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FİGEN ÇETİNKAYA

G. ECE SOYUTEMİZ

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# Microbiological and Chemical Changes throughout the Manufacture and Ripening of Kashar: a Traditional Turkish Cheese

Figen ÇETİNKAYA\*, G. Ece SOYUTEMİZ

Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Uludağ University, 16059 Görükle, Bursa- TURKEY

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**Abstract:** This study was performed to investigate the microbiological and chemical characteristics of Kashar cheese produced from raw milk according to traditional techniques. The counts of total aerobic bacteria, lactic acid bacteria (LAB) on MRS and M17 agar, psychrotroph organisms, yeast and molds, coliforms and the presence of *Escherichia coli* were determined during the manufacturing stages and ripening. The chemical properties determined were dry matter, fat in dry matter, pH and salt content. The stretching process, which appears to be the main method of destroying microorganisms, did not efficiently eliminate all the microorganisms but was sufficient to destroy *E. coli* that was present in 2 (16.7%) raw milk samples. No coliform bacteria were found during the ripening, whereas total aerobic bacteria, LAB on MRS and M17 agar, psychrotrophs, yeast and molds were present at levels of 5.36-6.44 log cfu g<sup>-1</sup>, 7.08-7.40 and 5.25-5.76 log cfu g<sup>-1</sup>, 5.04-5.59 log cfu g<sup>-1</sup>, 3.41-3.91 log cfu g<sup>-1</sup>, respectively. The data obtained from the present study suggest that specific manufacturing stages and ripening applied for the production of Kashar do not constitute an effective barrier to microorganisms.

**Key Words:** Cheese, kashar, microbial flora, chemical characteristics

## Geleneksel Türk Kaşar Peynirinin Üretimi ve Olgunlaşması Sırasında Görülen Mikrobiyolojik ve Kimyasal Değişiklikler

**Özet:** Çalışma, geleneksel tekniklerle çiğ süttten üretilen Kaşar peynirinin mikrobiyolojik ve kimyasal özelliklerini belirlemek amacıyla gerçekleştirildi. Üretim aşamaları ve olgunlaşma boyunca total aerob bakteri, MRS ve M17 agar'daki laktik asit bakterileri (LAB), psikrofil organizmalar, maya ve küf, koliform sayıları ve *Escherichia coli* varlığı belirlendi. Kimyasal olarak da kurumadde, kurumadde yağ, pH ve tuz içeriği saptandı. Mikroorganizmaların yıkılmasında en önemli bariyer olarak görülen haşlama işlemi, mikroorganizmaların tümünü elimine etmedi ancak çiğ süt örneklerinin 2'sinde (% 16,7) bulunan *E. coli*'nin yıkılmasını sağladı. Olgunlaşma boyunca koliform bakteri saptanmazken, total aerob bakteri, MRS ve M17 agardaki LAB, psikrofil organizmalar, maya ve küflerin sayıları sırasıyla 5,36-6,44 log kob g<sup>-1</sup>, 7,08-7,40 ve 5,25-5,76 log kob g<sup>-1</sup>, 5,04-5,59 log kob g<sup>-1</sup>, 3,41-3,91 log kob g<sup>-1</sup> seviyelerindeydi. Çalışmada elde edilen sonuçlar, kaşar peynirinin üretiminde uygulanan spesifik üretim aşamaları ve olgunlaşmanın mikroorganizmalar için etkili bir bariyer oluşturmadığını ortaya koymaktadır.

**Anahtar Sözcükler:** Peynir, kaşar, mikrobiyal flora, kimyasal özellikler

## Introduction

Kashar is the second most popular cheese in Turkey, with around 49,000 tons produced per year (1). It is produced from either sheep's or cow's milk, or a mixture of both (2), and shows similarity with other type of cheeses such as Caciocavallo, Provolone, Regusono, Kashkaval and partially with the 'Pasta Filata' type cheese such as Mozzarella (3). It contains an average of 24.2% protein, 4.2% ash, 41.9% moisture, 25.1% fat, and 4.6% salt (2), and its acidity (LA%) is 0.8-2.3 (4).

There is no standardized technique for the manufacture of Kashar and because of the utilization of raw milk some preventive measures have to be taken to assure elimination of undesirable bacteria. Stretching applied at 75 °C for 5 min during the manufacturing of Kashar is the main way of eliminating microorganisms. Antimicrobials and metabolic products produced by indigenous flora during ripening might also have detrimental effects on undesirable bacteria (4,5).

\* E-mail: figcetinkaya@yahoo.com

The aim of the present study was to evaluate the dynamic of the main groups of microorganisms and the chemical characteristics during the manufacture and ripening of Kashar.

## Materials and Methods

**Cheese making.** Kashar production is shown in a flow chart in Figure 1. Four independent cheese-making trials were carried out. For each trial 60 l of raw cow's milk was used. The milk was heated to 35 °C in a stainless steel cheese vat and 4 ml of calf rennet diluted in cold water was added to induce coagulation. The coagulum (usually 45-60 min after rennet addition) was cut in nearly 4 cm cubic forms and remained quiescent for ca. 15 min. The temperature of the vat content was gradually raised to 41 °C under continuous stirring. Thereafter, the coagulum was collected in cheesecloth and pressed for syneresis (2 h). Subsequently, the curd was cut into blocks (25-35 cm) and kept at room temperature for 16 h. At the end of this period, the curd took an elastic form and its pH reached 5.03-5.07. At this stage, heat treatment of curd at  $75 \pm 1$  °C for 5 min was performed. For this purpose, the curd was cut into long strips and placed in water bath in a stainless steel bucket containing several holes (5 mm diameter). After heat treatment of curd, it was manually kneaded for 5 min to eliminate air bubbles and molded in plastic molds (20 cm in diameter and 16 cm in height). The next day, cheese wheels (approximately 3 kg) were removed from the plastic molds and dry salted. Salt (average 13-15 g) was spread on the top surface of the wheels once every 2 days at room temperature over 10 days. The wheels were turned over during each salting process. They were thereafter stored at room temperature for 20 days and transferred to a ripening room ( $5 \pm 1$  °C) for 3 months (2).

**Sampling.** Duplicate samples (~ 200 ml or g each) were taken from milk, coagulum, acidified curd, heat-treated curd and cheese samples on days 1, 10, 30, 60, 90 and 120 of ripening. Samples were then transferred to the laboratory under refrigerated conditions (4 °C) and analyzed immediately.

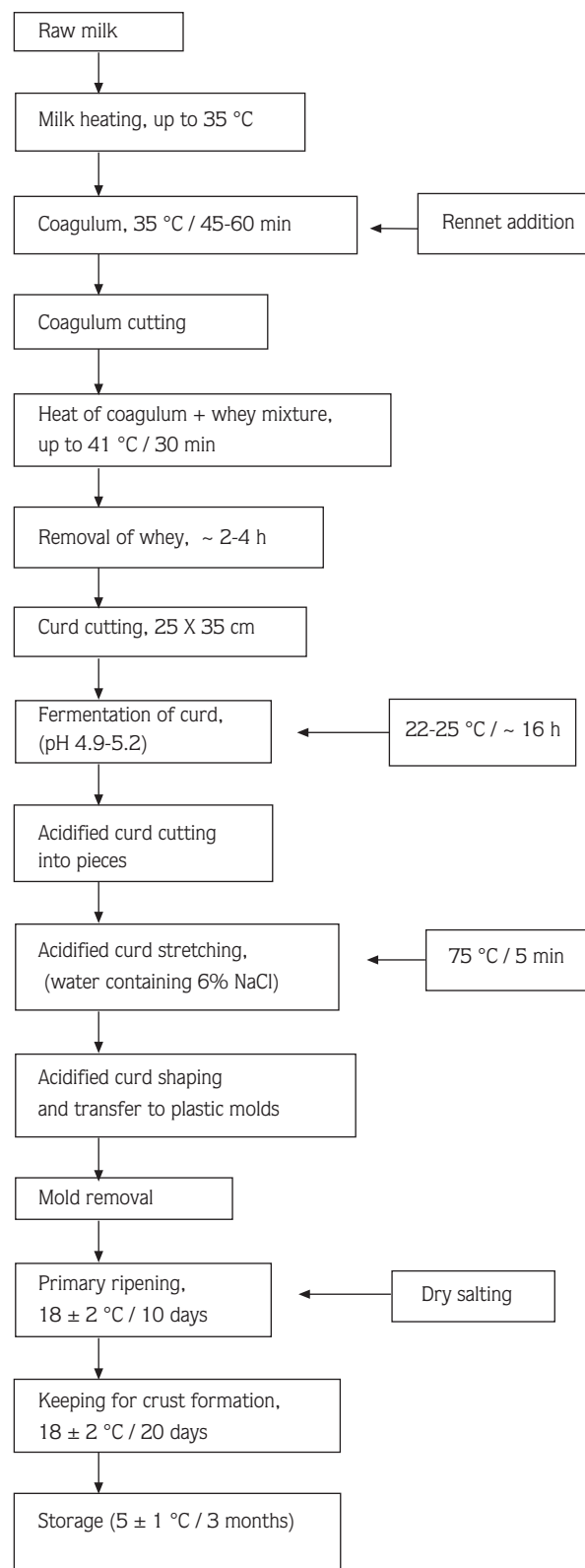


Figure 1. Flow diagram of Kashar production.

**Microbiological analysis.** Fractions 10 ml or 10 g from each sample were homogenized with 90 ml of sterile 0.1% peptone water solutions using a Seward Stomacher 80 Lab System for at least 2 min. Sequential decimal dilutions of the homogenate were prepared in sterile peptone water and plated in duplicates onto specific media. Total aerobic bacteria and coliforms were determined on Plate Count Agar (Oxoid, CM 325) at 37 °C for 48 h and on Violet Red Bile Agar (Oxoid, CM 107) at 37 °C for 24 h, respectively (6,7). For the determination *Escherichia coli*, 5 typical coliform colonies were inoculated on Lactose Broth in sterile tubes with Durham's tubes. Following incubating at 44.5 °C for 24 h, the cultures were monitored for gas (CO<sub>2</sub>) formation within Durham's tubes and also analyzed for IMVIC tests. The gas (CO<sub>2</sub>) formation and indole +, methyl-red +, Voges-Proskauer –, and citrate – isolates after 24 to 48 h incubation at 37 °C were assessed as positive (7). Lactic acid bacteria (LAB) were determined anaerobically (Gas-Generating Kit, Anaerobic System, Oxoid BR38) on MRS agar (Oxoid, CM 361) and on M17 Agar (Oxoid, CM 785) at 37 °C for 5 days, respectively (8,9). Psychrotroph organisms were determined on Plate Count Agar at 7 °C for 10 days (10). Yeast and molds were grown on Potato Dextrose Agar (Oxoid, CM 139) acidified with 10 ml/l of 10% tartaric acid at 22 °C for 5 days (8).

**Chemical analysis.** The pH was measured with an Orion pH-meter (Ionalyzer model 399A/F, Cambridge, MA, USA) at 25 ± 1 °C (8). Dry matter content in cheese was determined by heating at 105 °C until constant weight (11). Fat in milk was determined by the Gerber method (12). Fat in cheese was determined by the Van Gulik method (13) and fat in dry matter (FDM) was calculated as follows:

$$\text{FDM \%} = \text{F \%} \times 100 / \text{DM},$$

where F is the percentage of fat of cheese and DM is the percentage of dry matter of cheese.

The salt content was determined according to the method described by Case et al. (14), and salt in dry matter (SDM) content was calculated by the formula

$$\text{SDM \%} = \text{S \%} \times 100 / \text{DM},$$

where S is the percentage of salt of cheese.

## Results

Chemical analysis results of raw milks, acidified curd, heat-treated curd and cheese samples are presented in Figures 2 and 3. For all the cheeses, pH decreased to 5.03-5.07 after acidification of curd at room temperature for 16 h. Nevertheless, pH increased slightly during ripening from 5.13-5.17 to 5.30-5.43. The dry matter content, which was 56.79%-57.60% at the beginning of ripening, increased during ripening and at the end of the 120 days reached 66.28%-67.56%. The fat in dry matter was initially between 44.00% and 45.79%, and at the end of ripening ranged from 37.77% to 40.08%. The salt content in dry matter increased from 3.37%-3.72% to 4.97%-5.23% during ripening. This increase could be due to the increase in dry matter content.

The effect of the manufacture stages and time of ripening on total aerobic bacteria, coliforms, LAB on MRS and M17 agar, psychrotroph organisms, yeast and molds in Kashar are given in Figures 4, 5 and 6. For different microbial groups, microbial load increased 0.5-2 log by the syneresis stage. By the acidification of curd, the counts of total aerobic bacteria, coliforms, LAB, psychrotrophs, yeast and molds were 7.70-8.47 log cfu g<sup>-1</sup>, 5.52-6.62 log cfu g<sup>-1</sup>, 8.45-8.88 log cfu g<sup>-1</sup>, 7.82-8.07 log cfu g<sup>-1</sup>, 7.48-8.06 log cfu g<sup>-1</sup>, and 5.46-6.12 log cfu g<sup>-1</sup>, respectively. The heat treatment of the curd during the stretching process did not efficiently kill all the microorganisms. Depending upon the group of microorganisms a nearly 1-3 log decrease was observed after the stretching process. This suggests that a significant level of microorganisms resisted the stretching temperature or that the heat application time was not sufficient for the temperature in the core of the curd to reach the aimed level. Except for the psychrotrophs and the LAB, all tested microorganisms showed a slight decrease over the ripening. Interestingly, no coliforms were isolated at the end of ripening, suggesting total inhibition of coliforms during ripening. *E. coli*, which was present only in raw milk samples in the second trial, was totally eliminated by the stretching.

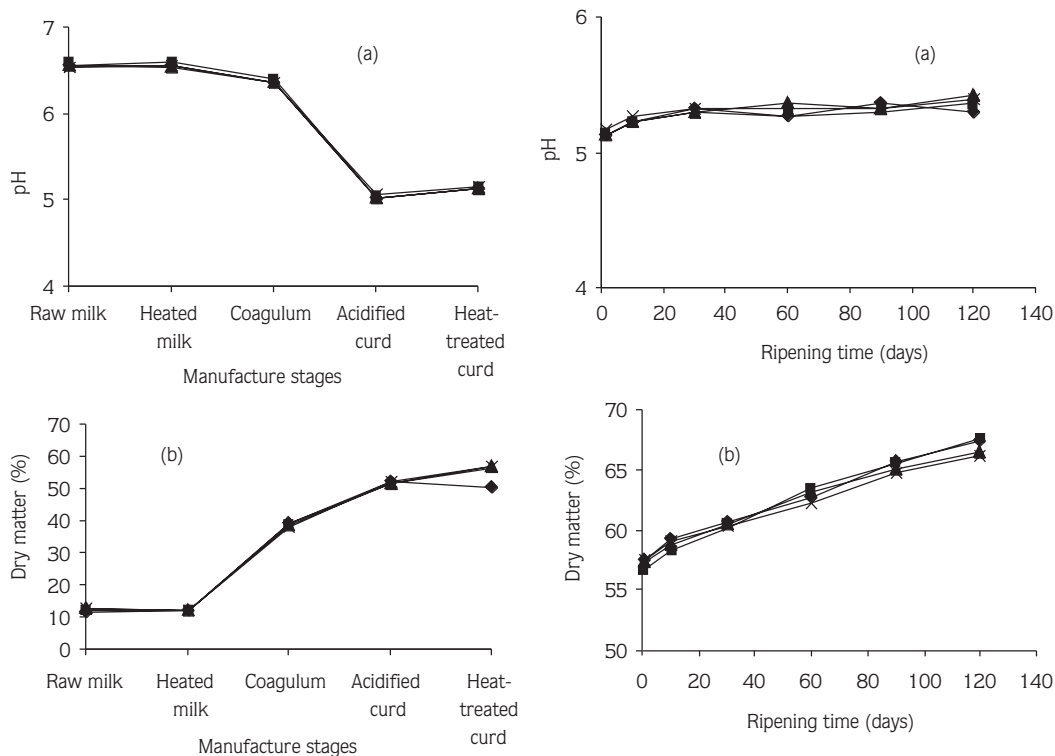


Figure 2. Changes in pH (a) and dry matter (b) during the manufacture and ripening of the cheeses (◆ Trial 1, ■ Trial 2, ▲ Trial 3, × Trial 4). Each point is the mean of duplicate analyses from 3 independent experiments.

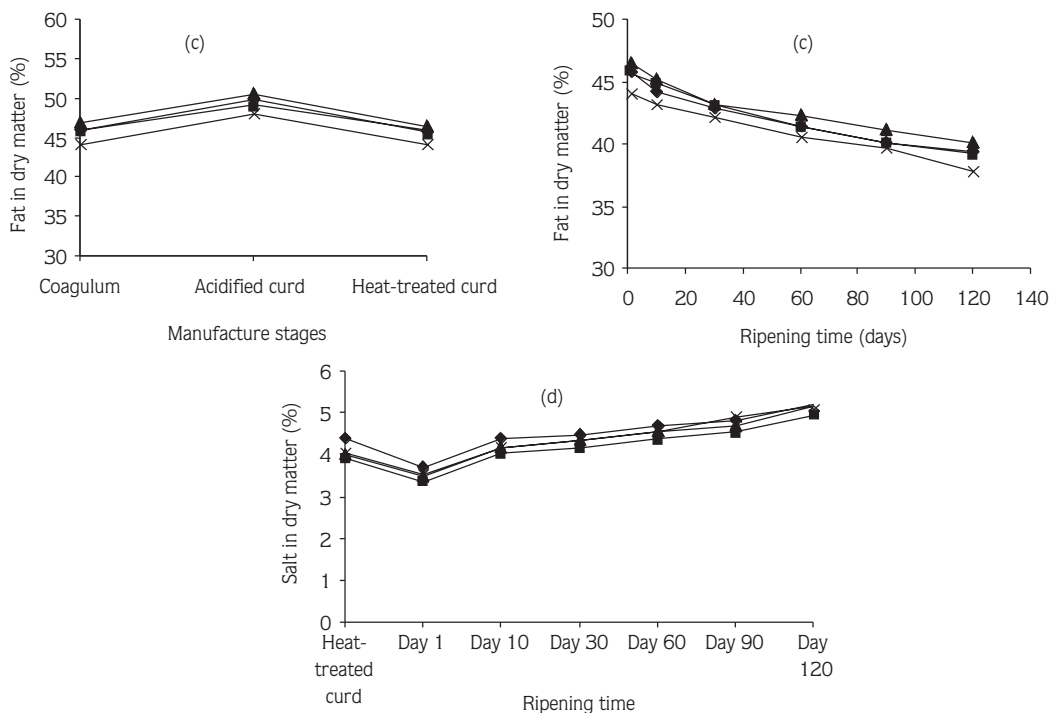


Figure 3. Changes in fat in dry matter (c) and salt in dry matter (d) during the manufacture and ripening of the cheeses (◆ Trial 1, ■ Trial 2, ▲ Trial 3, × Trial 4). Each point is the mean of duplicate analyses from 3 independent experiments.

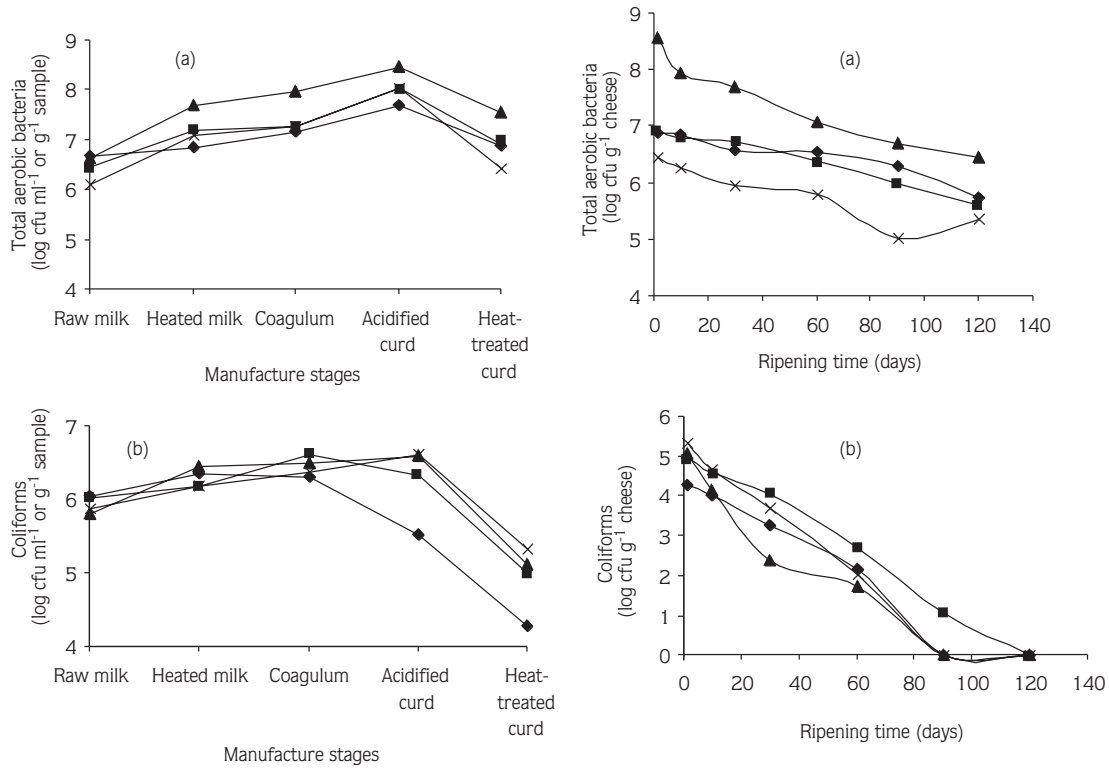


Figure 4. Microbial counts of total aerobic bacteria (a) and coliforms (b) during the manufacture and ripening of the cheeses (◆ Trial 1, ■ Trial 2, ▲ Trial 3, × Trial 4). Each point is the mean of duplicate analyses from 3 independent experiments.

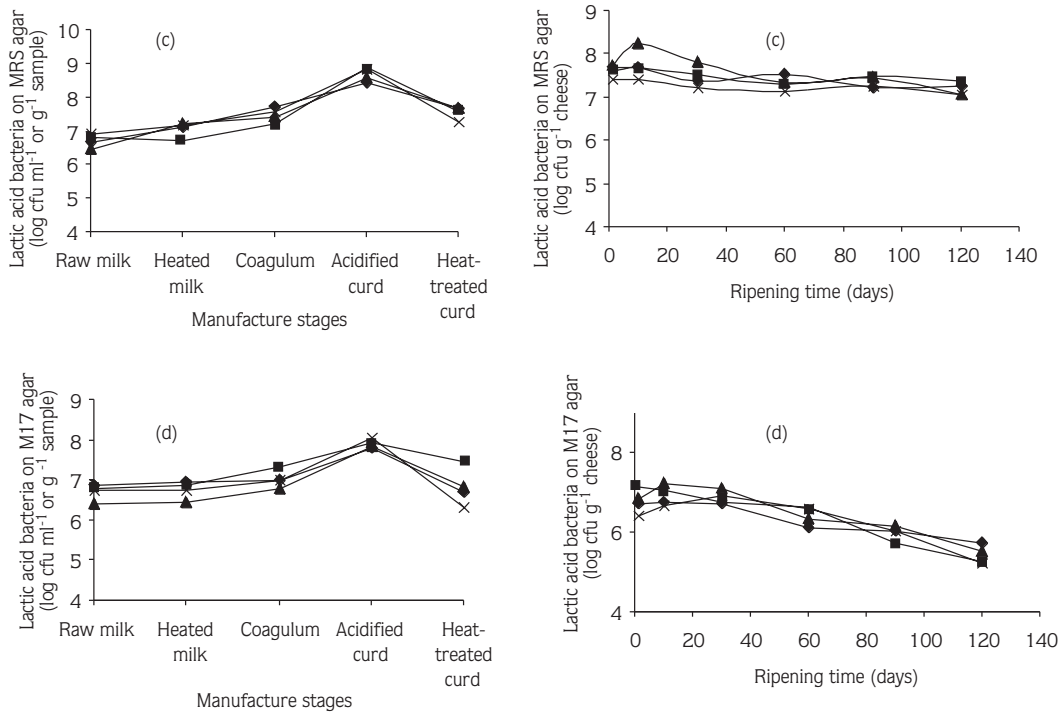


Figure 5. Microbial counts of lactic acid bacteria on MRS (c) and M17 (d) agar during the manufacture and ripening of the cheeses (◆ Trial 1, ■ Trial 2, ▲ Trial 3, × Trial 4). Each point is the mean of duplicate analyses from 3 independent experiments.

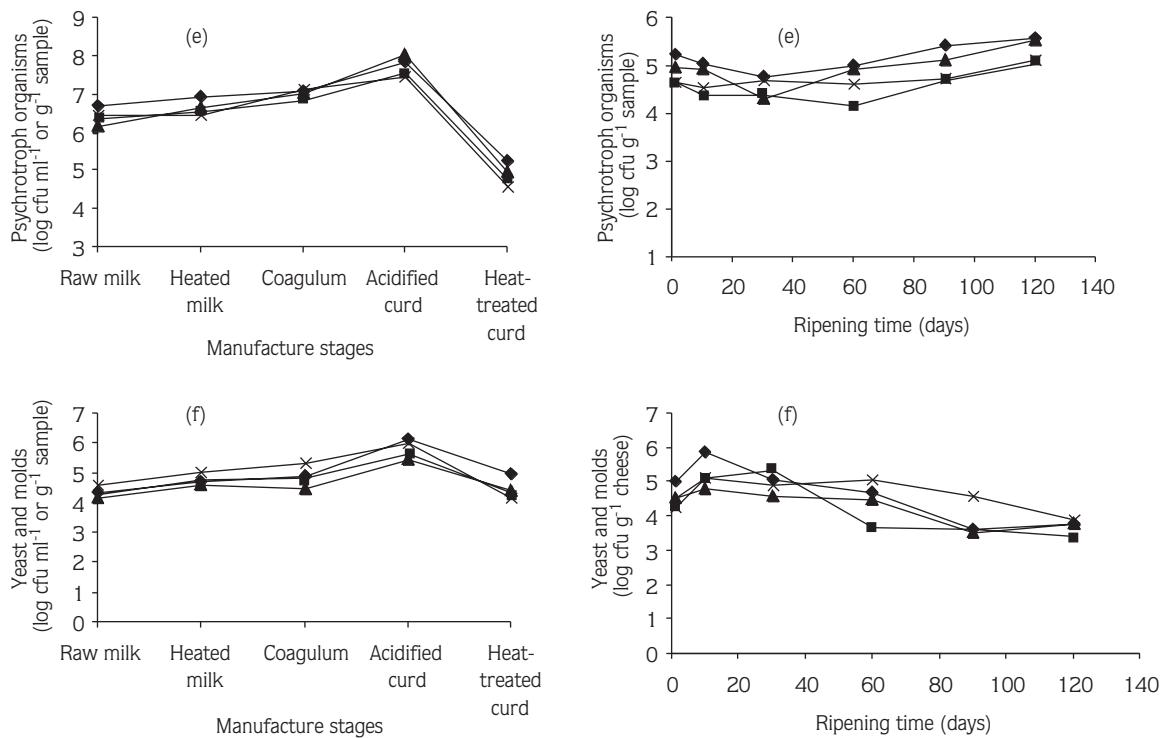


Figure 6. Microbial counts of psychrotroph organisms (e) and yeast and molds (f) during the manufacture and ripening of the cheeses (◆ Trial 1, ■ Trial 2, ▲ Trial 3, × Trial 4). Each point is the mean of duplicate analyses from 3 independent experiments.

## Discussion

According to the Turkish Standard Institute (TSE), the moisture content of Kashar must not be higher than 40%. The fat percentage in dry matter must be 45% and 20% for whole and half-fat cheeses, respectively. Salt content must range between 3% and 7% (15). The pH of Kashar was reported to range between 4.9 and 5.4 (4). Briefly, the chemical characteristics of Kashar produced and analyzed in this study met the necessary legal standards (Figures 2 and 3).

The microbiological quality of raw milks used in cheese making was not good and total aerobic bacteria counts varied between 6.13 and 6.67 log cfu ml<sup>-1</sup> (Figure 4a). This poor hygienic quality is likely the result of improper milking or storing conditions. Similar findings were also reported for the milks used in the production of other types of cheeses (16,17). Total aerobics increased constantly during the syneresis and manufacturing stages and reached 7.70-8.47 log units. These results are in good agreement with those reported by Halkman et al. (4) and Atamer et al. (18). By

stretching, a decrease of about 0.81-1.60 log units was noted. This level appears similar to those reported by Halkman et al. (4) and Soyutemiz et al. (5), but lower than those found (2.33 log units) by Akgün (19). At the end of the 120 days' ripening, the count of total aerobics decreased about 1.08-2.13 log units. Similar behavior has also been observed in goat's milk cheese (17) and in ewe's milk cheese (20).

The count of coliforms in raw milk samples was quite high and varied between 5.81 and 6.04 log cfu ml<sup>-1</sup> (Figure 4b). These are higher than those found by Fernanda Volken de Souza et al. (16). The decrease observed in coliforms after stretching varied between 1.24 and 1.49 log units. In the studies by Halkman et al. (4) and Akgün (19) these values were 3.93 and 2.38 log units, respectively. The numbers of coliforms decreased gradually throughout ripening and no growth was observed on the 90<sup>th</sup> day for the 1<sup>st</sup>, 3<sup>rd</sup>, 4<sup>th</sup> groups and on the 120<sup>th</sup> day for the 2<sup>nd</sup> group. Atamer et al. (18) and Akgün (19) in their experiments did not isolate coliforms on the 30<sup>th</sup> and 60<sup>th</sup> day of ripening of Kashar

respectively; this may have been due to the initially low coliform load of milk used as reported by the same authors. In a study realized on Pecorino del Poro, a ewe's cheese made from raw milk, the authors reported that coliforms decreased sharply throughout ripening (21). The decrease in bacterial counts may be related to the progressive increase in the lactic microflora, the increase in metabolites, or the increase in dry matter and the salt content of the cheese.

Only 2 out of 12 (16.7%) of the raw milk samples used in cheese making were found to be contaminated with *E. coli*. The stretching was highly efficient in the destruction of bacteria, whereas this was not the case in the study reported by Soyutemiz et al. (5), who found that only 20% of the *E. coli* were destroyed by the stretching process applied at 70 °C. This discrepancy could be explained by the difference in the temperature applied.

LAB play an important role in the development of required characteristics of dairy products. The counts of LAB in raw milk grown on MRS and M17 agar plates were 6.45-6.93 log cfu ml<sup>-1</sup> (Figure 5c) and 6.40-6.87 log cfu ml<sup>-1</sup> (Figure 5d), respectively. Higher and lower levels of LAB have been reported in several studies (16,17,22). Nearly a 1 log units decrease has been observed during the stretching process and this decrease was lower than that reported by Akgün (2.12 log) (19). As expected, for the first 30 days of ripening LAB count remained constant and a slight decrease was noted towards the end of ripening. The decrease of coccal-shaped LAB grown on M17 agar plates was more important than those observed on MRS agar plates. Similarly, in Caprino d'Aspromonte (23) and Pecorino del Poro (21) cheeses, coccal-shaped LAB decreased towards the end of ripening, while the lactobacilli increased. In another study performed on Kashar, the count of lactic streptococci was reported to decrease from 8.24 log cfu g<sup>-1</sup> to 3.10 log cfu g<sup>-1</sup> after 90 days of ripening (24). Furthermore, development of non-starter LAB throughout ripening was reported by several authors (17,25,26).

The psychrotrophs (Figure 6e) were quite high in the milk (6.18-6.72 log cfu ml<sup>-1</sup>). Similar findings were reported in some previous studies (16,17,27). A relatively important decrease was noted with stretching (2.61-3.09 log units). Thereafter, there was no remarkable change up to day 30 but a slight and constant increase was observed until the end of the ripening. In contrast, Fernanda Volken de Souza et al. (16) observed a constant drop-off of psychrotrophs in Serrano cheese until the end of the 60-day period; however, this reduction was more pronounced in summer (3 log) in comparison with winter (1 log), possibly due to environmental conditions. These observations were supported by Moatsou et al. (28), who reported a significant decrease in psychrotrophic bacteria counts after 60 days of ripening at 4 °C.

Like the other groups of microorganisms, the numbers of yeast and molds also exhibited an increase during the manufacturing steps until stretching (Figure 6f). A nearly 1.05-1.88 log units decrease was observed with stretching and these values are close to those described by Soyutemiz et al. (5), but lower than those obtained by Halkman et al. (4), who reported a 4.90 log units decrease. Throughout ripening their counts were nearly constant up to 30 days, thereafter gradually decreasing towards the end of the storing period. Similarly, decreases in yeast and mold counts during the ripening of Kashar were also reported in previous studies (18,24,29).

Stretching is the main stage for the elimination of non-desired bacteria during the manufacturing of Kashar. However, the results of the present study have shown that this step was not efficient for the production of healthier and safe Kashar. Therefore, in the future, studies should focus on the time versus temperature correlation notably in the core of the curd in order to optimize and standardize manufacturing conditions. The hygienic quality of raw milk also has an important effect on the final microbial load. To increase the hygienic quality of Kashar, it is necessary to use high hygienic quality and/or heat-treated milk in cheese making.



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