

# MICROBIOLOGICAL, BIOCHEMICAL AND RHEOLOGICAL CHANGES THROUGHOUT RIPENING OF KURDISH CHEESE

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## ABSTRACT

Kurdish cheese has been traditionally prepared from raw milk in the northeast of Iran. It is ripened in goat's skin bags. This study was conducted on microbiological diversity and physicochemical properties of Kurdish cheese during ripening (1, 20, 40 and 60 days). The results indicated that the type and counts of microorganisms were mainly influenced by ripening time. Lactic acid bacteria and Enterobacteriaceae were predominant during the first 20 days of ripening, and lactobacilli were the most common microorganisms found during ripening. The initial numbers of coliforms and *E. coli* decreased rapidly, whereas a gradual increase in counts of moulds and yeasts was noticed in the early days of ripening. Coliforms, *Salmonella* and coagulase-positive *Staphylococcus* spp. were undetectable in ripened cheese. The optimal time for consuming is after 60 days because the undesired biota decreased below dangerous limits. pH, moisture content and aw decreased during ripening. No significant variations were observed in protein values during ripening. The textural parameters such as hardness, chewiness and gumminess increased as ripening progressed, but springiness decreased. The level of microelements in cheese samples during maturation were in the following order Na > Ca > K > Zn > Cu.

## PRACTICAL APPLICATIONS

Kurdish cheeses have been traditionally prepared, and the market for them has existed in many parts of Iran for many years. Its microbial diversity originates from natural microbiota present in the raw milk, and environmental exposures are important points to consider for public health. There is an increased demand from consumers for high-quality natural food, free from contaminating microorganisms. Hence, indigenous lactic acid bacteria were identified successfully for production of fermented milk products with increased safety. Given the significant economic importance of Kurdish cheeses to a number of small domestic and industrial producers, particularly in the northeast of Iran, this study can be used to help implement strategies designed to enhance the product's microbiological and physicochemical quality.

## INTRODUCTION

Traditional cheeses made from raw milk display considerable variability, forming a complex microbial ecosystem among dairies (Pinto *et al.* 2006). Iran has a very long tradition of producing varieties of cheese such as Lighvan and

Koozeh (Navidghasemzad *et al.* 2009; Edalatian *et al.* 2012). Kurdish cheese locally named "Panere-Kurdi" has been traditionally made from cow or sheep whole raw milk or a mixture of both. It has a white to creamy color and usually considered as a semi-hard cheese. The cheese has a relatively high fat content (especially when made from sheep milk),

a crumbly texture and a buttery flavor. Goat's skin bag is used to package and ripen the cheese. Goat's skin bags are porous and therefore permeable to water and air. In old days, the goat's skin was used for cheese packaging probably because of the lack of alternative materials (Hayaloglu *et al.* 2007).

Diversity of lactic acid bacteria (LAB) isolated from Kurdish cheese was investigated by Mortazavi *et al.* (2007). They concluded that a wide diversity of LABs, mainly lactobacilli (65.7%), were found to be present in the cheese, which give various attributes to the product.

Studies on Kurdish cheese are very limited, but a number of studies have been reported on Tulum, which is ripened and kept in almost a similar way (Ceylan *et al.* 2003; Erdogan *et al.* 2003; Guler and Uraz 2004).

Generally speaking, consumers prefer Kurdish cheese made in the traditional style than those made in the modernized or modified approach since most believe that cheese ripened in goat's skin bag is superior to cheese ripened elsewhere, e.g., in polyethylene bags.

No starter culture is used and the fermentation solely relies on natural microbiota of the milk. It is matured in brine while kept in goat's skin bags, the most distinctive aspects of the process. Food infections associated with the consumption of raw milk cheese pose a threat to public health, leading to big economic losses (Jaai Kim and Lee 2013). Besides, these pathogen microorganisms as well as LAB originated from the raw milk influence the ripening process in various ways including production of lactic acid, proteolysis and lipolysis and a decrease in the oxidation-reduction potential and pH, which contribute to its characteristic flavor, taste and texture (Cetinkaya and Soyutemiz 2006).

It is without doubt that for consumers, texture, particularly in cheese, is one of the most important attributes that help to determine the identity of a product as well as describes the attribute of a food material resulting from a combination of physical and chemical properties (Buffa *et al.* 2001 and Quigleya *et al.* 2011).

The demand for traditional cheeses has increased in Iran, like in many other parts of the world, and therefore studies are needed to develop a better understanding of the technology, chemistry and microbiology of such cheeses. Like many traditional cheeses, Kurdish cheese is understudied and therefore the aim of the present study was to evaluate microbial status, especially hygienic aspects, physicochemical and rheological characteristics of the Kurdish cheese over the ripening period.

## MATERIALS AND METHODS

### Cheese-Making

Four batches of cheese were made in the traditional manner. Raw ovine milk was thermized at approximately

$40 \pm 5$  C and transferred to cheese vats. Then it was coagulated by adding commercial calf rennet, the mixture was incubated for 45–60 min to allow clotting. Afterwards, dry salt was added to batches. The curd is then cut into small grains and pressed (1–2 kg for each kg of curd) to have the whey drained out. The pressed curd was placed in brine (14%, w/v) and transferred to the refrigerator for 1 h and finally placed inside the goat skins, through the neck. The skins were filled with brine (10–12%, w/v), hermetically sealed and stored at a constant temperature of  $5 \pm 1$  C for 2–3 months. The skins were disinfected in boiling water for 20 min prior to cheese making.

### Sampling

Cheese sampling was performed on days 0, 20, 40 and 60 of ripening, according to Anon (1996). For microbiological analyses, 20 g of each cheese sample, taken from the interior, were homogenized (Stomacher 400, Seward Laboratory Blender, BA 7021, London, U.K.) for 2 min with 180 mL of sterilized sodium citrate (2%, w/v; Merck KGaA, Darmstadt, Germany). Decimal dilutions were prepared in sterilized ringer solution (Oxoid, Hampshire, U.K.) and aliquots of 100  $\mu$ L were plated on selective media in duplicate as described below. Except for enterococci, the media for plating bacteria were supplemented with cycloheximide at 170 mg/kg. Results from plate counts were confirmed by microscopic observation. The colonies with characteristic properties were counted in appropriate dilutions and reported as colony forming units (cfu/g) of cheeses.

### Media

The following media were used for enumeration of different groups of microorganisms: M17 agar for presumptive mesophilic and thermophilic coccid-shaped LAB at 37 and 45C for 48 h; MRS agar (pH 5.4; Oxoid, CM361) for presumptive mesophilic and thermophilic lactobacilli at 30, 37 and 45C for 48 h and anaerobically. Enterococci were counted on Kanamycin Esculin Azide Agar at 37C for 48 h aerobically (IDF 1998). Total mesophilic bacteria were counted on Plate count Agar at 37C for 72 h (IDF 1994). YGC was used for enumeration of yeasts and moulds at 25C for 5 to 7 days (IDF 1990a,b). Total and fecal coliform bacteria were calculated using the most probable number (MPN) technique. Coliforms were enumerated on Violet Red Bile Agar at 37C for 24 h. Brilliant green lactose bile broth with Durham tube was used to determine coliforms at 45C for 48 h, according to IDF (1994); *Escherichia coli* was measured in lauryl sulfate broth, following 48 h of incubation at 37C. Positive tubes were transferred to tubes containing Brilliant Green Bile Broth (BGLB) and incubated

for 48 h at 37C (Hayaloglu *et al.* 2007). The number of test tubes giving positive results with the BGLB was noted. Samples from the positive BGLB tubes were transferred into EC broth and incubated in a water bath at 45C for 24 h for determining fecal coliform bacteria and *E. coli*, as described by Downes and Ito (2001). Contents of the positive tubes were transferred into sterile tryptone broth for 48 h at 44C and Indol tests was conducted (IDF 1994). Baird Parker agar supplemented with egg yolk tellurite emulsion was used for the isolation of *Staphylococcus aureus* at 37C for 48 h. For identification of *S. aureus*, black colonies surrounded by a white clear zone were gram-stained. Colonies were selected and inoculated in brain heart infusion broth at 37C for 18 h and transformed in rabbit plasma after incubation at 37C for 6 h; the broth was tested for the biochemical identification of coagulase-positive species (Downes and Ito, 2001). *Salmonella* spp. detection was carried out after pre-enrichment in 1% buffered peptone-water (24 h at 37C); 25 g cheese samples were added to 225 mL of pre-enrichment solution and homogenized according to IDF (1995). After the pre-enrichment process, the culture was put on a selective enrichment base. Selenite cystine broth was used for selective enrichment. The culture was incubated for 48 h at 37C after adding 10 mL pre-enrichment culture to 100 mL selenite cystine broth medium. Following the selective enrichment process, the culture was plated on a selective medium, Bismuth Sulphite Agar, and incubated at 37C for 24 h. Typical colonies with *Salmonella*'s characteristics were inoculated on Nutrient agar medium and kept for 24 h at 37C. Pure colonies were then gram-stained and examined under the microscope. Typical colonies were plated out on Triple Sugar Iron Agar and were tested for urease tests (Oxoid), Voges-Proskauer test, Indole test and Lysine decarboxylase test after 24 h incubation at 37C confirmatory tests were done for *Salmonella*.

### Physicochemical Analyses

Samples of cheese were analyzed for fat content by Gerber method and total proteins by macro-Kjeldahl. The moisture, NaCl and ash contents were measured according to AOAC (2007); the salt content was expressed as salt in moisture concentration. The pH was measured using a pH meter (Metrohm, Herisau, Switzerland). Water activity ( $a_w$ ) at 25C was determined on triplicate samples using the AQUA LAB Water Activity Measurement (Decagon, Pullman, WA).

### Mineral and Trace Metal Levels in Cheese

Cheeses were analyzed chemically for macrominerals including Na, K, Ca and microminerals (trace elements) such as Zn and Cu by atomic absorption spectrophotometer

(Biochrom, Cambridge, U.K.), according to Mendil (2006). Readings were made on dilutions obtained from recovery of the solubilized ashes, by getting aliquots directly from them for the spectrophotometric analysis (Gonzalez *et al.* 2009).

### Texture Profile Analysis

Cheese cubes (20 × 20 × 20 mm) were tempered to 12 ± 0.5C and used for texture profile analysis using the Texture Analyser (TA1000, CNS-Farnell, England). The TA17 probe (30 × 25 mm diameter) was used and the test was carried out at a crosshead speed of 1 mm/s and penetration distance of 10 mm. Hardness, gumminess, springiness and chewiness were evaluated in triplicate, according to the definitions given by Awad (2006).

### Statistical Analysis

The data were presented as the mean values of four batches of cheese analyzed in duplicate. Statistical analysis was performed using ANOVA. MINITAB 14 statistical software (Minitab Inc., State College, PA) at the significance level of 0.05 ( $P < 0.05$ ) was used for evaluation. All experiments were performed in duplicate. Duncan multiple range tests with a confidence interval of 95% was used to compare the means.

## RESULTS AND DISCUSSION

### Microbiota of the Kurdish Cheese and Changes in Microbial Populations Over Ripening

The total mesophilic counts decreased from 9.93 log cfu/g (on day 0) to 9.06 at 60 days of ripening (Table 1). The high total bacterial counts at the first day might be due to the observed high coliform count in the cheese. These counts were similar to those obtained from several cheeses, such as San Simon cheese (García Fontán *et al.* 2001; Mangia *et al.* 2011). Milk is usually contaminated by yeasts and moulds from the surrounding environment during lactation, cheese-making or through contact with equipment. During ripening of Kurdish cheese, counts of yeasts and moulds increased, the maximum counts was found to be 5.95 log cfu/g (20th days of ripening) and minimum of 5.60 (day 0) (Table 1). This may be closely related to depletion in lactose content and the simultaneous utilization of LAB numbers, while their contribution into the ripening process is due to their proteolytic and lipolytic activities. However, one should keep in mind that the counts of yeasts enumerated can be attributed to variation in salt concentration and temperature as well as the standards of hygiene prevailing during cheese making (El-Owni and Hamid 2008).

**TABLE 1.** CHANGES IN MICROBIAL POPULATIONS (LOG CFU/G) ± STANDARD DEVIATION (SD) AT DAY 0, 20, 40 AND 60 OF RIPENING OF KURDISH CHEESE

Parameter	Ripening time (days)			
	0	20	40	60
Total mesophilic microflora	9.93 <sup>a</sup> ± (1.21)	9.48 <sup>ab</sup> ± (0.79)	9.28 <sup>ab</sup> ± (0.25)	9.06 <sup>ab</sup> ± (0.39)
Yeasts and moulds	5.60 <sup>ab</sup> ± (0.98)	5.95 <sup>a</sup> ± (0.44)	5.79 <sup>a</sup> ± (1.22)	5.71 <sup>a</sup> ± (0.81)
Coliforms	8.22 <sup>a</sup> ± (0.12)	7.99 <sup>ab</sup> ± (1.19)	5.13 <sup>bc</sup> ± (0.32)	1.05 <sup>ef</sup> ± (0.51)
<i>Escherichia coli</i>	5.27 <sup>a</sup> ± (0.23)	5.09 <sup>a</sup> ± (0.29)	3.30 <sup>c</sup> ± 0.16	<1.00 <sup>ef</sup>
<i>Salmonella</i>	2.11 <sup>a</sup> ± (1.03)	1.12 <sup>ab</sup> ± (1.14)	0.518 <sup>b</sup> ± 0.89	<1.00 <sup>c</sup>
Staphylococci	3.06 <sup>a</sup> ± (0.63)	1.22 <sup>bc</sup> ± (0.47)	1.02 <sup>bc</sup> ± (0.16)	<1.00 <sup>c</sup>
Mesophilic lactococci	7.53 <sup>ab</sup> ± (1.41)	8.12 <sup>b</sup> ± (0.15)	7.39 <sup>b</sup> ± (0.81)	6.85 <sup>b</sup> ± (0.66)
Thermophilic lactococci	7.61 <sup>a</sup> ± (0.33)	7.47 <sup>a</sup> ± (0.03)	7.17 <sup>ab</sup> ± (0.47)	6.94 <sup>b</sup> ± (0.83)
Mesophilic lactobacilli	7.68 <sup>a</sup> ± (0.18)	7.72 <sup>a</sup> ± (1.34)	7.36 <sup>a</sup> ± (1.23)	7.21 <sup>a</sup> ± (1.16)
Thermophilic lactobacilli	8.27 <sup>a</sup> ± (1.32)	7.53 <sup>a</sup> ± (0.17)	7.26 <sup>ab</sup> ± (0.07)	7.07 <sup>b</sup> ± (0.29)
Enterococci	6.98 <sup>a</sup> ± (0.09)	7.17 <sup>a</sup> ± (0.55)	6.96 <sup>a</sup> ± (0.73)	6.91 <sup>a</sup> ± (0.77)

Means in the same row with different letters are significantly different ( $P < 0.05$ ).

Values in parentheses indicate standard deviation related to three replicates ( $n = 3$ ).

Numbers of microorganisms such as coliforms and *E. coli* are indicative of hygiene quality. Iranian cheese Standard 5486-2 regulates that white cheese should contain coliforms at no more than 100 cfu/g, and no *E. coli* should be present in 1 g of cheese. In our study, ripening time had a significant ( $P < 0.05$ ) influence on the numbers of coliforms and *E. coli*, with counts in fully ripened cheeses at 5–6 log units lower than that of day 0 cheeses (Table 1). In addition, the largest decrease was in coliforms, which decreased by 8 log unit in the period studied. Aly and Galal (2002) and Kayagil and Candan (2009) stated that coliforms grow only during the early stages of cheese-making, when conditions such as pH and temperature are favorable. Based on Caridi *et al.* (2003) results, it has been demonstrated that strains of *Lactobacillus paracasei* have an intense antagonistic activity against *E. coli* through bacterocin production. Our results were in accordance with Ceylan *et al.* (2003) results, who reported a significant negative correlation between yeast–mould contents and coliform counts due to acid-forming ability. Ceylan *et al.* (2003) concluded that the majority of cheeses start ripening with a high number of coliforms and *E. coli*. Nevertheless, almost all the cheeses are *E. coli*-free at the end of the ripening time. To explain the rapid disappearance of *E. coli* during the ripening of cheese, it may be probably due to the decline of pathogenic microorganisms during ripening. They include antagonistic action of lactic acid basically by causing a decrease in pH and an increase in lactic acid concentration, effect of high salt level on their growth, low aw and low temperature of storage (Ceylan *et al.* 2003).

Salmonellosis continues to persist as a problem in the food industry. Based on our results, pathogenic species of *Salmonella* were not detected in any sample at the end of ripening period (Table 1; also refer to the initial level). Mangia *et al.* (2011) reported that *Salmonella* survived very poorly in Pecorino cheese. Although this cheese had a

relatively high pH (5.6), acid-adapted cells were detected after 14 days but were not detected in subsequent samplings. Bintsis and Papademas (2002) concluded that in Feta cheese made from raw milk, *Salmonella* was completely inhibited because the strain could not survive the high salt concentrations (70 g/L NaCl). The numbers of *Salmonella* decreased at a rate depending on the salt concentration, starter activity and the storage time.

The presence of coagulase-positive *Staphylococcus* and the occurrence of enterotoxins in food are important parameters for the evaluation of food safety for the consumers (Leloir *et al.* 2003). In our study, the initial population of *Staphylococcus* decreased from 3.06 log cfu/g (on day 0) to 1.12 at 40 days of ripening; however, no coagulase-positive *Staphylococcus* was detected in any of the samples at the end of ripening period (Table 1). Our findings agreed with El-Owni and Hamid (2008). It is well known that ripening acts as a natural selector, during which LAB normally inhibit pathogens (Cetinkaya and Soyutemiz 2006). Some LAB not only are resistant to acid but require a low pH to grow; Lactobacilli can lower their internal cytoplasm to 4.4 to 4.8 and survive well at an external pH of 3.5 (Hayaloglu *et al.* 2007). The low internal growth-limiting pH probably contributes to the ability of lactobacilli to lower the external pH to values lethal to *Staphylococcus* and related pathogens during food fermentation. These beneficial organisms have a clear advantage for survival in these products, and their metabolism contributes to the elimination of pathogens (Oner and Karahan 2006).

Table 1 shows the distribution rate of lactic bacteria identified throughout Kurdish cheese ripening times. In this study, LABs were the leading biota during ripening of Kurdish cheese. As well as the lactic biota, there is an adventitious biota which can also contribute to the ripening of cheeses (Cichoscki *et al.* 2002). High LAB counts were also reported in different varieties of cheese made from raw milk

(Las Casas Lima *et al.* 2008; Nespolo *et al.* 2010; Mangia *et al.* 2011).

During ripening, mesophilic lactococci were maximized earlier and then slightly decreased. As a consequence, from 0 to 20 days, this group increased only by 1.0 log unit for the samples. In other studies on raw milk cheeses, the predominance of lactococci during the early stages of ripening has also been reported (Manolopoulou *et al.* 2003; Quigleya *et al.* 2011).

The predominance of lactobacilli throughout the ripening process is shown in Table 1. The same phenomenon was observed by Cichoski *et al.* (2002), Navidghasemzad *et al.* (2009); According to their documents, during the fermentative process of cheese, the formation of several metabolites such as lactate, citrate, glycerol and amino acid among others, which are better utilized by lactobacilli, take place. Mangia *et al.* (2011) reported that in Teleme, lactococci were found to be present only in the curd; the low pH and high salt content seems to favor the growth of lactobacilli.

Ripening time had a significant influence over the counts of these populations. Also, their numbers decrease during ripening at a rate dependent to some degree on the sensitivity of different species of LAB to salt, water activity and autolysis power of the strains. The ability of LAB to produce lactic acid helps to reduce the pH, which in turn increases the expulsion of whey from the curd, thus lowering the moisture content; this diminishes the propensity for microbial spoilage (Caridi *et al.* 2003).

### Changes in Chemical Composition During the Ripening of Kurdish Cheese

Mean values for chemical composition of cheeses and changes throughout ripening are shown in Table 2. In this

study, the pH of the cheese showed a decreasing trend until the end of the ripening period. The highest value (5.29) was obtained at the beginning of the storage period, while the lowest (4.69) at the end of the storage period. Oner and Karahan (2006) stated that in produced cheeses according traditional methods, pH was found to be between 4.38 and 5.94. The pH of cheese is influenced by the growth of both starter and non-starter LAB in raw milk cheeses. In general, lactic acid is the major inhibitor to the acid-sensitive microorganisms, because their proportion is higher than the other acids during coagulation and ripening of the cheeses (Ceylan *et al.* 2003). According to some studies results, there is an increase in pH at the end of cheese ripening; it has been attributed to the utilization of lactic acid, formation of nonacidic decomposition products and liberation of alkaline products of protein decomposition (Manolopoulou *et al.* 2003; Mucchetti and Neviani 2006; Quigleya *et al.* 2011).

Moisture content of cheeses steadily decreased throughout ripening (nearly 23–24%) due to water surface evaporation (Table 2). Aly and Galal (2002) reported that moisture declined as storage time progressed. It might be attributed to degradation of protein and fats.

Fat content of samples increased slightly during ripening. Fat content was found to reach a maximum of 28.1% after 60 days, from a minimum of 20.5% at the first day when cheeses were manufactured. The values were similar to Mangia *et al.* (2011) results for Pecorino cheese. The high average fat contents in this study could be due to high fat content in sheep milk used for cheese-making.

As shown in Table 2, protein content gradually increased during cheese ripening ( $P < 0.05$ ). The lowest protein content (20.29%) was obtained at the beginning of the storage period, while the highest (26.03%) was recorded at

Parameter	Ripening time (days)			
	0	20	40	60
pH	5.29 ± (0.52) <sup>a</sup>	4.82 ± (0.56) <sup>b</sup>	4.77 ± (0.16) <sup>b,c</sup>	4.69 ± (0.09) <sup>c</sup>
Moisture (%w/w)	62.27 ± (0.49) <sup>a</sup>	43.5 ± (0.60) <sup>b</sup>	40.03 ± (0.41) <sup>b,c</sup>	33.91 ± (1.23) <sup>c</sup>
Total solid (%)	38.73 ± (0.09) <sup>a</sup>	56.85 ± (0.56) <sup>b</sup>	60 ± (0.85) <sup>b,c</sup>	64.07 ± (0.47) <sup>c</sup>
Fat (%)	20.5 ± (0.17) <sup>a</sup>	24.2 ± (1.00) <sup>b</sup>	27.13 ± (0.49) <sup>b,c</sup>	28.1 ± (0.32) <sup>c</sup>
Protein (%)	20.29 ± (0.25) <sup>a</sup>	22.3 ± (0.38) <sup>a</sup>	24.1 ± (1.17) <sup>ab</sup>	26.03 ± (1.26) <sup>ab</sup>
$a_w$	0.974 ± (0.30) <sup>a</sup>	0.953 ± (0.21) <sup>b,c</sup>	0.941 ± (0.26) <sup>c,d</sup>	0.927 ± (0.32) <sup>d</sup>
NaCl-in-moisture%	3.18 ± (0.39) <sup>a</sup>	5.55 ± (0.11) <sup>b</sup>	5.64 ± (0.31) <sup>b</sup>	5.71 ± (0.41) <sup>b,c</sup>
Ash (%)	3.92 ± (0.29) <sup>a</sup>	6.24 ± (0.09) <sup>b</sup>	8.86 ± (0.72) <sup>c</sup>	9.53 ± (0.57) <sup>c</sup>
Ca (mg/g Ash)	158/60 ± (1.01) <sup>ab</sup>	165/60 ± (0.26) <sup>a</sup>	163/60 ± (0.36) <sup>a</sup>	168/60 ± (0.05) <sup>a</sup>
K (mg/g Ash)	36/91 ± (0.04) <sup>b</sup>	41/00 ± (1.14) <sup>a</sup>	39/34 ± (0.66) <sup>a</sup>	40/11 ± (0.002) <sup>a</sup>
Na (mg/g Ash)	177/93 ± (0.58) <sup>b</sup>	180/6 ± (1.00) <sup>ab</sup>	183/21 ± (0.13) <sup>a</sup>	184/14 ± (0.06) <sup>a</sup>
Zn (mg/g Ash)	0/59 ± (0.16) <sup>ab</sup>	0/622 ± (0.91) <sup>a</sup>	0/651 ± (1.12) <sup>a</sup>	0/671 ± (0.01) <sup>a</sup>
Cu (mg/g Ash)	0/136 ± (0.79) <sup>a</sup>	0/133 ± (1.17) <sup>a</sup>	0/133 ± (0.57) <sup>a</sup>	0/141 ± (0.21) <sup>a</sup>
Fe (mg/g Ash)	0/910 ± (0.21) <sup>a</sup>	0/891 ± (1.11) <sup>a</sup>	0/919 ± (0.63) <sup>a</sup>	0/950 ± (1.02) <sup>a</sup>

Means in the same row with different letters are significantly different ( $P < 0.05$ ).

Values in parentheses indicate standard deviation related to three replicates ( $n = 3$ ).

**TABLE 2.** CHANGES IN PHYSICO-CHEMICAL PROPERTIES AND MINERAL CONTENTS ± STANDARD DEVIATION (SD) AT DAY 0, 20, 40 AND 60 OF RIPENING OF KURDISH CHEESE

the end of the storage period. The increase in the protein content was mainly due to loss of moisture. Our findings were in agreement with those reported by Hayaloglu *et al.* (2007), Mucchetti and Neviani (2006) and Quigleya *et al.* (2011).

In this study, aw values showed a gradual decrease ( $P < 0.05$ ) in the first month of ripening, remaining relatively constant after this point until reaching final average values of 0.927 (Table 2). This parameter is directly proportional to the moisture content of the cheese and inversely to the concentration of NaCl and other low molecular weight compounds (García Fontán *et al.* 2001). Our results are similar to those reported by Viana *et al.* (2009) and Mangia *et al.* (2011) for cottage and Pecorino Romano cheese respectively. These values were inferior to those obtained for the San Simon cheeses (García Fontán *et al.* 2001), but higher than those found in Armada cheese (García Fontán *et al.* 2001; Las Casas Lima *et al.* 2008). Whereas Buriti *et al.* (2005) reported that no significant ( $P > 0.05$ ) decrease in values of water activity (aw) during storage.

The final quality of a cheese is to a great extent determined by the salt concentration due to its influence on the development of LAB, enzyme activity and the biochemical relationships during the ripening of the cheese (Oner and Karahan 2006). The salt content showed a significant increase ( $P < 0.05$ ) during ripening. The lowest value for salt content was 3.18% (on day 0), while the highest value was 5.71% at the end of 60 days of ripening (Table 2). These values were similar to those described by Oner and Karahan (2006). Ceylan *et al.* (2003) reported that the salt content at 1 and 30 days to be 4.25 and 4.29%, respectively, in white cheeses produced by traditional methods. The results in this study were lower than those reported by Aly and Galal (2002) who reported salt percent of 7.24 to 8.43. This result was higher than those reported by Ceylan *et al.* (2003) who stated 2 and 5.03% for minimum and maximum salt content.

Ash content showed a significant increase ( $P < 0.05$ ) during ripening. The highest value (9.96%) was obtained at the end of the storage period, while the lowest (3.92%) being at the beginning of the production (Table 2). Similar results were obtained by El-Owni and Hamid (2008) who

found that ash content of Sudanese cheese increased during storage period. The ash content in cheese is due to the salt added to the curd and to the mineral components, most of which come from chlorides, phosphates, and sodium, potassium, calcium and magnesium citrates; they are a constitutive part of the milk salts and intervene in the formation of organic and inorganic compounds.

As shown in Table 2, the average concentrations of main elements and trace elements (expressed as mg/g ash) gradually increased during cheese ripening, but not significantly ( $P > 0.05$ ).

The mineral content of cheese is variable due to factors such as origin of the milk (cow, ewe and goat), geographical area and possible contamination derived from the equipment (Tarakci and Temiz 2009). During ripening period, Mean Ca, K, Na, Zn and Fe concentrations were 163.2, 38.3, 180.96, 0.651 and 0.921 mg/g ash, respectively. The average Cu concentration was 0.137 mg/g; our findings were similar to those obtained by Mendil (2006). Based on results, the mineral levels showed a similar behavior and no significant differences throughout the ripening of Kurdish cheese were observed. This phenomenon agreed with Cichoski *et al.* (2002) results for Prato cheese. Our results were comparable to those reported by Gonzalez *et al.* (2009). He stated that the effect of ripening time was statistically significant in all cases, except for Zn; he found that during cheese ripening some of the mineral salts may migrate from the central part towards the external layer of the cheese.

### Texture Profile Changes During Kurdish Cheese Ripening

The changes in texture parameters (hardness, gumminess, springiness, cohesiveness and chewiness) were observed (Table 3). The increase in hardness during 60 days of ripening was reflected in decreasing moisture, which acts as a plasticizer in the protein matrix, thereby making it less elastic and more susceptible to fracture upon compression (Fox *et al.* 2004). Extent of primary proteolysis influence the rheological properties of cheese; As the cheese matrix is made up of interacting protein molecules, a firmer curd is anticipated with more protein (Fox *et al.* 2004). Also, an

**TABLE 3.** CHANGES IN TEXTURE PROFILE ANALYSIS  $\pm$  STANDARD DEVIATION (SD) AT DAY 60, 120 AND 180 OF RIPENING OF KURDISH CHEESE

Parameter	Ripening time (days)			
	0	20	40	60
Hardness (N)	11.27 $\pm$ (0.23) <sup>de</sup>	14.24 $\pm$ (0.56) <sup>d</sup>	35.58 $\pm$ (0.16) <sup>ab</sup>	40.9 $\pm$ (0.09) <sup>a</sup>
Gumminess (N)	1.02 $\pm$ (0.49) <sup>e</sup>	2.3 $\pm$ (0.60) <sup>d</sup>	6.93 $\pm$ (0.41) <sup>b</sup>	9.17 $\pm$ (1.23) <sup>a</sup>
Springiness (mm)	7.24 $\pm$ (0.09) <sup>a</sup>	6.41 $\pm$ (0.56) <sup>b</sup>	4.76 $\pm$ (0.85) <sup>c</sup>	4.77 $\pm$ (0.47) <sup>c</sup>
Cohesiveness	0.235 $\pm$ (0.17) <sup>a</sup>	0.215 $\pm$ (1.00) <sup>a</sup>	0.185 $\pm$ (0.49) <sup>a</sup>	0.14 $\pm$ (0.32) <sup>a</sup>
Chewiness	7.43 $\pm$ (0.25) <sup>d</sup>	14.83 $\pm$ (0.38) <sup>c</sup>	32.99 $\pm$ (1.17) <sup>b</sup>	46.35 $\pm$ (1.26) <sup>a</sup>

Means in the same row with different letters are significantly different ( $P < 0.05$ ).

Values in parentheses indicate standard deviation related to three replicates ( $n = 3$ ).

increase in the protein concentration causes an increase in the viscosity leading to a firmer bodied cheese (El-Owni and Hamid 2008).

There was significant reduction in springiness (elasticity) as the ripening period progressed up to 60 days. Ozer *et al.* (2002) and Awad (2006) concluded that cheeses exhibited a loss of elastic characteristics with ageing. In general, it is expected that with an increase in hardness, the springiness should also change accordingly (Awad 2006), but degradation of the protein, reduction of free water and firming up of the fat all tend to reduce springiness (Tarakci and Temiz 2009).

The chewiness and gumminess of aged Kurdish cheese was higher ( $P < 0.05$ ) than fresh cheese, due to the high proteolysis in the former type and the lower moisture content. Considerable increases in the gumminess and chewiness values of the cheeses were also noted by Ozer *et al.* (2002) and Awad (2006). Because the chewiness and gumminess are calculated from assigned hardness, the variation in these parameters during the storage was due largely to the variations in the scores for hardness.

The cohesiveness values did not change during storage. It can be said that while the number of bonds making up of the protein matrix increased, the strength of these bonds was not affected by the time of storage (Ozer *et al.* 2002; Awad 2006).

## CONCLUSION

The chemical and microbial evaluation of Kurdish cheeses examined in the present study was found to be beneficial for not only the consumers but also the manufacturers since it will help them to standardize their production practices and allow them to produce higher quality and more stable products with well-defined characteristics.

Knowledge of the organisms involved in Kurdish cheese maturation may lead to a better understanding of the development of its biochemical and rheological properties, which in turn are modified by their own microbial metabolism. In the present study, ripening time had the largest impact on cheese composition, significantly affecting the microbial, chemical and textural parameters. According to our results, the optimum time for consuming Kurdish cheese would be after passing ripening times (60 days) due to a decrease in pH, undesirable biota and increase in desirable LAB counts.

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