properties of Lighvan cheese made with bovine milk and a mixture of bovine and ovine milk were compared with those of traditional cheese made with ovine milk.

MATERIALS AND METHODS

Cheese making and sampling

Lighvan cheeses were prepared from raw bovine milk (3.6 ± 0.7% fat, 12.6 ± 1.1% total solids, 3.2 ± 0.3% protein) and a 1:1 mixture of raw ovine milk (7.9 ± 0.8% fat, 19.7 ± 0.9% total solids, 6.2 ± 0.5% protein) and bovine milk (1:1 mixed milk composition: 5.8 ± 0.4% fat; 16.2 ± 0.1% total solids and 4.7 ± 0.2% protein) according to a method previously described by Kafili et al. (2009). Production of cheese consisted of several steps:

1. Coagulation of milk at 28–32 °C (depending on the season) by adding lamb rennet.
2. Cutting the coagulum into walnut-sized pieces and transferring them to rectangular cotton bags for whey drainage.
3. Compressing the cotton bags with a four-kilogram mass for 2–4 h (depending on season) for better water drainage.
4. Cutting the curd mass into 25 × 25 × 25 cm cubes and placing the cubes in a 22% brine stream for 6 h.
5. Transferring the curd cubes to a basin, sprinkling sodium chloride crystals on their surfaces and storing for 3–5 days. In this stage, the cubes were turned upside down between nine and 15 times for better whey removal.
6. Keeping the cubes in 12% salt brine for 3 months at an average temperature of 10 ± 2 °C.

   The fat content of prepared Lighvan cheese produced from ovine, bovine milk and mixed milks were 6.5 ± 0.3 and 10.9 ± 0.5%, respectively. The fat content of ovine Lighvan cheese determined in a previous study (Aminifar et al. 2010) was 15.2 ± 0.31. Ten samples (25 × 25 × 25 cm cubes) were collected on days 1, 30, 60 and 90 and analysed in triplicate.

Physicochemical analysis

The cheese samples were analysed for moisture content according to the standard (4A) IDF (1982). Their salt content was measured by Kirk and Sawyer’s method (1991). The pH of cheese samples was determined using a Knick 766 calimatic pH-metre (Niels, Bohrweg, Utrecht, Netherlands). Total nitrogen was determined according to the Kjeldahl method (IDF 1993). Kuchroo and Fox’s method (1982) was used to determine water-soluble nitrogen (WSN) as a proteolysis indicator. According to this method, 20 g cheese were homogenised with 40 g water in a stomacher for 10 min at 20 °C. The suspension was heated to 40 °C and held there for 1 h, then centrifuged at 3000 × g for 30 min. The supernatant was then filtered through glass wool and the WSN was measured using the Kjeldahl method (IDF 1993). All physicochemical properties (moisture content, salt, salt-in-moisture, total nitrogen in dry matter (TN/DM), WSN in total nitrogen (WSN/TN), pH and acidity) were measured in triplicate, and SAS statistical software (version 8.2, SAS Institute Inc., Cary, NC, USA) was applied for the statistical analysis of the experimental data. Multifactor analysis of variance (ANOVA) with the least significant difference (LSD) test (P < 0.05) was used to evaluate the effect of time and type of milk on the physicochemical characteristics of cheese samples. Microsoft Excel 2007 was used for table drawing.

Microstructure

Preparation of cheese samples for scanning electron microscopy (SEM) analysis after 1, 30 and 90 days of ripening was performed according to the method previously described by Drake et al. (1996), with modifications suggested by Madadlou et al. (2007). This method had been used previously by Aminifar et al. (2010) to study the microstructure of traditional Lighvan cheese. According to this method, 5–6 mm³ cheese cubes were prepared by cutting the cheese blocks with a sharp razor. The cheese cubes were immersed in 2.5% (w/v) gluteraldehyde fixative (Merck Chemical Ltd., Darmstadt, Germany) for 3 h, then washed in six changes of distilled water (1 min each). The cubes were dehydrated in a graded (40, 55, 70, 85, 90 and 96%) series of ethanol baths for 30 min at each grade and defatted with three changes of chloroform (10 min each).

The prepared cubes were covered with ethanol and refrigerated until SEM analysis. The cubes were freeze-fractured in liquid nitrogen into approximately 1-mm pieces according to the method described by Sipahioglu et al. (1999). After the cheese pieces had been dried to a critical point, a sputter-coater (Balzers, Type SCD 005; BalTec Inc., Pfäffikon, Switzerland) was used to apply gold coating for 6 min. The samples were examined with a scanning electron microscope (XL Series, model XL30; Philips, Eindhoven, the Netherlands) operated at 15.0 kV. Photomicrographs were recorded at 500, 1000, 2000 and 4000 magnification levels.

3D images were prepared with an interactive 3D surface plot function from image analysis software (ImageJ; National Institutes of Health, Bethesda, MD, USA). Appropriate 3D images were created with these settings: z-ratio: 0.18, smoothing: 12.0, lightening: 0.2, scale: 1.34 and grid size: 512. This software has been used previously in the analysis of cheese microstructure and SEM images (Aminifar et al. 2010).

Texture profile analysis

The fracturability, or brittleness, and hardness of cheese were determined after 1, 30, 60 and 90 days of ripening using uniaxial force compression performed with a texture analyser (Testometric M350-10CT, Lancashire, UK) fitted
with a 0–50-kg load cell. Prior to analysis, the cheese cube samples (20 × 20 × 20 mm) were tempered to 12 ± 5 °C. They were compressed to 70% of their initial height with a plunger, 40 mm in diameter, moving at 30 mm/min. The first break of the compression curve was considered as fracturability (Fredrick et al. 1986). The force required to compress the sample to 70% of its initial height determined hardness (Szczesniak 1963). All samples were analysed using three replications in each stage of ripening.

RESULTS AND DISCUSSION

Changes in the pH, moisture content, salt, salt-in-moisture, TN/DM and WSN/TN during the ripening period in cheeses made from sheep’s, cow’s or mixed milk (1:1) are shown in Table 1.

The pH of the cheese samples is affected by two main factors: lactose fermentation and production of amino groups by yeast and bacteria (Fox 1993). Regardless of the kind of milk, the pH of the samples did not change significantly in the 60 days of ripening. This could be related to the balance between the two determinant pH factors. Because of the inhibitory effect of salt on yeast and proteolytic bacteria (Guinee and Fox 1993), the pH of bovine and mixed Lighvan cheeses, like that of ovine Lighvan cheese, decreased between the 60th and 90th days due to the production of lactic acid. Because the pH of cow’s and sheep’s milk does not differ significantly (Jenness et al. 1974), the pH of the Lighvan cheese was not affected by the type of milk. As after 90 days lactobacilli were the dominant group of bacteria in the Lighvan cheese (Kafli et al. 2009), the pH of all samples was around 4.45 due to lactose fermentation.

From the statistical analysis of the moisture content of Lighvan cheeses made with the different kinds of milk at different stages of ripening, a sharp decrease was observed between days 1 and 30. This depletion could be related to storage of cheese cubes in 10–12% salt brine and the movement of water from cheese to brine due to the high osmotic pressure of NaCl (Zerfiridis 2001). As a result of equilibrium between the adsorption and desorption of water, the moisture content was constant between 30 and 90 days. Adsorption of water could be related to the water-holding ability of proteolysis products (Creamer and Olson 1982). In all stages of ripening, bovine Lighvan cheese had higher moisture content than cheeses made from ewe’s or mixed milk cheeses. This difference could be attributed to the higher level of total solids in sheep’s milk in comparison with cow’s milk. Pappa et al. (2006) found that Telemi cheese made from cow’s milk had higher moisture content than cheese made from ewe’s and goat’s milk. Similar results were reported by Kehagias et al. (1995) for white-brined cheese. At day 1, the moisture content of cheese made from mixed milk was higher than that of ovine cheese, but after 90 days the moisture content of both types was the same.

Table 1 Changes in physiochemical properties of Lighvan cheese made from sheep’s (S), cow’s (C) or mixed milk (M) during storage (mean ± SD of three trials)

<table>
<thead>
<tr>
<th>Physicochemical characteristics</th>
<th>Kind of milk</th>
<th>Ripening time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>C</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>5.45±0.26</td>
<td>5.70±0.33</td>
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<tr>
<td></td>
<td>5.75±0.29</td>
<td>5.69±0.23</td>
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<tr>
<td></td>
<td>5.60±0.14</td>
<td>4.43±0.02</td>
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<tr>
<td>Moisture content (%)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>68.59±0.48</td>
<td>61.37±0.21</td>
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<tr>
<td></td>
<td>62.15±0.30</td>
<td>62.14±0.26</td>
</tr>
<tr>
<td></td>
<td>68.59±0.48</td>
<td>61.37±0.21</td>
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<tr>
<td></td>
<td>62.15±0.30</td>
<td>62.14±0.26</td>
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<tr>
<td>Salt (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.45±0.19</td>
<td>13.13±0.43</td>
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<tr>
<td></td>
<td>13.76±0.45</td>
<td>14.60±0.26</td>
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<td></td>
<td>14.76±0.45</td>
<td>14.60±0.26</td>
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<tr>
<td></td>
<td>13.13±0.43</td>
<td>13.68±0.23</td>
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<tr>
<td>TN/DM (%)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>5.68±0.16</td>
<td>5.44±0.10</td>
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<tr>
<td></td>
<td>5.18±0.12</td>
<td>5.15±0.09</td>
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<tr>
<td></td>
<td>5.21±0.15</td>
<td>5.23±0.03</td>
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<tr>
<td></td>
<td>10.07±0.11</td>
<td>18.04±0.19</td>
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<tr>
<td>WSN/TN (%)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>13.01±0.11</td>
<td>18.04±0.19</td>
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<tr>
<td></td>
<td>14.38±0.26</td>
<td>14.56±0.20</td>
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<tr>
<td></td>
<td>16.92±0.71</td>
<td>16.75±0.09</td>
</tr>
</tbody>
</table>

a,b Means of each parameter in the row with different letters are significantly different (P < 0.05).

1,2 Means of each parameter in the same column of the same day with different numbers are significantly different (P < 0.05).

S For better comparison, results from Lighvan cheese made from sheep’s milk were picked up from our previous research (Aminifar et al. 2010).
Regardless of the kind of milk, the NaCl content of Lighvan cheese increased significantly during the first month of ageing due to the difference in osmotic pressure between brine and cheese water (Guinee and Fox 1986). Because equilibrium had been reached after 30 days, the salt content did not change from the 30th to the 90th day. Salt content was affected by the kind of milk used. Cheese made from bovine or mixed milk had higher salt content than cheese made from ewe’s milk. Our results agreed with those reported by Geurts et al. (1974), who found that the diffusion coefficient and the quantity of the salt absorbed by Gouda-type cheeses during brine salting increased as the moisture content of the curd increased. Byers and Price (1937) reported similar results for brine-salted Brick cheese.

Regardless of the type of milk, the percentage of TN/DM decreased during ageing as a result of proteolysis and diffusion of soluble nitrogenous compounds into the brine (Abd El-Salam et al. 1993). Because ovine milk has a higher level of protein (casein and whey) and other nonprotein nitrogenous compounds than bovine milk (Gil and Sanchez Medina 1981), the percentage of TN/DM in ovine Lighvan cheese was higher than in cheeses made from bovine or mixed milk. Blending of bovine milk with ovine milk did not alter the percentage of TN/DM significantly.

WSN/TN (%) values increased significantly in the first month of ageing, regardless of the kind of milk. This increase could be attributed to proteolytic activity of raw milk proteases and microorganisms (Ardo 1999). Between 30 and 90 days, due to the inhibitory effect of salt on proteolysis, the ratio of WSN to total nitrogen in all samples did not change significantly. The higher WSN/TN (%) values in cheese produced from ovine milk compared with cheeses from bovine or mixed milk in all stages of ripening could be attributed to the composition of ewe’s milk. Lower levels of WSN/TN (%) in Teleme cheese made from bovine milk in comparison with cheese produced from ovine milk at the age of 60–180 days was reported by Pappa et al. (2007).

When bovine milk is mixed with ovine milk, its ratio of WSN to total nitrogen changed significantly.

Microstructure

Figures 1, 2 and 3 show SEM micrographs of the Lighvan cheese samples produced from sheep’s, cow’s and mixed milk at 1, 30 and 90 days of ripening. For better comparison, SEM micrographs of ovine Lighvan cheese were compiled from our previous study (Aminifar et al. 2010). Tri-dimensional images of the cheese SEM micrographs are shown below the original 2D images. At day 1, while ovine Lighvan cheese had a compact protein matrix with a few void spaces, the protein matrix of Lighvan cheeses made from bovine or mixed milk packed less densely, with cavities filled with whey (Figure 1). The higher density of the ovine cheese structure relative to cow’s or mixed milk could be related to its lower moisture content.

A low-density casein network results in a high-moisture cheese (Kalab and Modler 1985); therefore, the SEM micrographs suggest that bovine and mixed Lighvan cheeses contain more moisture than sheep’s cheese. This was borne out by physicochemical analysis. Regardless of the type of milk, physical homogenous aggregation of casein produced pores in cheese texture after 30 days (Figure 2). Homogeneity in ovine cheese was more pronounced than that of cow’s and mixed milk cheese during ripening (Figures 2 and 3). This homogeneity could be related to the higher salt content of ovine cheese than bovine and mixed cheese. Salt plays an important role in the homogeneity of brined cheese. Chloride ions produced from NaCl diffusion enhance hydrophobic interactions, which increase casein linkages and result in a more homogenous texture (Rowney et al. 2004). Pores were shown with white holes in 3D images. As Lighvan cheeses are made from raw milk, microbial fermentation was the main source of gas production and pore formation during ripening. Due to the rearrangement of the casein network as a result of water expulsion during cheese ageing in brine, cheese made with cow’s and mixed milk became denser after 30 days. Changes during brining in the microstructure of Lighvan cheese manufactured from different kinds of milk could be attributed to curd syneresis (Fox 1993).

Regardless of the type of milk, a homogenous casein network surrounded a large number of holes in the cheese after 90 days (Figure 3). Micrographs of ovine Lighvan cheese showed a large hole which was not found in bovine or mixed samples (marked in Figure 3a). This large cavity could be attributed to a fingerprint of large fat globules that was removed by chloroform during sample preparation. As ovine Lighvan cheese had a higher fat content than cow’s or mixed milk cheeses, its fat globules were larger as a result of clumping and coalescence. Guinee et al. (2000) demonstrated that when fat content of cheese increases, clumping and coalescence of the fat globules becomes pronounced. Disintegration of some fat globules from lipolysis during cheese ripening increases serum release and therefore raises protein matrix density. As a result of this process, the intact fat globules might join into one larger globule and act as a protein matrix breaker (Lopez et al. 2007).

Figures 1–3 show a greater difference between the microstructures of cheeses made with different kinds of milk at the start of ripening than at its end; brining could lessen the difference. 3D images after 90 days (Figure 3) showed that compaction is similar between cheeses made with sheep’s and mixed milk, and greater than that of bovine cheese. This difference could be related to the higher moisture content of Lighvan cheese made with cow’s milk relative to the other two kinds of cheese.

Texture profile analysis

Figure 4 shows changes in the hardness of Lighvan cheeses made with different kinds of milk during ripening.
Figure 1 SEM micrographs of Lighvan cheese made from sheep’s (a, d), cow’s (b, e) and mixed (c, f) milk after 1 day of ripening. (a), (b) and (c) are magnified 500 × and (d), (e) and (f) are magnified 1000 ×. SEM Micrographs of ovine milk cheese were compiled from our previous study (Aminifar et al. 2010). 3D images are shown below the original 2D images.

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Figure 2  SEM micrographs of Lighvan cheese made from sheep’s (a, d), cow’s (b, e) and mixed (c, f) milk after 30 days of ripening. (a), (b) and (c) are magnified 500× and (d), (e) and (f) are magnified 1000×. SEM Micrographs of ovine milk cheese were compiled from our previous study (Aminifar et al. 2010). 3D images are shown below the original 2D images.
Regardless of the type of milk, hardness decreased during first month of ageing. Generally, hardness of cheese during storage in brine depends on two main factors: decreases in moisture during brining, which increases hardness (Kaya 2002), and proteolysis, which decreases hardness due to casein breakdown (Fox et al. 1996). Because in our study Lighvan cheeses were manufactured from different kinds of raw milk, proteolysis was dominant. After 1 month, due to the inhibitory effect of salt on proteolysis, hardness became constant (Guinee and Fox 1993). Substitution of cow’s milk instead for sheep’s milk in Lighvan cheese production decreases its hardness significantly. Pappa et al. (2007) showed that type of milk affected Teleme cheese hardness. They reported that goat’s cheese was significantly harder (5.6–8.9 kg) than cow’s (3.3–5.0 kg) and sheep’s (3.7–6 kg). Tsigkros et al. (2003) demonstrated that textural

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**Figure 3** SEM micrographs of Lighvan cheese made from sheep’s (a, d), cow’s (b, e) and mixed (c, f) milk after 90 days of ripening. (a), (b) and (c) are magnified 500× and (d), (e) and (f) are magnified 1000×. SEM Micrographs of ovine milk cheese were compiled from our previous study (Aminifar et al. 2010). 3D images are shown below the original 2D images.
properties of Feta cheese are affected by milk composition, and its hardness is increased when caprine milk is added to ovine milk. Kalatzopoulos (1993) showed that differences in textural properties of cheese produced from different kinds milk could be related to different casein structures or their concentration. In their study, when ovine milk was added to bovine milk in a 50:50 ratio, the hardness of bovine milk cheese increased significantly as result of an increase in its total solids. At the start of ripening, sheep’s milk cheese was 12 N harder than mixed milk cheese, but at the end of ripening this value had fallen to 7 N. This phenomenon could be related to their moisture contents, which were not significantly different at the end of ripening, in contrast to the beginning.

Figure 5 shows the effect of types of milk and ripening time on Lighvan cheese brittleness. According to Larmond (1976), when the brittleness value of a cheese sample decreases, it becomes more brittle. Regardless of type of milk, all types of Lighvan cheese became more brittle during the first month of ripening. This phenomenon could be related to pore formation and decreases in moisture content during ripening. A similar trend for the elastic modulus of Halloumi cheese made with bovine and ovine milk during ripening was reported by Raphaelides et al. (2006). It seems that migration of water, which could play a plasticising role, from the cheese into the brine reduced the elasticity of all types of Lighvan cheese. Raphaelides et al. (2006) showed that migration of water into empty meshes of Halloumi cheese reduced its matrix rigidity. The greater elasticity of ovine Lighvan cheese in comparison with the two other kinds could be related to its fat content, which could also play a plasticising role. The texture of mixed milk cheese became more elastic as a result of its increased fat content.

Figure 4 Effect of milk origin and ripening time on Lighvan cheese hardness. For better comparison, sheep’s milk Lighvan cheese data were compiled from our previous study (Aminifar et al. 2010).

Figure 5 Effect of milk origin and ripening time on Lighvan cheese brittleness. For better comparison, sheep’s milk Lighvan cheese data were compiled from our previous study (Aminifar et al. 2010).

CONCLUSIONS
This study investigated the effect of substituting ovine milk with bovine milk or mixed ovine and bovine milk in Lighvan cheese production on physicochemical characteristics, microstructure and textural properties of product during ripening. The physicochemical characteristics of Lighvan cheeses produced from different kinds of milk showed significant changes in fat content, moisture content, salt content, TN/DM and WSN/TN changes, also microstructure and porosity, particularly in later stages of ripening, but the rate of these changes differed. Ovine Lighvan cheese was significantly harder than bovine or mixed milk, and the hardness of bovine Lighvan cheese increased when half of its milk was replaced with ovine milk. Substitution of ovine milk with bovine or mixed milk led to a more brittle product.

REFERENCES


