



The Existence of *Enterococcus* spp. In Civil Cheese

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ABSTRACT

Enterococcus species with lipolytic and proteolytic properties can pass directly from animal faeces or indirectly from contaminated water, equipment or storage tanks into milk. The present study focused on isolation and identification of *Enterococcus* species and to determine the microbiological characteristics of traditionally produced civil cheese. TMAB, Coliform, *Enterococcus* and yeast-mold counts of 40 civil cheese samples made by traditional method were determined. In addition, *Enterococcus* species was isolated and identified with Vitek2 compact system. total mesophilic aerobic bacteria counts were 8.22 log cfu/g; the number of the number of *Enterococci* were 5.77 log cfu/g, Coliform bacteria count were of 4.18 log cfu/ g and the number of yeast and mould were 6.35 log cfu/g. It was determined that the isolated 75 *Enterococcus* strains consisted of 33 *Enterococcus faecalis*, 23 *Enterococcus durans*, 12 *Enterococcus faecium* and 6 *Enterococcus gallinarum* and 1 *Enterococcus avium*. As a result, because of the production of fresh civil cheese under unhygienic conditions, its microbiological quality is low. This situation poses a potential health hazard to consumers.

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Introduction

Enterococcus spp. is very common not only in animal foods such as feces, soil, surface waters and plant materials, but also meat and dairy products (Franz et al., 1999). *Enterococci* are a group of important lactic acid bacteria (Franz et al., 2003, Psoni et al., 2006). *Enterococci* are bacteria, that resistant to pasteurization temperature, which can be adapted to different substrates and growth conditions. Although *Enterococcus* spp has a positive effect on the production and maturation of some traditional cheeses, a high amount of *Enterococcus* contamination in fresh and soft cheeses often results from insufficient sanitation during the production phase (Giraffa, 2003). Some *Enterococcus* spp. contribute to the formation of ripening and aroma in cheese as Cheddar, Feta, Mozerella, Cebreiro, Venaco and Hispanico (Centeno et al., 1999, Sarantinopoulos et al., 2002). The proteolytic and peptidolytic enzymes of *Enterococci* can break down the casein and cause the development of cheese texture and aroma, but causing undesirable bitter peptides to form (Giraffa, 2003, Foulquié Moreno et al., 2006). In addition, *Enterococcus* spp. it also increases the amount of water-soluble nitrogen as it contributes to the breakdown of casein (Centeno et al., 1999).

In a study of goat milk and cheese, 27 species were isolated and 22 of these isolates were identified as *Enterococcus faecium* and 5 of them were *Enterococcus faecalis*. (Rivas et al., 2012). In another research conducted in Italy, in semicotto caprino cheeses made from goat milk by traditional methods were isolated *Enterococcus faecalis* and *Enterococcus faecium*, *Enterococcus durans*, *Enterococcus hirae* and *Enterococcus gallinarum* (Suzzi et al., 2000).

In a study conducted in Spain, in the three different cheeses made from sheep's milk were isolated 95 *Enterococcus* and then identified by PCR as *E. faecalis*, *E. hirae*, *E. avium*, *E. devriesei* and *E. malodoratus* (Martín-Platero et al., 2009). In another research, 282 *Enterococcus* strains were isolated and identified from the milk, Roncal and Idiazabal cheeses. *Enterococcus faecalis* were the dominating species both in the milk as well as in the two types of cheese, and *Enterococcus faecium*, *Enterococcus durans*, and *Enterococcus avium* which were lower level (Arizcun et al., 1997).

The aim of this study is to determine the microbial quality of civil cheese are produced from raw milk by traditional methods. It is also to detect the presence of *Enterococcus* species, some of which are known to be used as starter cultures, in civil cheese.

Materials and Methods

The 40 civil cheese samples (25 g) were homogenised in 225ml of sterile ringer solution. Afterwards, the other solutions were prepared. Inoculation was performed by the pour plate method. The number of total aerobic mesophilic bacteria was determined on Plate Count Agar (PCA, Merck). The plates were incubated under aerobic conditions at $37\pm 1^\circ\text{C}$ for 24 h. For the determination of Coliform counts, 1 ml of the appropriate dilutions was inoculated into Violet Red Bile Agar (VRBA, Merck). The petri dishes were incubated at 30°C under anaerobic conditions for 2 days. Yeast and mold counts were determined using Rose Bengal Chloramphenicol (RBC) agar. The petri dishes were incubated under aerobic conditions at $25\pm 1^\circ\text{C}$ for 5 days. *Enterococcus* spp. counts were determined on Enterococci Agar (Merck) incubated under aerobic conditions at $37\pm 1^\circ\text{C}$ for 48 ± 1 h. Bacterial counts were expressed in Log cfu /g.

Enterococcus Identification

From the primary culture's different colonies with identical morphological appearance were selected for further purification. The selected colonies were inoculated on a Blood Agar medium and after incubation at $37\pm 1^\circ\text{C}$ for 24 h. Strains for identification were selected based on the morphological and physiological characterisation. The suspension prepared with pure *Enterococcus* spp. was loaded into Gram Positive cards. Finally, all the *Enterococcus* isolates were subjected to identification using the Vitek2 Compact system.

Results and Discussion

Microbial quality of civil cheese samples were total mesophilic aerobic bacteria counts were $8.22 \log \text{cfu/g}$; the number of *Enterococcus* spp. were $5.77 \log \text{cfu/g}$, *Coliform* bacteria count were of $4.18 \log \text{cfu/g}$ and yeast-mould count were $6.35 \log \text{cfu/g}$. The 75 *Enterococcus* strains isolates that were obtained from civil cheese samples were 23 *Enterococcus durans*, 33 *Enterococcus faecalis*, 12 *Enterococcus faecium* and 6 *Enterococcus gallinarum* was 1 *Enterococcus avium*.

The genus *Enterococcus* spp. important bacteria in the food microbiology. They participate and contribute in different fermentation process in dairy products (Giraffa, 2003). Similar levels of Enterococci have also been reported for feta chesses (Manolopoulou et al., 2003), white-brined cheeses (Litopoulou-Tzanetaki and Tzanetakis, 1992) and civil cheese (Tekinşen and Elmalı 2006). It was found *Enterococcus faecalis* (Arizcun et al., 1997, Suzzi et al., 2000, Ghrairi et al., 2008, Martín-Platero et al., 2009), *Enterococcus durans* (Arizcun et al., 1997, Suzzi et al., 2000), *Enterococcus faecium* (Arizcun et al., 1997, Suzzi et al., 2000, Ghrairi et al., 2008), *Enterococcus gallinarum* (Suzzi et al., 2000) and *Enterococcus avium* (Arizcun et al., 1997, Martín-Platero et al., 2009) isolates in this study are compatible with previous research.

It was observed that the number of TMAB determined in our study was compatible with the values determined by some researchers in civil cheese (Sert and Kıvanç, 1985, Tekinşen and Elmalı, 2006) The number of yeast-mold in cheese samples was found to be compatible with some studies lower than some (Sert and Kıvanç 1985), and higher than some (Manolopoulou et al., 2003). *Coliform* bacteria number were found higher than the other researches (Sert and Kıvanç 1985, Manolopoulou et al., 2003, Tekinşen and Elmalı, 2006).

The reason for the different microbial parameter results obtained in studies may be due to the fact that raw milk used in cheese production is not standard. In addition, the use of low-quality raw milk, improper production and storage conditions can be considered as the reason for the high microorganism counts. As a result, because of the production of fresh civil cheese under unhygienic conditions, its microbiological quality is low. This situation poses a potential health hazard to consumers.

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