Microbiological Quality Evaluation of Various Types Of Traditional Romanian Cheese Through Advanced Methods

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Abstract

Raw milk represents a nutritive environment for a number of pathogens, like *Salmonella* spp., *Escherichia coli* O157: H7, *Staphylococcus aureus* etc. This fact can cause a serious of foodborne outbreaks associated to the consumption of contaminated milk and derivate products. The traditional processing of raw milk in poor hygiene conditions can pose a serious microbiological risk. The study aimed at evaluating the incidence of pathogen bacteria in ripened traditional cheese by advanced biochemical and molecular methods in order to reveal the possible risk of consumer exposure. The study was applied on 150 samples of ripened cheese from the following types: salted teleme cheese and „Burduf” cheese and „Năsal” cheese. The traditional teleme cheese presented an average value of the total *E. coli* count in between 11.06 ± 0.52 - 38.33 ± 2.76 CFU/g. The risk represented by the presence of *E. coli* and *Staphylococcus aureus* is low within the first steps of ripening, being absent after 28 months of ripening in the teleme cheese samples. The *Staphylococcus aureus* load was in between 3.82 ± 0.12 log CFU/g for the first period of ripening in „Burduf” cheese and 0.27 ± 0.56 log CFU/g after the second period of maturation, following a descendant pathway towards the last period of ripening. In „Năsal” cheese we isolated the specific *Brevibacterium linens*, which gives the characteristics of this type of cheese, but also *Micrococcus* spp., in 35% and lactic streptococci in 20%. The traditional cheese evaluated represent a low risk of contamination given that no sample investigated has exceeded the maximum limits allowed by the legislation and no pathogen bacteria isolated.

Keywords: traditional Romanian cheese, milk, hygienic quality, PCR

INTRODUCTION

Consumer's demand for safer and higher quality food products is continuously growing on a global scale. For this reason, quality management systems have been developed, so as to monitor constantly the hazard occurrence and prevent possible outbreaks (Sandrou and Arvanitoyannis, 2000). The most dangerous and severe hazards occurring daily in animal origin products such as

cheese are the biological ones (Tranter, 1990), the dairy industry increasing constantly the efforts to ensure the quality and safety through the development and implementation of proactive programs such as the HACCP (Ito, 1974).

Cheeses are the most popular dairy products in the entire world, processed under various types and shapes according to country and traditionality (Fox *et al.*, 2000). The world cheese production

has increased, with an annual rate of 4.2% during the last 20 years (FDA, 2001). Researches concerning the food poisoning cases caused by contaminated cheese consumption show that the scarces processing conditions contributes the most to the development of the causative agent (FDA, 2001). Normally when such episodes occur a large number of people are affected. Thus, the application of a strict surveillance within cheese production line can minimize the risk of foodborne illnesses occurrence (Hill, 2000, Temelli *et al.*, 2006).

Unfortunately, the traditional processing of cheese does not comprise these strict surveillance systems, which sometimes make it very difficult to control the occurrence of pathogens. These types of products are a very nutritive environment for numerous pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, *Staphylococcus aureus* etc. The traditional processing can represent a higher risk of contamination due to poorer hygiene conditions and lack of strict surveillance system. Until now there have been a serious of foodborne infection episodes stated to be caused by consumption of contaminated cheese with *Salmonella*, *Campylobacter*, *Staphylococcus aureus* and *E. coli* O157: H7 (Zottola and Smith, 1991; De Buyser *et al.*, 2001). The aim of this study was to evaluate the incidence of pathogens in ripened cheese obtained in industrial and traditional systems with an emphasis on the risk exposure assessment.

MATERIALS AND METHODS

Sample collection and preparation

The material subjected to research was represented by 150 of ripened telemea cheese products obtained in the industrial system and salted "telemea" cheese obtained in a traditional system. All the samples collected were kept in

their original package and kept at 0...4°C until their further analysis. The analysis was made within 48 hours from their reception in the Food safety laboratory of Faculty of Veterinary Medicine Cluj Napoca.

Identification of *Staphylococcus* spp.

The isolation and identification of *Staphylococcus* spp. was made according to the current standard protocol (SR EN 6888/2005). Briefly, the specific solid media used was Baird – Parker (Oxoid; Germania) and the incubation made at 37°C for 24 hours. The confirmation was made by cultural examination, evaluating the colonies' aspect on the selective media (color, size, shape, aspect and margins) and morphological exam (Gram staining). The representative colonies were subjected to polymerase chain reaction (PCR) confirmation technique.

Identification of *E. coli*

The isolation and identification of *E. coli* was made according to the protocol described in the current standard (SR EN 16649-2/2007). Briefly, the solid culture media used was TBX (Tryptone Bile X-Gluconoride) (Oxoid; Germania). The incubation was made at 37°C for 24 hours and the confirmation followed the same protocol as for *Staphylococcus* spp., described in detail above.

PCR method for bacteria genera confirmation

The DNA extraction was made from morphologically confirmed *E. coli* colonies. The DNA was extracted from approximately 10-12 specific colonies using a specific extraction kit (Isolate II DNA kit, Bioline, Germania) following the protocol described by the producer.

The DNA quantities and purities were evaluated by spectrophotometry, using a Nanodrop ND-1000 apparatus (NanoDrop Technologies, Inc., Wilmington, DE, USA). Each sample was brought to a concentration of 20 ng/μl with elution buffer. The PCR technique was made in a final volume of 25

Tab. 1. The primers used for the confirmation of the isolated *E.coli* and *Staphylococcus* strains

Gene	Primer sequences (5'- 3')	Size (bp)	T (°C)	Reference
23S	F: AAGGAATCACCTTGCAGATAAACTC R: TTTCCGAGTACATTGGCATCGT	736	56	Malorny <i>et al.</i> , 2004
NUC2	F: ATGAAGTCAAATAAATCGCT R: TTTGGTGAAAAATACTTCTC	458	58	Gandra <i>et al.</i> , 2011

μL, using the following reagents: MyTaq (Bioline, England) polymerase mix (12.5μL/sample) and 1 μL of Forward/ Reverse Primer specific for each bacteria (Tabel no.1). The amplification was made in a G-Storm thermocycler (LabTech, England), using 35 temperature cycles as it follows: denaturation 94°C (1 min.), alignment temperature according to specific primers (table 1) and elongation at 72 °C (1 min.). The migration of the amplified products was made in an agarose gel of 2.5% concentration stained with EvaGreen DNA dye (1.7μl – 35 ml/gel; 7μl – 140 ml/gel). The electrophoresis was made at 100 V (20 min.) and 70V(40 min). The reading and interpretation was made in a Trans UV imaging system (BioRad, Germany).

Statistical interpretation

Statistical interpretation of data was performed using the operating system Windows 2007, program Origin 8.5.

RESULTS AND DISCUSSION

The results obtained at the bacterial load analysis of telemea cheese obtained in an industrial system

Due to the fact that this type of cheese is practically always covered in brine, it is protected on the surface against bacteria. *E. coli* and *Staphylococcus aureus* are pathogens with high risk on the public health.

For the safety of the telemea cheese sent for consumption it is mandatory to perform analysis on the milk destined for processing (Donnelly, 2004). In conformity with the regulations (Reg. CE 2073/2005, modified by Reg. CE 1441/2007), in case of ripened cheese subjected to thermal treatment, it is mandatory to identify the presence of staphylococci enterotoxin, the *E. coli* load and the number of staphylococci coagulase-positive. Regarding the presence of *E. coli*, we have found in the analyzed samples that only 2 were negative (Fig.1) (samples 7, 14), respectively 7.69%. The rest of the samples have shown an average number in between 8.66 ± 1.52 - 16.33 ± 7.76 CFU/g, values relatively low, which are in accordance with the legislation (Reg. 1441 CE/2007), of maximum 100 CFU/g. Similar values have been obtained by Suler *et al.* (2010), the study mentioning also that the presence of *E. coli* in this type of dairy products is due mainly to poor hygiene conditions along the technological process.

In what concerns the *Staphylococcus* genus, the frequency of positive samples was much lower, 8 samples being negative (20.51%).

The statistics showed an average value of the positive samples in between 1 ± 1.73 and 45.66 ± 6.02 CFU/g, values that are in conformity with the legislation (max. 100 CFU/g) (Fig.2). Given the fact that the coagulase-positive staphylococci load was in between the maximum limit imposed

Fig.1. The representation of *E. coli* contamination of the teleme cheese obtained in the industrial system

by the legislation (Reg. 1441 (CE)/2007), we did not test the samples for staphylococci enterotoxin. Similar values were reported by Suler *et al.* (2010), highlighting values in between 0.48 and 7.08 CFU/g at the same type of dairy products. In

contrast, a study made by Duminică (2009), has shown that the ripened telemea cheese holds a high prevalence percent for *E. coli* 56.57% and average for *Staphylococcus* (11.76%).

Fig. 2. The representation of the contamination with coagulase-positive staphylococci in the telemea cheese obtained in the industrial system

Fig. 3. The representation of *E. coli* contamination in the telemea cheese obtained in a traditional system

A number of studies have shown high variation within the load of these bacteria according to the ripening stage. For example, Mojsova *et al.* (2009), has shown that in the fourth day of ripening, the average value of *E. coli* colonies is of 5.3 log CFU/g (200.000 CFU/g), and in the tenth day the value is situated in between 2 and 4.77 log CFU/g. After 90 days of ripening, no bacteria was identified which

reveals that the low value of the pH has inhibit the presence of these germs.

The results obtained at the bacterial load analysis of telemea cheese obtained in a traditional system

Regarding the presence of *E. coli* in traditional telemea cheese we found that no sample had a lower value than 10 CFU/g. All samples have shown an average value of the total number of

Fig. 4. The representation of contamination with coagulase-positive staphylococci in the teleme cheese obtained in a traditional system

Fig. 5. Molecular confirmation of *E. coli* strains isolated from the industrial system „teleme” cheese; M – ethalon scale (100 bp); 1 – positive control; 2 – negative control; 3 – 9 – positive PCR confirmed samples

Fig. 6. The molecular confirmation of the *E. coli* strains isolated from the traditional teleme cheese; M – the ethalon scale (100 bp); 1 – positive control; 2-9 confirmed positive samples

colonies in between 11.06 ± 0.52 and 38.33 ± 2.76 CFU/g, values that are higher than those revealed at the samples collected from the hypermarkets (fig 3).

The high load of *E. coli* in traditional telemea cheese can be attributed to the fact that this type of dairy product is normally processed from raw milk and the technological manufacturing lacks the hygiene conditions.

The *Staphylococcus* showed a lower prevalence, 4 samples being negative. Although the number of negative samples was higher than in *E. coli* case compared to the industrial type of telemea cheese the percentage of negative samples was much lower (13.33% vs. 20.51%). The statistical evaluation has shown an average of *Staphylococcus* load in between 4 ± 1.73 and 23.12 ± 2.02 CFU/g, values that are in conformity with the legislation (max. 100 CFU/g) (Fig. 4). The values obtained in our study were much higher than those reported by Suler *et al.* (2010), which revealed numbers in between 0.48 and 7.08 ufc/g.

The molecular testing of *E. coli* and *Staphylococcus* strains isolated from telemea cheese

The strains of *E. coli* and *Staphylococcus* isolated from the ripened cheese samples were molecularly tested for confirmation

All *E. coli* isolated were confirmed also by PCR, being positive at the specific DNA amplification with the common sequence (fig. 5, 6). The PCR protocol tested in this study was implied the identification of the common sequences for *E. coli*, sequence 23S, which has a molecular weight of 736 base pairs.

From the total of 43 ripened telemea cheeses, *E. coli* was confirmed by PCR in 42 samples. This high prevalence (97.57%) is concerning given that other data from field literature states a lower prevalence. For example, Soomro *et al.* (2002) have only 57%, and Farzan *et al.* (2012), 30.28 %. A similar percent to our was revealed by Paneto *et al.* (2007) in the traditional italian ripened cheese. Other types of cheese like cottage, has shown a much lower prevalence (12.9%) (Singh and Prakash, 2008). No sample tested positive at the PCR for *Staphylococcus aureus*.

CONCLUSIONS

Our study shows that traditionally obtained telemea cheese has a higher prevalence for *E. coli* and *Staphylococcus*. This higher load may be due to the particular processing but also to lack of strict hygiene conditions during processing. We recommend a better monitoring of the good manufacturing practices and (GMP) and good hygiene practices (GHP) within this particular processing chain.

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