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Microflora and Pathogen Bacteria (*Salmonella*, *Klebsiella*, *Yersinia*, *Pseudomonas*, *Aeromonas*, *Escherichia coli*, *Staphylococcus aureus*) In Urfa Cheese (A Traditional White-Brined Turkish Cheese)

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Abstract: Urfa cheese is normally produced from ovine or bovine milk or appropriate mixture of these two especially in Urfa. Urfa cheeses are being manufactured, as regional, by the use of raw milk or scalded milk that has been heated up to 30-35°C. This cheese use to be consumed in the region, without waiting the completion of its maturation period. Gastroenteritis case number in Urfa is considerably high as compared to the other regions. For these reasons 11 different Urfa regional cheeses have been purchased within their brine concentration. They were studied during 0-7th, 30th and 60th day as regard to micro flora and pathogen microorganisms. At the end of micro flora research of Urfa cheeses, *Escherichia, Salmonella, Enterobacter, Klebsiella, Pseudomonas, Yersinia, Aeromonas, Hafnia, Serratia, Morgenella* and also *Cedecea, Citrobacter, Hafnia* have been detected. Besides, *Staphylococcus* (*S. aureus)* has been determined therein. *Steptococcus, Lactococcus, Lactobacillus, Pediococcus, Leuconostoc* were identified as well. In our research we have detected that the variety of micro organism are quite a lot in Urfa cheeses. We identified various pathogen bacteria also. It has recommended that the region's people not consume fresh Urfa cheese.

Key words: Urfa cheese, microflora, bacteria enumeration, *Staphylococcus aureus, Escherichia coli, Yersinia enterocolitica, Aeromonas, Salmonella typhi*

Introduction

The use of pasteurized milk and milk products is not a health risk for human. After pasteurization milk products can be contaminated by pathogen microorganisms. In Turkey milk products do not manufactured on an industrial scale, some as still being produced traditionally at a small diary level. These kinds of products every time carry risks for pathogen microorganisms. Cheese is an important integral part of diet consumed in Turkey. Urfa cheese is a traditional white cheese produced in mainly South-east of Turkey. It is normally produced from ovine or bovine milk or appropriate mixture of these two. In the traditional method of manufacture of Urfa cheese, microbiological safety was provided by keeping cheese in a very dense brine solution. Sometimes manufacturers scald the fresh cheese blocks in boiling whey for about 2-3 min. However scalding is not satisfactory enough to provide microbial safety.

Materials and Methods

Cheese: Eleven different Urfa cheeses were purchased from 11 different markets in Urfa on May 2002. They were transported to the Ankara Gazi University, Biology Faculty laboratory under 4°C for microbiological analyses. Isolations and identifications were carried out in Gazi University Biology Faculty, microbiology laboratory.

There isn't any Standard cheese-making method of Urfa cheese but the traditional cheese-making procedure is outlined in Fig 1.

Microbiological analyses

Cheese processing and sampling: The preparation of samples was determined accordance to Turkish Standards TS 591 (Anonymous, 591, 1989). Samples were taken from cheese pieces after brining and at days 0-7, 30 and 60 of storage. Sampling for microbiological examinations was done according to White *et al*. (1993). Representative 10 g. samples of the cheese were taken aseptically using an ethanol-sterilized cheese borer. One percent (wt/v) peptone water was used for the preparation of the decimal dilutions. The solid samples (10 g.) were blended with 90 ml. of peptone water using a stomacher 10^{-1} of dilution and 0.1 ml. of cheese brine were poured onto duplicate plates.

Enumeration and assessment of total alive mesophyll bacteria: To utilize total alive mesophyll bacteria counts and isolation, the cheese samples of which dilution has

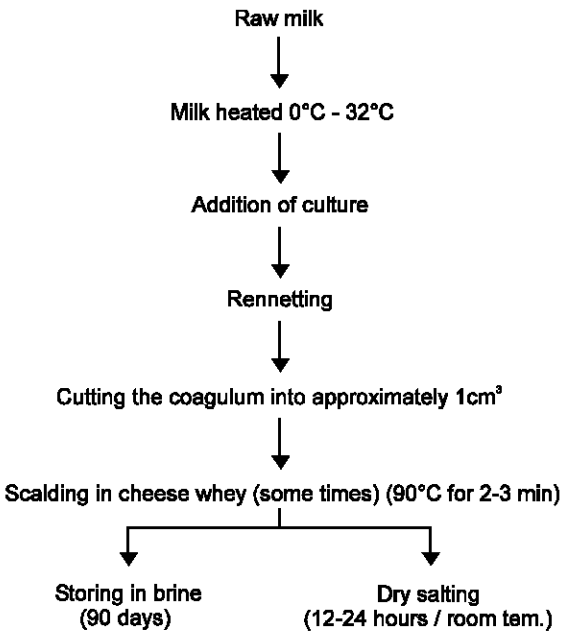

Fig 1: Flow diagram of Urfa cheese-making.

been prepared plated on Plate Count Agar (PCA). Plates were incubated at 30°C for a period of 48 hours. Afterward, the total microorganism count in 1 gram and 1 ml. sample was separately calculated by taking the colonies at the plates and the dilution rates into account. (Anonymous, 7725,1989).

Enumeration and assessment of total alive coliforms: To utilize total alive coli form bacteria counts and isolation, the cheese samples of which dilution has been prepared plated on Eosin Methylene Blue agar (EMB: Difco, Detroit, Michigan), Brillant green agar (BG: Difco, Detroit, Michigan) and Mc Conkey (Difco, Detroit, Michigan) agar .Plates were incubated at 37°C for a period of 48 hours. For identification of Gram negative colonies they were examined for mannitole, sucrose, glucose, lactose fermentation, indole production lisine and ornithine decarboxylase, aesculine hydrolysis. They were screened using triple sugar iron agar (TSI: Oxoid, Basingstoke, Hampshire, UK), Simmons citrate agar (Merck, Darmstadt, Germany) and urea agar. Biochemical characterization of the strains was performed with bioMerieux API 20 E (Marcy-l'Etoile, France). Afterward, the total microorganism count in 1 gram and 1 ml. sample was separately calculated by taking the colonies at the plates and the dilution rates into account. (Anonymous, 7725, 1989).

Enumeration and assessment of *Salmonella* and *Shigella:* To utilize total alive coli form bacteria counts and isolation, the cheese samples of which dilution has been prepared plated on *Salmonella* and Shigella Agar

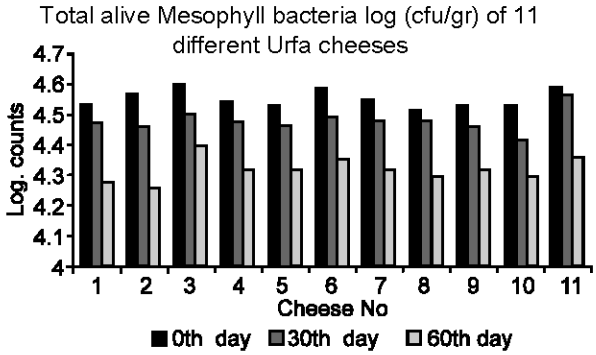

Fig. 2: Total alive mesophyll bacteria count (cfu/gr) of 11 different cheese samples.

(SS: Oxoid, Basingstoke, Hampshire, UK). Plates were incubated at 37°C for a period of 24 hours. Typical colonies were subjected to tests which include the lactose, mannitole, raffinose, sucrose, xylose, indole, urea, citrate, H_2S, arjinine, ornithine, lisine and aesculine. Biochemical characterization of the strains was performed with bio Merieux API 20 E (Marcy-l' Etoile, France).

Enumeration and assessment of lactic acid bacteria (LAB): To utilize total alive coli form bacteria counts and isolation, the cheese samples of which dilution has been prepared plated on MRS (Difco, Detroit, Michigan) Plates were incubated at 30°C for a period of 48 hours. The LAB strains were identified following the criteria of Sharpe *et al.* (1966), for isolates of *Lactococci*, the criteria of Kandler and Weiss (1986) for Lactobacilli and the criteria of Garvie (1986) for Leuconostocs, as described by Centeno *et al.*, (1996). Biochemical characterization of the strains was performed with bioMerieux API 50 CHL (Marcy-l' Etoile, France). Afterward, the total microorganism count in 1 gram and 1 ml. sample was separately calculated by taking the colonies at the plates and the dilution rates into account.

Enumeration and assessment of *Staphylococcus aureus:* To utilize *S. aureus* isolation, the cheese samples of which dilution has been prepared plated on Baired Parker Agar BP (Oxoid, Basingstroke and Hampshire, UK). The plates were incubated at 25°C for a period of 48-72 hours. Afterward, the total micro organism count in 1 gram and 1 milliliter sample was separately calculated by taking the colonies at the plates and the dilution rates into account. (Anonymous, 6582, 1989) First of all, Gram painting was applied on the colonies which have been grown on the plates and then they were subjected to catalase, DNase production, mannitol fermentation and coagulase tests.

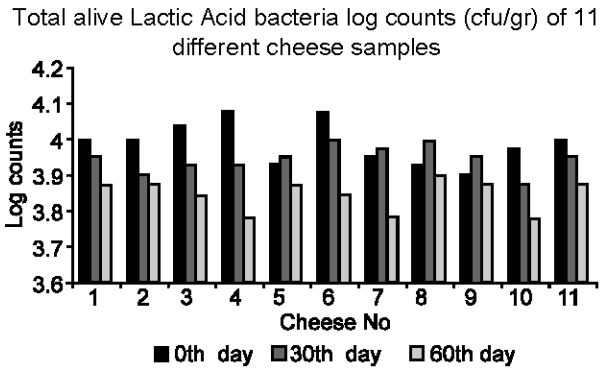

Fig. 3: Total alive lactic acid bacteria log. count (cfu/gr) of 11 different cheese samples.

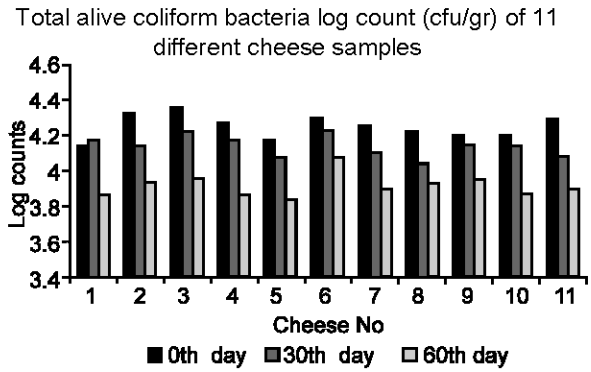

Fig. 4: Total alive coliform bacteria count (cfu/gr) of 11 different cheese samples

Results and Discussion

In our research, 11 different Urfa regional cheeses have been purchased within their brine concentration. It is concentrated on these cheese samples thereof, as regard to micro flora and pathogen micro organisms, during 0-7th, 30th and 60th days.

Bacteria counts in 11 different cheese samples: Total alive mesophyll bacterium count (cfu/gr) in 11 different cheese samples is shown in Fig. 2.

As it is shown in Figure 2 in 2nd, 3rd, 6th and 11th Urfa cheese serials, the Total alive mesophyll bacteria counts are very high in 0-7th and 30th days. But in these Urfa cheese serials a sudden decrease in 60th day was observed. The fresh cheeses and the cheeses of 30th day, on all regions' cheeses, the mesophyll live bacterium count has been observed as around 3.2×10^4 cfu/gr. Because all regions' cheeses are the cheeses that have been made from unscalded or scalded milk heated up to 30, 35°C. But also the total alive mesophyll bacterium counts in all 11 serials have been reduced. In 0-7, 30th. and 60th. days, total alive mesophyll bacterium count of 2nd, 3rd. 6th and 11th cheese samples are 2.8×10^4 cfu/gr, 3.2×10^4 cfu/gr, 3.1×10^4 cfu/gr and 3.2×10^4 cfu/gr respectively.

In Fig. 3, total lactic acid bacterium count (cfu/gr.) in 11 different Urfa cheeses is given.

Total lactic acid bacterium count (cfu/gr.) in 11 different regions' cheeses was enumerated as minimum as $9,8 \times 10^{-3}$ in 0-7th day. In 30th day, an increase in total lactic acid bacterium counts has been determined of the 7th, 8th and 9th serial Urfa cheeses. In 60 th day, the average of total lactic acid bacterium counts is $7,4 \times 10^{-3}$ cfu/gr.

The total coliform bacteria count (cfu/gr.) of 11 different Urfa cheeses is given in Fig. 4.

Although, when it is looked to the coliform bacteria counts of 11 different serial Urfa cheeses, the Total coliform bacteria counts were found very high in 2st, 3nd, 6rd, 9th and 11th serial cheeses.

Lactic acid bacterium isolation and their identifications: In our research, 80 lactic acid bacteria have been isolated from 11 different serial Urfa cheese samples. The varieties and species numbers and their per cents are given in Fig. 5.

The maximum lactic acid bacterium in 11 different variety Urfa cheeses is *Enterococcus faecalis* with a ratio of 33%. The next higher one, *Enterococcus faecium*, with a ratio of 13 % has been isolated.

Gram negative and *Staphyllococcus* isolation and its identification: In the cheese samples that were used in the research, Gram negative bacteria identifications had been made. In Fig. 6, the Gram negative bacteria percent values that were identified from 11 different Urfa cheeses.

In Urfa cheeses, *Salmonella* (*S. choleraesuis*, *S. typhi*) has been isolated from 2nd, 3rd and 6th cheese samples. During 60 days while *S. choleraesuis* has been observed in 2nd cheese sample, it was monitored in 3rd cheese sample for 30 days. *S. typhi* which was available in the 1st cheese sample has also been isolated for a period of 60 day. It is thought that the same bacterium is contaminant due to it was unable to be isolated from the cheese of same sample.

While *Morganella morganii* has been isolated in 2nd. and 3rd. Urfa cheese samples up to 30 days, but in the meantime it was not isolated from the brines.

Pseudomonas is the bacterium species established mostly in the Urfa cheeses. It was detected in 9 out of 11 Urfa cheeses. (81,8%). *P. fluorescence* was specified in the samples of 1st, 3th, 5th, 6th, 7th, 8th 9th and 11th cheese samples. While it survived for 60 days in 1st, 2th, 3th, 5th, 6th, 7th and 11th cheese samples it remained viable only 30 days in 8th and 9th. *P. maltophilia* has also been found in 3nd, 8rd, 9th, 10th Urfa cheese experiments.

Klebsiella is determined in the 8 Urfa cheese sample. *K. pneumoniae* has been isolated along 60 and 30 days

The distribution of total lactic acid bacterium in 11

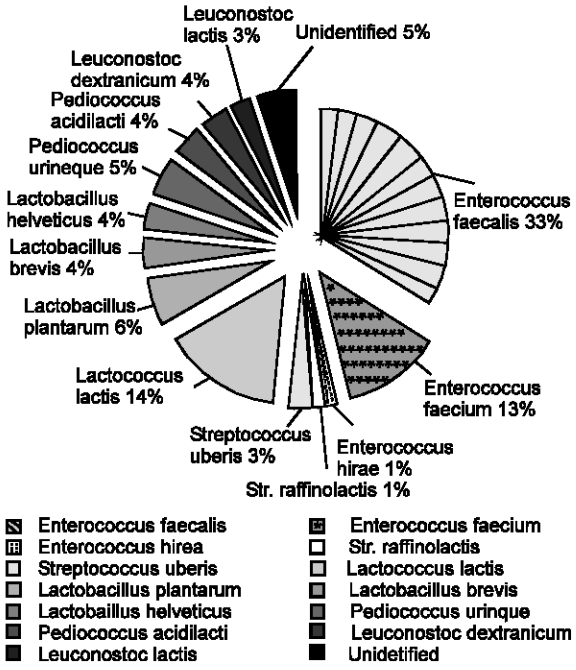

Fig. 5: The distribution of total lactic acid bacterium in 11 different Urfa cheese.

different Urfa cheese from 1st, 3rd and 8th cheese samples respectively. *K. oxytoca* survived for 60 days in the 2nd, 5th, 6th cheese samples. *K. oxytoca* survived 30th day in the 2nd sample and 60th days in the others. *K. ozane* has been determined in the cheese samples of 9th and 11th.

3 Different species of *Citrobacters* are determined in the specimen of eleven Urfa cheeses. *C. freundii* has been isolated along 60 days, from 1st and 11th cheese samples. In one of the 2nd ad 6th cheese samples, *C. youngae* survived 60th day *C. diversus* was isolated for 30 days in the cheese samples of 4th and 7th and for 60 days in the 5th cheese sample.

Yersina was established in 4 Urfa cheeses. In the 1st cheese sample, *Y. mortareti* stayed alive up to 0-7th day. *Y. intermedia* was detected in the 6th cheese sample throughout of 60 days and in 10th one for 30 days. As regards to 11th cheese sample, *Y. enterocolitica* was able to be isolated up to 60 days.

Aeromonas salmonicida was isolated for 30 days in the cheese samples of 9th and 11th as well as in the brines thereof. On the other hand, *Aeromonos sobria* sustained its viability up to 30th day in the 7th cheese sample.

Serratia liguefaciens was isolated for 60 days in the samples of 1st, 2th, 4th, 5 th, 6th and 11 th cheeses as well as the brines.

At the end of micro flora research of Urfa cheeses, *Escherichia, Enterobacter, Cedecea, Citrobacter,* Persentages of Gram negatif bacteria in 11 different Urfa cheeses *Klebsiella, Pseudomonas, Yersinia,*

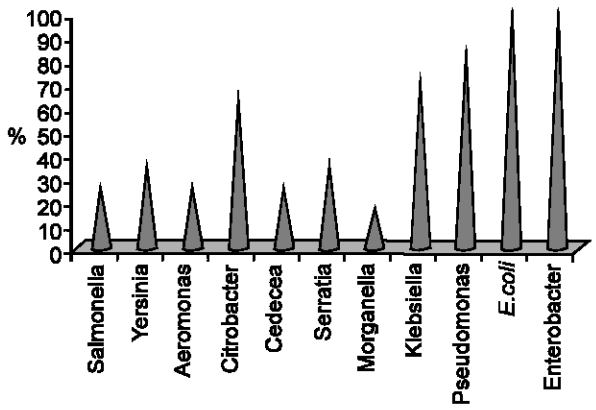

Fig. 6: The percentages of Gram negatif bacterium isolated from 11 different Urfa cheeses.

Aeromonas, Serratia and *Morgenalla* have been detected. Besides, *Staphylococcus* has been determined therein. *Steptococcus, Lactocossus, Lactobacillus, Pediococcus*, *Leuconostoc* were identified as well.

Average total alive mesophyll count of eleven different Urfa cheeses between 0-7th days is 3,5 x 10^4 cfu/gr. The average of 30th day is 3,1 x 10^4 cfu/gr. The average of 60th day is 2,0 x 10^4 cfu/gr. Total mean coliform bacteria count is, between 0-7th days, 1,9 x 10^4 cfu/gr. The average of 30th day is 1,3 x 10^4 cfu /gr while the average of 60th day is 7,6 x 10^3 cfu/gr. Especially in 4 (2nd, 3rd, 6th and 11th) out of the all Urfa cheese experiments, total mesophyll count and total alive coli form bacteria counts during 0-7th, 30th and 60th days are pretty high. In the direction of our studies, the previous researches that have been applied on the Urfa cheeses experiments sold in the local market indicates that *E. coli, S. aureus*, coli form bacteria counts being specified in these cheeses are extremely high from the limits given in the International Standards. (9; 10; 11). Average 0-7th days of total yeast and mold count in our experiments is 1,7 x 10^3 cfu/gr, while the average of 30th day is 3,2x10^3 cfu/gr. In addition the average of 60th day is found as 2,7 x 10^3 cfu /gr. The average of total lactic acid bacterium count during 0-7th days is 7,0 x 10^3 cfu/gr., the average of 30th day is 8,9 x 10^3 cfu/gr and the average of 60th day is found as 5,5 x 10^3 cfu/gr.

E. coli, among the Gram negative bacteria, that have been isolated from the Urfa cheese experiments in this study has been detected in all Urfa cheese samples.

In accordance with various researches, *Pseudomonas* are the contaminant bacteria in the raw milk flora. (Muir *et al.*, 1979; El - Bassiony *et al.*, 1985). Urfa cheese is produced from raw milk or the milk heated to 30-35°C. In our study, *Pseudomonas*, among the Urfa cheese experiments, is the most extensive bacterium, after *E. coli. Pseudomonas* has been proliferated at a ratio of 81.8% at 11 Urfa cheese experiments and *P. fluorescens* was determined in the 1st, 3th, 5th, 6th, 7th,

8th 9th and 11th cheese samples. While it survived throughout 60 days in 1st 3th and 11th cheese experiments, it has been capable of living up to 30 days in the other cheese samples. *P. maltophilia* has also been found in 3nd, 8rd, 9th, 10th Urfa cheese experiments. It was unable to survive after 0-7th day in the 8th cheese sample; it stayed alive up to 60th days in the 3th, 9th, 10th cheese experiments. Apart from importance of *Pseudomonas* in the sense of the public health, it also adds bitterness to cheese (Francoise *et al*., 2004).

In the research, *Salmonella* (*S. choleraesuis* and *S. typhi* at the rate of 18.1% and 9.09% respectively) at the rate of 27.2% exists among the bacterium species proliferated in Urfa cheeses. Both specie of *Salmonella*, gastroenteritis were effective. *S. choleraesuis* was observed during 60 days in the 2nd cheese sample and 30 days in the 3rd Cheese sample. *S. typhi*, that was available in 1st cheese experiment, isolated from cheese for 60 days. While *S. choleraesuis* having isolated from the brine of 2nd cheese sample throughout 60 days, *Salmonella* has been isolated up to 30th days in the brines of other samples. In the other countries, *Salmonella* has caused, by dependant to cheese, to a great deal of epidemic diseases. (Bergdoll, 1989; Zottola and Smith, 1991). Some researchers have been mentioned about "Salmonellosis" events originated from cheese at the Urfa and its proximities. (Ozer *et al*., 2003).

In our study, 8 out of 11 Urfa cheese experiments (72, 7%) *Klebsiella* (*K. pneumoniae*, *K. ozaneae*, *K. ornithinolytica*, *K. oxytoca* at rates of 27.2%, 27.2%, 9.09% and 27.2% respectively) has been isolated. *K. pneumonia* was able to survive up to 30th -60th day in some Urfa cheeses. *K. oxytoca* stayed alive for 60 days in 5th and 6th cheese experiments. *K. ozanea* was determined in 5th and 6th samples for 60 days. *K. ozaneae* has been found in the 9th and 11th cheese samples brines for 60 days. *K. ornithinolytica* survived 30 days in 2nd. cheese sample.

Yersinia (*Y. intermedia*, *Y. enterokolitica* and *Y. mortareti* at rates 18.1%, 9.09%, 9.09% respectively) has been obtained from 4/11 (36.3%) of our samples. *Y. mortareti* could be stayed alive in the 1st cheese sample up to 0-7th day. *Y. intermedia* has been detected for 60 days in the 6th cheese sample and 30 days in 10th and 11th ones. *Yersinia* species either might be available in pasteurized milk owing to insufficient pasteurization process, mixing of raw milk into pasteurized milk or contamination of pasteurized milk with *Y. enterokolitica* afterward with the other *Yersinia* species or also it is available in the cheese due to being decontaminated during the production of cheese. (Hamama *et al*., 1992; Ozer *et al*., 2004).

Likewise, in another study that has been made on Urfa cheeses, *Y. enterocolitica* at a rate of 1% (wt/v) was added to the milk during the production process of Urfa cheese, which was manufactured by using pasteurized milk and survival period thereof has been investigated. The half of them, after the production of the cheeses, has been subjected to the scalding process for 2-3 min. whiles the other half not. *Y. enterocolitica* was able to be survived maximum 30th day in the both 2 species of cheeses rested in the brines having salt concentration with 15% and 17, 5% salt. (Freitas *et al*., 1993).

In our research, the availability of *Aeromonas* as percentage in 11 different Urfa cheese samples is 27.2% (3/11). (18.1% *A. salmonicida* and 9, 09% *A. sobria*). *Aeromonas* were able to be remained alive up to 30th day, in the cheeses. *Aeromonas* is a pathogen causes a lot of diseases in human and animals. It infects by means of water and various foods and resulting the diseases together with gastroenteritis, indicating very different symptoms. (Araujo *et al*., 2002).

At our study, *S. aureus* was determined in 3 / 11 cheeses. *S. aureus* is a micro organism being available very often in raw milk and in the vicinity of manufacturing plant at the cheese industry. It has tolerance against salt and has a wide growth spectrum. Having insufficient amount of acidity in the cheese increases proliferation of *S. aureus* on one hand and causes proliferation of enterotoxin on the other hand. (Bergdoll, 1989). In a similar study made in Brazil, 45 white cheese samples that were offered to market for consumption, have been purchased and the evidence of *S. aureus* and enteropathogen bacterium was investigated. *E. coli, S. aureus* and *Aeromonas* were found at the rates of 97, 7%, 77.7% and 17.7% respectively in the said 45 white cheeses (Araujo *et al*., 2002).

At our research, in all of the Urfa cheeses, *Enterobacter* was found. The following amounts were identified therein; *E. intermedium* (18.1%), *E. nimipressuralis* (18.1%), *E. cancerogenus* (45.5%) and *E. gergovia* (18.1%).

At our study, within 11 cheese samples, 33% *Enterococcus faecalis* and 13% *Enterococcus faecium* were found in the lactic acid bacterium identifications. In a similar study being made by Aleksieva (1983) *E. faecalis* and *E. faecium* rates were found as 22% and 33% respectively.

Parallel to our study, a study has been made in Spain in regard to Roncal and Idiazabal cheeses those are being produced from raw ovine milk. These two different cheese samples were investigated, subsequent to a maturation period of 120 days, as microbiologically. No conspicuous difference, in the both cheeses, was observed from the micro organism species (Arizcun *et al*., 1977).

In the Urfa cheeses those have been manufactured from raw milk or the milk, which has not been treated by sufficient pasteurization processes, pathogen bacteria with gastroenteritis agent were detected. During 30-60

days isolated Gram negative bacteria were observed. Urfa cheeses are being manufactured, as regional, by the use of raw milk or scalded milk that has been heated up to 30-35°C. Therefore the number and variety of micro organism are quite a lot. This cheese use to be consumed in the region, without waiting the completion of its maturation period. Gastroenteritis case number in Urfa is considerably high as compared to the other regions. In conclusion it has recommended that the region's people not consume fresh Urfa cheese.

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