THE ASSESSMENT OF MICROBIOLOGICAL QUALITY OF SOME TRADITIONAL ROMANIAN CHEESES

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Summary

It has been analyzed a total number of 954 cheese samples. The samples have been harvested from 9 types of cheese in different market places. The obtained statistical analyzed results have led to the conclusion that all types of cheese have been recorded unconformingly samples. It has been noticed either the getting over of some microbial parameters (coliforms, coagulase-positive staphylococci, sulphite-reducing bacteria) or the presence of some pathogen and opportunistic bacteria (Clostridium perfringens has been isolated from 1.68% samples; Proteus sp. has been isolated from 0.84% samples).

Key words: cheeses, bacterial, yeast, moulds, parameters

Microbiological quality of cheeses is a part of food safety field, due to the fact that there can be generated a range of diseases to consumers by the presence into the alimentary products of pathogen and opportunistic bacterial species, or by the degradation of nutritive substratum resulting toxic metabolites (2, 4, 5). Based on this, in the recent years there have been run large programs of milk processing units modernization and there has been purchased high-quality equipment in order to ensure a decreasing of the contaminants number in the final product, for the same purpose being imported and adapted new technologies and processing chains, more efficient and conformingly to the up-to-date hygienic requirements in processing (5, 8). Firstly, the direct effect of these measures was the milk products quality improvement generally, as well as the improvement of the microbiological and physical-chemical parameters of the intermediary and final products, but there has been no significant influence on the quality of the final products analyzed in the selling points. Especially the cheeses are marketed, even in some super-market selling points, in inadequate conditions: different types of cheese are presented (with or without moulds in the paste) with no complying with minimal hygienic standards of cross-contamination preventing, there are presented products without being prepackaged, many products are displayed in open shop windows, the products slicing is made with no complying with minimal hygienic standards.

It is important that the economical representatives with interest in food industry assimilate the new concepts in alimentary hygiene and focus on the entire food-chain: raw material – final product – merchandise. Only a dynamic analysis of milk products will provide to the market healthy products, which would totally preserve their hygienic characteristics.
Materials and methods

There have been harvested cheeses samples from various selling points in Bucharest markets, the present study aiming to cover many different types of cheese, made by different economic units and marketed in different selling units. The types of cheese were: curds cheese, brined cheese (cottage cheese) and cheese with scalded and spun paste, fermented cheese. The harvested samples were delivered in sterile containers in optimal temperature conditions. The samples processing was made in the alimentary microbiology laboratory according to the microbiological STAS regulations and standardized ISO methods (10, 11).

The study was made during a period of one year (during 2008), being analyzed 954 samples (125 curds cheese samples, 114 brined cow cheese samples, 96 brined sheep cheese samples, 89 brined mixed cheese samples, 128 Dalia pressed cheese samples, 157 Penteleu pressed cheese samples), 79 mozarella samples, 54 Tilsit samples and 112 samples of smoked pressed cheese).

In order to establish the total aerobic heterotrophic mezophilic bacteria (TAHMB) there have been made decimal dilutions in peptonated water. From each dilution it was distributed, with sterile dropper, 1 ccm in each of the 2 Petri plates. In each Petri plates was mold agar melted and cooled at 40 – 45°C, it was stirred and incubated for 24 hours at 37°C. It was assessed the mean of CFU/g product (1, 3, 11).

In order to establish the probable number of coliform bacteria there were made decimal dilutions; from each of them 1 ml was put in 3 tubes with BGBB medium (brilliant curds bile broth) and 1 Durham tube. It has been incubated for 24-48 hours at 37°C. The interpretation was made based on gas production and following 3 numbers mean calculation (according to the score for each of the three tubes with BGBB medium), the obtained mean being read following Mac Grady table. In addition, there has been used the method of establishing coliform bacteria number by colonies counting (ISO 4832). From the decimal dilutions it is distributed 1 ml in two Petri plates. In each plate is mold VRB agar (agar and lactose, bile salts, crystal violet and indicator), melted and cooled at 45°C. The above prepared plates are incubated at 35°C for 24 hours. After the incubation the red-purple colonies are counted and the mean value is calculated with the formula: \( N = \frac{\Sigma c}{n_1 + 0.1 n_2} \) (where \( \Sigma c \) is the sum of counted colonies; \( n_1 \) is the number of plates to be counted from the first dilution; \( n_2 \) is the number of plates to be counted from the second dilution; \( d \) is the dilution rate corresponding to the first used dilution) (1, 3, 11).

In order to establish the pathogen and opportunistic germs species there were used standardized ISO methods: SR ISO 6597 for identifying Salmonella bacteria, STAS ISO 6888 for identifying coagulase-positive staphylococci, SR 2356/1 for identifying Proteus bacteria, STAS ISO 4832 for identifying Escherichia coli bacteria, SR ISO 7251 for establishing the probable number of E. coli, SR ISO 7932 for identifying Bacillus cereus species, STAS 12966-91 for establishing the moulds number, STAS 12964-91 for establishing the yeasts number etc. (1, 11). All the isolated
bacterial strains presumptively identified as pathogenic or opportunistic were biochemically tested for confirmation diagnosis; the scientific data obtained being thus rigorously demonstrated.

Results and discussions

The results obtained following the complete bacteriological analysis of each harvested and processed sample were statistically processed and average values are calculated for each type of product. The total aerobic heterotrophic mesophilic bacteria had mean value between relatively large limits: curds cheeses – $2.4 \times 10^3$; cottage cheeses made from diary unpasteurized milk – $1.7 \times 10^4$; ewe cottage cheeses – $1.2 \times 10^4$; fresh cottage cheeses made from mixed milk - $2.9 \times 10^3$; Dalia pressed cheese – $2.1 \times 10^3$; Penteleu pressed cheese – $4.8 \times 10^4$; mozzarella – $6.7 \times 10^5$; Tilsit cheese – $2.1 \times 10^2$; smoked pressed cheese – $2.7 \times 10^2$. Although for the different types of analyzed cheese this parameter is not regulated, we consider that the obtained values reveal an important number of contaminants.

The microbiological analysis for assessing the number of coliform bacteria, *Escherichia coli* and coagulase-positive staphylococci has proved a range of exceeds comparatively to the maximum allowed values. The microbiological analysis for assessing the number of sulphite reducing bacteria and *Bacillus cereus* has proved the presence of these bacterial species in the studied samples; although the above germs numbers are not regulated, the samples that recorded higher values than admitted limits in EU were considered inadequate.

The number of samples which recorded exceeds and the percent of unconforming samples are shown in table 1.

### Table 1

<table>
<thead>
<tr>
<th>Samples type</th>
<th>Coliform bacteria</th>
<th>E. coli</th>
<th>CPS</th>
<th>B. cereus</th>
<th>SR bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>%</td>
<td>No. of samples</td>
<td>%</td>
<td>No. of samples</td>
</tr>
<tr>
<td>Curds cheeses</td>
<td>12</td>
<td>9.68</td>
<td>4</td>
<td>3.22</td>
<td>15</td>
</tr>
<tr>
<td>Brined cheeses (cow’s milk)</td>
<td>17</td>
<td>14.78</td>
<td>7</td>
<td>6.09</td>
<td>21</td>
</tr>
<tr>
<td>Brined cheeses (sheep milk)</td>
<td>5</td>
<td>5.10</td>
<td>1</td>
<td>1.02</td>
<td>5</td>
</tr>
<tr>
<td>Brined cheeses (mixed milk)</td>
<td>4</td>
<td>4.59</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>
The statistical analysis of the results led to the conclusion that the majority of products recorded a number of unconforming samples. From all the analyzed samples there have been not isolated bacteria belonging to *Salmonella* genus. Alternatively, there have been isolated bacteria belonging to *Proteus* genus, *Clostridium* genus and some samples were overloaded with yeasts and moulds (more than $1 \times 10^4$). There has been noticed also, in the case of some cheeses, the presence of certain moulds species either producing toxic metabolites (*Aspergillus*), or degrading the biochemical structure of proteins and lipids and altering organoleptic indicators (*Mucor* sp.). The number of positive samples and their percent from all the analyzed samples for each type of aliment are shown in table 2.

### Table 2

<table>
<thead>
<tr>
<th>Aliment type</th>
<th>Proteus</th>
<th>Clostridium perfringens</th>
<th>Total Number of Fungi</th>
<th>Aspergillus sp.</th>
<th>Mucor sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>%</td>
<td>No. of samples</td>
<td>%</td>
<td>No. of samples</td>
</tr>
<tr>
<td>Curds cheeses</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1.61</td>
<td>-</td>
</tr>
<tr>
<td>Brined cheeses (cow’s milk)</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>3.48</td>
<td>-</td>
</tr>
<tr>
<td>Brined cheeses (sheep milk)</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1.02</td>
<td>-</td>
</tr>
<tr>
<td>Brined cheeses (mixed milk)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Dalia pressed cheese</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CPS – coagulase – positive staphylococci
SR bacteria – sulphite-reducing bacteria
Based on the obtained results following the bacteriological analysis it is proved that a range of products are contaminated either during transportation, or directly in market, due to the inadequate storage conditions or temperature for a certain product.

The presence of the *Aspergillus* and *Mucor* species was noticed in Penteleu and smoked pressed cheese maintained on refrigeration temperatures (0 – 6°C), which leads to the protection layer moisture and allows moulds spore spreading (frequently noticed in pressed cheese kept in low temperatures for several weeks) (4, 5, 6, 7).

**Conclusions**

All types of cheese analyzed have recorded unconforming samples concerning microbiological parameters. There are 3 types of cheese to be noticed: curds cheeses, brined cheeses (cottage cheese) made from cow's milk, and Penteleu pressed cheese, which have recorded the highest number of unconforming samples, for the majority of the analyzed microbiological parameters.

Following microbiological analysis none of the products has recorded samples contaminated with germs belonging to Salmonella genus. Microbiological examination conducted in order to identify bacteria belonging to *Proteus* genus revealed such bacteria presence in eight samples, four of them in a single type of product (smoked pressed cheese).

*Clostridium perfringens* sp. was identified in 16 samples (1.68% from the total number of analyzed samples), the highest number of positive samples being recorded in the following types of cheese: brined cheeses (cottage cheese) made from cow's milk (3.48%), and mozzarella (5.13%).

**References**


