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Original Article

Characterization of Poosti Cheese, a Traditional Raw Sheep Cheese during Ripening: Physicochemical, Microbial and Micro-structural Aspects

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ABSTRACT

Background and Objectives: This study is the first research on the physiochemical characteristics, microbial population and microstructure of Poosti cheese over 90-days of ripening. The main difference between Poosti cheese and other types of traditional cheese is the skin, which is used for its storage.

Materials and Methods: Physicochemical characteristics including moisture, salt, pH, acidity, fat, and rate of water-soluble nitrogen to total nitrogen were measured during the ripening. Total population of mesophilic and psychrotrophic bacteria, *Escherichia coli*, *Coliform*, lactic acid bacteria, yeasts and moulds were determined during the aging. Micro-structural properties of Poosti cheese were evaluated by scanning electron microscopy (SEM). Three-dimensional (3D) images, binarised micrographs and surface plot were obtained by analyzing the two-dimensional (2D) SEM micrographs by Image J software.

Results: The results showed that the moisture content, pH and fat percentage were decreased during the aging; however, salt, acidity, and ratio of water-soluble nitrogen to total nitrogen were increased during this time. Besides, the total count of bacteria was decreased during the aging. Finally, the SEM images indicated that the density of casein network, the number and depth of pores, and also roughness of the structure were increased during the ripening.

Conclusions: According to this study, physicochemical characteristics of Poosti cheese were changed during the aging (salt and fat were increased, while pH and moisture were decreased) as a result of ripening in the skin and high microbial population of raw milk. The microstructure of Poosti cheese was changed during the ripening due to water and salt movement, and the fermentative activity of microbial population.

Keywords: Traditional cheese, Physicochemical properties, Microbial properties

Introduction

Sheep products are appreciated by consumers around the world due to their special characteristics; also they play an important role in the dietary of people who have allergy to bovine milk protein and could not tolerate bovine milk. Many researchers believe that traditional dairy products, especially raw sheep cheeses, should be considered as a cultural and economical product. Although there are different types of home-made sheep cheese, only a few are made in industrial scale (1). There are several types of raw sheep cheese, which are produced in different climates of Iran such as Lighvan in mountainous areas of Azerbaijan (2)(3), Siamezgi in north Iran (4), and Koozeh cheese in west Iran (5). These types of cheese have high importance in Iranian dietary due to their special characteristics; however, there are only a few studies conducted in this field.

Poosti cheese is one of the Iranian traditional cheeses with special properties such as powdery texture with some holes and yellowish appearance. It

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is also considered as a semi-hard, starter-free, and long-ripened cheese. This kind of cheese is traditionally produced from raw sheep milk in the mountains of Sangesar area, suburbs of Semnan province (Iran).

Ripening is one of the most important stages in the formation of special characteristics of traditional cheese (6). It is a slow and complex process, which is comprised of a sequence of microbiological and biochemical reactions. Different aspects of cheese such as microbiological, physicochemical, textural and micro-structural properties are affected by these reactions (7). Unique aroma and texture of traditional raw sheep cheese could be attributed to the microbiological properties of raw milk, the traditional way of production, and the ripening period (8, 9).

In spite of various investigations on the physicochemical and micro-structural properties, as well as the microbial population of different kinds of cheeses during the ripening by several researchers (2, 10-16) there is a lack of information on Poosti cheese in this regard. For this purpose, in the present study, the physiochemical, microbial and micro-structural properties of Poosti cheese, a traditional raw sheep cheese, were investigated over a 90 days of ripening.

Materials and Methods

Cheese making and sampling: Production of Poosti cheese consists of the following steps: At first, the milk was clarified by the thin tissue. Then it was coagulated by lamb rennet at 28-35°C and kept warm to complete its coagulation (about 2 hours). The resulted coagulum was stirred and transferred into rectangular cotton bags for whey drainage. For better whey removal, the cotton bag was compressed by a heavy rock (about 5-6 kg) for 10 days. During this period, salt was added (25%), and the cheese was rubbed every day. The prepared cheese was transferred into four prepared sheepskins and packed tightly. For ripening of Poosti cheese, the filled sheepskins were kept in a man-made cave (Sangesar, suburbs of Semnan, province of Iran) for three months at an average temperature of 4-6°C. In each stage of ripening (1, 30, 60 and 90 days), one of the filled sheepskin was selected for sampling.

Sheepskin container preparation: The intact sheep or goat skin, which has no perforation or rupture, was selected, and then its endoderm was thoroughly cleaned by washing and scraping by knife. To prevent mold formation, it was dried by solar energy. The cleaned and dried skin was rubbed by yogurt and salt to make it white. The prepared sheepskin container is shown in Fig. 1.

Fig 1. Prepared sheepskin container for preservation of Poosti cheese

Physicochemical analysis: The moisture content of Poosti cheese samples was determined according to the IDF Standard 4A (17), and Kirk and Sawyer's method (18) was used to assess its salt content. An Elmetron pH meter and AOAC (17) method were applied for pH measurement and determining the acidity of Poosti cheese samples, respectively. The fat content was also measured following the Gerber's method (19).

In addition to the mentioned characteristics, the total nitrogen was also determined according to the Kjeldahl's method (20). The ratio of water-soluble nitrogen (WSN) to total nitrogen (TN) was used as a proteolysis indicator (21), where WSN was determined in agreement with the Kuchroo and Fox's method (22). According to this method, 20g of the cheese was homogenized with 40 g water in a stomacher for 10 minutes at 20°C. The suspension was heated at 40°C for 1 hour, and then centrifuged at 3000 × g for 30 minutes. The supernatant was then filtered through glass wool and the WSN was measured using the Kjeldahl method (20). All physicochemical properties (moisture content, salt,

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fat, total nitrogen in dry matter (TN/DM), WSN in total nitrogen (WSN/TN), pH and acidity) were the average of at least three separate experiments at each stage of ripening (1, 30, 60, and 90 days).

Microbiological assays: Microbiological properties of Poosti cheese samples were determined after 1, 30, 60 and 90 days of ripening. Ten grams of the cheese were homogenized with 90 ml of 2% (w/v) sodium citrate (Merck Chemical Ltd., Darmstadt, Germany) solution at 45°C in a Stomacher 400 (Seward Ltd., London, UK) for 3×1 minute (23). Total mesophilic aerobic and psychrotrophic bacteria were grown on plate count agar (Merck Chemical Ltd., Darmstadt, Germany) after incubation at 30°C for 72 hours. To confirm the presence of *Escherichia coli*, laurylsulfate-tryptose broth (Merck Chemical Ltd., Darmstadt, Germany) was used within 48-hour incubation at 37°C. Enumeration of coliforms was also carried out by using a violet red bile lactose agar (VRBL, Merck Chemical Ltd., Darmstadt, Germany) medium after 24 hours incubation at 37°C. For determination of lactic acid bacteria, the MRS agar media (Merck Chemical Ltd., Darmstadt, Germany) was incubated at 42°C for 72 hours. In addition, the selective medium as dichloran-rose bengal chloramphenicol agar (DRBC, Merck Chemical Ltd., Darmstadt, Germany), was applied for determination of yeasts and molds at 25°C for 5-7 days. The counts were expressed as common logarithm of colony forming units per gram (CFU/g) of sample.

Microstructure: Microstructure of Poosti cheese samples was studied by scanning electron microscope (SEM) (XL Series, model XL30, Philips, Eindhoven, Netherlands) after 1, 30 and 90 days of ripening according to the Drake's method (24) with some changes in details, which were proposed by Madadlou (25). According to this method, in the first step, cheese cubes (approximately 1.5×1.5×1.5 mm) were prepared by cutting the cheese sample blocks. Then the cubes were fixed by immersion in 2.5% (w/w) gluteraldehyde (Merck Chemical Ltd., Darmstadt, Germany) for 3 hours. Afterwards, the fixed cubes were washed in six changes of distilled water (1 minute each) and dehydrated by 40, 55, 70, 85, 90 and 96% series of ethanol, each for 30 minutes. Then the dehydrated cubes were defatted in three changes of chloroform (10 minutes each). Next, the cubes were covered with ethanol and kept in 3-6°C until SEM analysis. According to Sipahioglu's method (26), before SEM analysis, the cubes were freeze-fractured in liquid nitrogen, dried to critical point, and coated with gold for 6 min in a sputter-coater (Balzers, Type SCD 005, BalTec Inc., Switzerland). The SEM instrument was adjusted at 15.0 kV, and photomicrographs were recorded at 500, 1000, 2000 and 4000 magnification levels. Three-dimensional (3D) images of the micrographs were drawn by interactive 3D surface plot function from the image analysis software (Image J; National Institutes of Health, Bethesda, MD, USA). To obtain better 3D micrographs, settings as z-ratio: 0.18, smoothing: 12.0, lightening: 0.2, scale: 1.34 and grid size: 512 were applied. Surface plots of Poosti cheese were also drawn by *analyze tab menu, surface plot function,* and binarised-SEM images of Poosti micrographs were obtained by the *plugins* tab of the software.

Statistical analysis: The experiment was conducted with three replications. All experimental data were statistically analyzed using a PROC general linear model (GLM) procedure of SAS statistical software (version 8.2, SAS Institute, Inc., Cary, NC), by analysis of variance (ANOVA) followed by Duncan's test during ripening. All assessments were based on a significance level of $p<0.05$ and all the experiments were performed based on one factor repeated designed.

Results

Physicochemical parameters: Moisture content, fat, TN/DM, salt, pH, WSN/TN and titratable acidity values of Poosti cheese samples are shown in Table 1. In the first period, from 1 to 60 days, there was a significant change in pH levels. In the next period, from 60 to 90 days, a significant decrease was observed in pH.

From the 30th day of the ripening, titratable acidity was increased, where no significant difference was observed between the acidity values between 30, 60 and 90 days of aging.

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A significant decrease in moisture content of Poosti cheese was observed during the 90 days of ripening.

The salt content of Poosti cheese was increased from 1 to 60 days. Then, constancy in salt content was observed between 60 and 90 days.

The data from total nitrogen in dry matter (TN/DM) experiments revealed no significant difference between TN/DM (%) values during the aging period but there was an increase in TN/DM(%) at the first month of the ripening.

The WSN/TN ratio had the highest value after one month of ripening and then it did not change significantly.

Finally, the fat percentage was increased during the first month of the ripening. However, this amount was decreased from 30 to 90 days.

Microbial characteristics: The counts of total mesophilic and psychrotrophic bacteria, yeasts and mould, and the presence of *Escherichia coli*, *Coliform*, and lactic acid bacteria during the manufacturing and ripening of Poosti cheese are shown in Table 2. In the first 30 days of ripening, the counts of total mesophilic and psychrotrophic bacteria were high (1.2×10^8 cfu/ml) but these counts were reduced to 1.9×10^7 cfu/ml after 30 days.

From day 1 to day 30, the yeast and mould population was decreased and then completely disappeared until the end of the ripening.

Table 1. Physicochemical properties of Poosti cheese during the ripening (Means ± SD)[e]

Physicochemical characteristics	Ripening period (days)				
	Flocculation	1	30	60	90
pH	5.298±0.01[c]	5.333±0.01[c]	5.566±0.02[b]	5.600±0.05[a]	5.277±0.01[c]
Titratable acidity (%, w/w)	0.85±0.005[c]	0.87±0.01[b]	1.488±0.06[a]	1.544±0.01[a]	1.466±0.01[a]
Moisture content (%, w/w)	49.677±0.5[a]	39.176±3.7[b]	27.533±0.7[c]	20.412±2.5[c]	12.944±0.4[d]
Salt (%, w/w)	4.08±0.01[d]	3.523±0.05[c]	6.549±0.1[b]	7.766±0.06[a]	7.733±0.08[a]
TN/DM (%, w/w)	18.893±0.3[c]	10.27±0.4[b]	27.193±0.9[a]	29.00±1.9[a]	27.99±2.4[a]
Fat(%, w/w)	21.933±0.4[c]	24.766±0.3[b]	27.666±0.3[a]	24.233±0.5[b]	24.166±0.4[b]
WSN/TN (%, w/w)	30.933±0.04[c]	35.89±0.05[b]	40.666±1.4[a]	39±1.1[a]	38.72±1.4[a]

[a-d] Means within the same row with different superscripts differ significantly ($p < 0.05$).
[e] Response shows as mean ± standard deviation for 3 replications.

Table 2. Effect of ripening on microbial characteristics of Poosti cheese

Microbial characteristics	Ripening period (days)				
	Curd	1	30	60	90
Total microbial count (cfu/g)	+	++++	+++	++	++
Yeasts and mould (cfu/g)	+++	++	-	-	++
Escherichia coli (cfu/g)	+	+	-	-	+
Coliform (cfu/g)	+	+	-	-	+
Lactic acid bacteria (cfu/g)	+	++	+	+++	+++

The sign of + indicates the relative activity of microorganism and – indicates non activity of the microorganism.

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Microstructure: The SEM micrographs with 500, 1000, 2000, and 4000 magnifications and the 3D images of the Poosti cheese samples at 1, 30, and 90 days of ripening are shown in Figures 2-4. In the first day of ripening, the cheese textures comprised of casein network along with a few pores (Fig. 2 a-d). At this time, a homogenous network with low depth pores was also observed in the 3D images (Fig. 2 e-h).

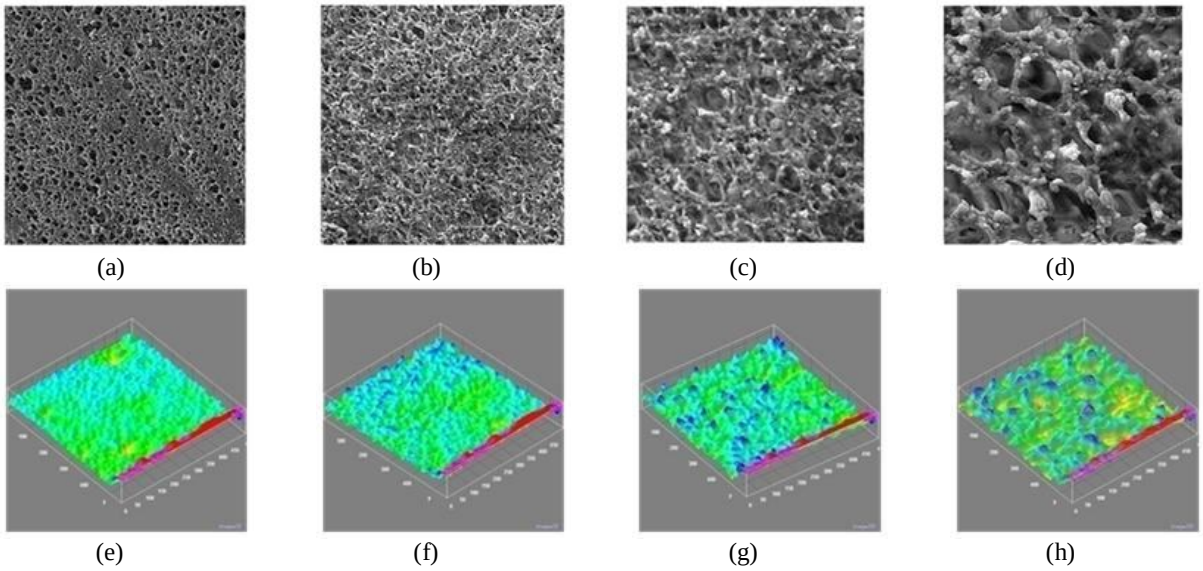

Fig 2. Scanning electron microscopy (SEM) micrographs of Poosti cheese samples at day 1 of ripening with (a) 500, (b) 1000, (c) 2000, and (d) 4000 magnifications. Three-dimensional (3D) images of SEM micrographs with (e) 500, (f) 1000, (g) 2000, and (h) 4000 magnifications are shown below the original 2D images.

During the 30 days of ripening, the microstructure of Poosti cheese samples was significantly changed as shown in Fig. 3 (a-d). At this time, the density of protein network was increased (increase in the compaction of 2D and 3D-images). In addition to the mentioned changes, 3D images of 30 days microstructure also showed that the size of pores increased during the first month of ripening (Fig. 3 e-h).

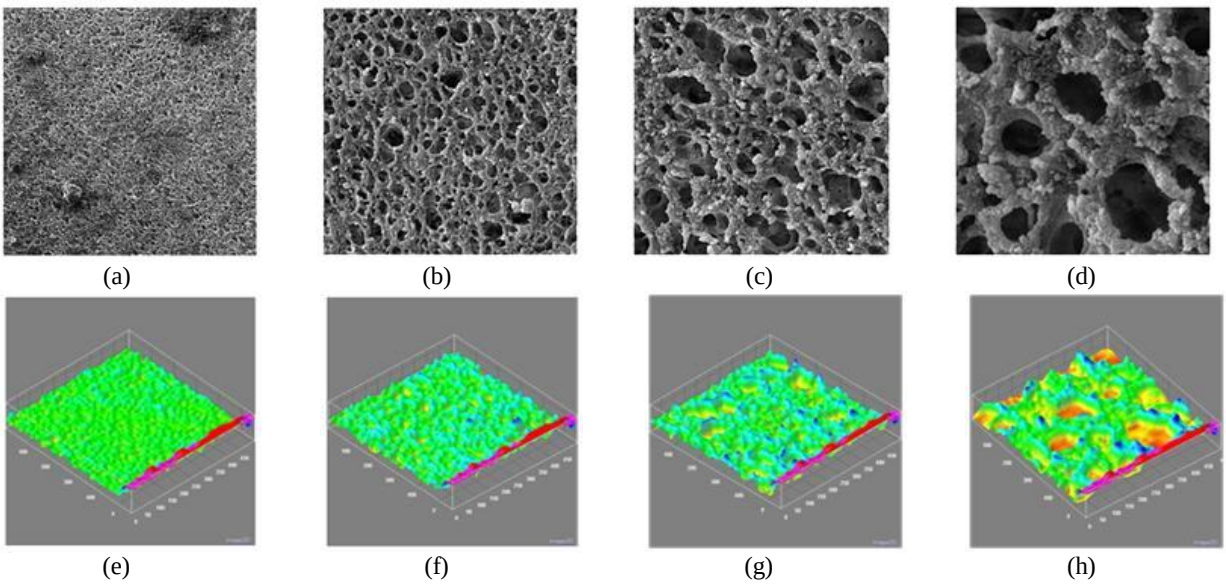

Fig 3. Scanning electron microscopy (SEM) micrographs of Poosti cheese samples after 30 days of ripening with (a) 500, (b) 1000, (c) 2000, and (d) 4000 magnifications. Three-dimensional images (3D) of SEM micrographs with (e) 500, (f) 1000, (g) 2000, and (h) 4000 magnifications are shown below the original 2D images.

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From day 30 to 90 of ripening, the density of the casein network was increased (Fig. 4 a-d). It is obviously seen that the change in the microstructure of Poosti cheese in the first month of ripening is more than this period. According to the 3D images (Fig. 4 e-h), the size of pores was increased during 30 to 90 days of ripening. Increase in pore depth is accompanied with the appearance of orange and red patches in the 3D images (4000x).

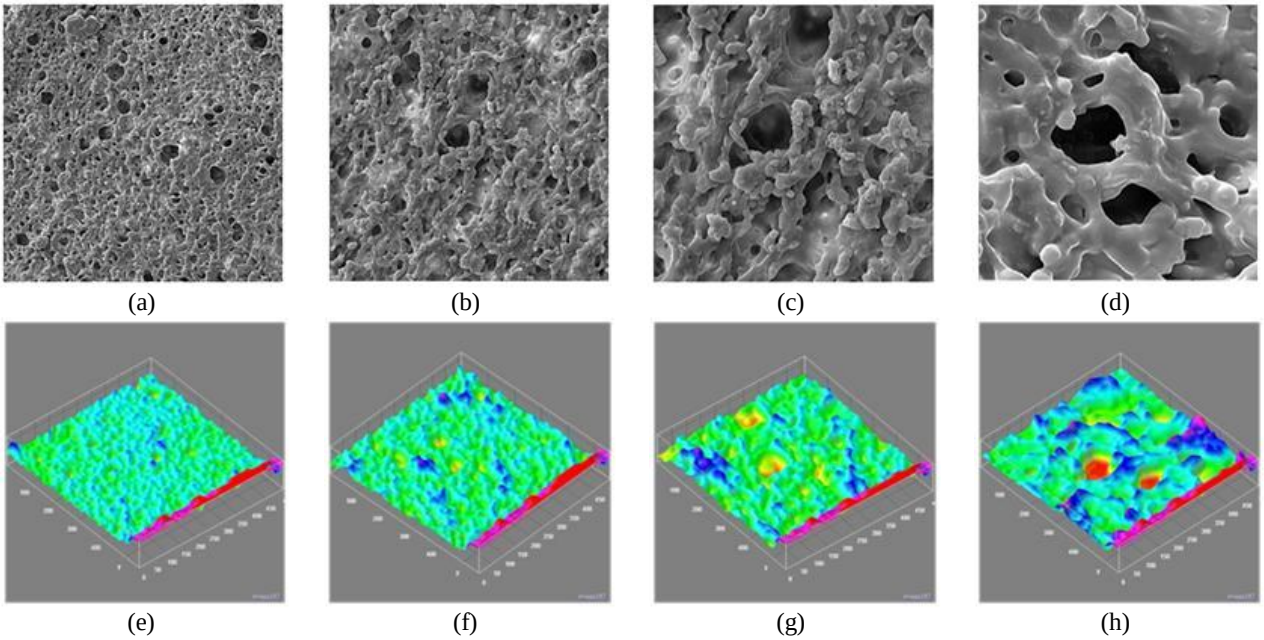

Fig 4. Scanning electron microscopy (SEM) micrographs of Poosti cheese samples after 90 days of ripening with (a) 500, (b) 1000, (c) 2000, and (d) 4000 magnifications. Three-dimensional images (3D) of SEM micrographs with (e) 500, (f) 1000, (g) 2000, and (h) 4000 magnifications are shown below the original 2D images.

The surface plot and binarised-SEM images of Poosti cheese samples at 1, 30, and 90 days of the ripening at 4000 magnification are shown in Fig. 5 (a-f). As shown in Fig. 5 (a-c), the roughness of the structure gradually increases during 90 days of ripening. Binarised-micrographs showed an increase in black patches (Fig. 5d-f) during the aging.

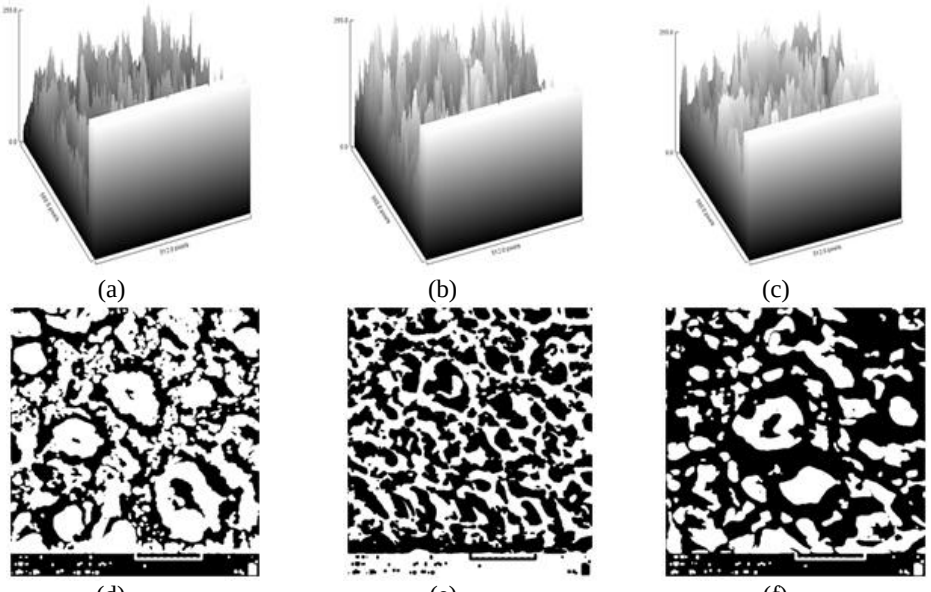

Fig 5. Surface plot after (a) 1, (b) 30, and (c) 90 days and binariesd SEM micrographs after (a) 1, (b) 30, and (c) 90 days of Poosti cheese ripening.

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Discussion

Physicochemical parameters: The changes in pH levels of cheese were dependent on the acids and amino groups resulting from the fermentation of lactose and protein degradation by the microorganisms, respectively (27). The negligible change in pH from 1 to 60 days could be related to the balance between acids and amino groups produced through the activity of diverse microorganisms in the raw milk and sheepskin. Decrease in pH level within 60-90 days could be attributed to the decrease in protein degradation by the microorganisms as a result of salt adsorption and dominance of the lactic acid bacteria (27).

Increase in the acidity in the first month of ripening was due to the activity of mesophilic bacteria and lactobacillus (23), and further non-significant change could be considered as a result of balance in the microbial population.

A significant decrease in the moisture content of Poosti cheese in the 90 days of ripening could be related to the presence and high osmotic pressure of salt in the surrounding of cheese texture (28), and also due to the adsorption of moisture by the skin used for cheese container. Interestingly, there is a difference between the trend of changes in the moisture content of Poosti and Lighvan cheeses, which could be attributed to their surrounding media during the ripening; as salted Poosti cheese was kept in sheepskin but the other aged in brine (29).

The salt content of Poosti cheese increased during 60 days of ripening as a consequence of salt diffusion into the cheese texture. No significant difference in salt content within 60-90 days of ripening could be due to the formation of equilibrium between salt diffusion into the texture and water expel from the cheese (28). Additionally, the results showed that increase in the salt content of Poosti cheese was accompanied with pH changes due to the inhibitory effect of salt on the proteolytic microorganisms (30).

Non-significant difference between TN/DM (%) values during the aging are not in agreement with the TN/DM (%) values obtained from the Lighvan cheese study, which decreased during the ripening (29). This disagreement could be related to the medium used for keeping the cheese; as in Lighvan cheese, the products of proteolysis were diffused into the brine but they remained intact in the Poosti cheese texture.

The highest value WSN/TN ratio after one month of ripening could be attributed to the activity of the proteolytic microorganisms, degradation of large protein, and formation of soluble peptides (31). The WSN/TN values of Poosti cheese were higher than those of Lighvan cheese (29). The higher population of proteolytic microorganisms in Poosti cheese as a result of raw milk and the skin container could cause this. The non-significant change in WSN/TN ratio during 60-90 days of ripening was due to the inhibition of proteolytic microorganisms by salt (30).

Finally, increase in the fat percentage during the first month of ripening was resulted from water expel from the texture; however decrease in its content from during 30-90 days could be due to lipolysis process and conversion of fat into volatile compounds (5).

Microbial characteristics: In the first 30 days of ripening, the high counts of total mesophilic and psychrotrophic bacteria could be explained by the diverse microbial population of raw milk, the sheepskin container, and also the lack of thermal processing during the manufacturing procedure. After 30 days, the reduce in these counts was due to the inhibitory effect of salt (23).

From day 1 to day 30, decrease in the yeast and mould population could be related to the inhibitory effect of salt (33), and also the dominance of lactic acid bacteria (34). Disappearance of *Coliforms* and *Escherichia coli* is due to the inhibitory effect of salt (35), change in pH levels, and the dominance of lactic acid bacteria (34).

Dominance of lactic acid bacteria at the end of the ripening process could have several reasons. The main source of these bacteria is the raw milk and the sheepskin container. Furthermore, since the most types of microorganisms were inhibited by salt, lactic acid bacteria had more chance to populate (5).

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Microstructure: The increase in the density of protein network (increase in compaction of 2D and 3D-images) was related to the salt and water movement during the ripening process in the skin. The use of NaCl crystal in Poosti cheese production process was also responsible for curd synersis. Diffusion of salt into the texture provides chloride ions, which are responsible for increase in the hydrophobic interactions among the proteins (36); as other researchers have demonstrated similar mechanism during the cheese ripening (25) This phenomenon was due to increase in the repulsive force between the negative ions in the cheese texture, which leads to increase in the hydrophobic interactions between the proteins. Besides, the compaction of Poosti cheese is more than that of other traditional cheeses such as Lighvan cheese (29) due to the difference in their ripening circumstances. Results obtained from other studies revealed that accumulation of protein and more homogeneity in the cheese texture could be observed by increasing the salt concentration (32). Increase in the pore size in the first month of ripening could be attributed to the fermentative activity of the raw milk's and sheepskin's microbial population (31).

Curd synersis is responsible for the increase in casein network density from day 30 to day 90 of ripening. It is clear that the change in the microstructure of Poosti cheese in the first month of ripening is more than in other stages of this process. This matter could be related to the equilibrium in water and salt movement (which was reached after one month of ripening) and also the inhibition of proteolytic microorganisms as a result of salt adsorption (29). Moreover, such a result was in accordance with the findings from the physicochemical and microbial analyses. Increase in pore size within 30-90 days of ripening could be attributed to the increased lactic acid bacteria fermentation.

Increase in the roughness of the structure was due to the increase in the size and the depth of the pores. A similar change in the depth of pores at the last stage of ripening was also reported by Madadlou et al. (25).

Increase in black patches in the binarised micrographs comes from the increase in casein network density around the pores, and could be due to water expel from the cheese texture (37).

This study aimed to investigate the physicochemical properties, microbial population, and microstructure of Poosti cheese produced from the raw sheep milk during 90 days of ripening. The results showed that the physicochemical characteristics were changed all along the aging as a result of storage of cheese in the sheepskin and the microbial population of raw milk. Additionally, the changes in microstructure images during the ripening of this traditional cheese also revealed an increase in casein network density and pore formation.

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