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MICROBIOLOGICAL EXAMINATION OF TELEMEA CHEESE IN CONSUMPTION NETWORK

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Abstract

In order to prevent the appearance of consumer diseases, any food products need to respect bacteriological standards; to not contain pathogenic germs, and the saprophytes ones have to be under maximal norm limits. In this study there were harvested 30 samples of Telemea cheese, from two different production units, in two different counties. The samples were collected in two different seasons: summer and winter and were microbiological analysed, in order to determine the following parameters: coliform bacteria, E. coli, Coagulase-positive staphylococci, Salmonella, total number of yeasts and moulds. The result were statistical evaluated, establishing each microbiological index dynamic, depending on season.

Key words*: cheese, consumer, pathogenic germs, saprophyte germs.*

INTRODUCTION

Telemea cheese is a traditional product made from milk (Georgescu, 2000). The characteristics of this range of cheeses consist in: manufacturing technology, relatively high salt content and the fact that they are matured and preserved until consumption in brine (Costin, 2003). The assessment of the microbiological quality of cheeses is difficult, because of the technological microflora used in their manufacture. Microbiological control of cheeses involves the identification of pathogenic germs or the establishment of the causes of defects. (Bărzoi, 2002).

MATERIALS AND METHODS

The microbiological control was performed on a number of 30 Telemea cheese samples, collected from two processing locations (Ilfov County - P3 and Câmpulung Muscel - P5), in the warm and cold seasons.

The working procedure used consists in determining the following microbiological parameters: coliform bacteria, *E. coli*, *Salmonella*, *Staphylococcus* coagulase positive, Yeasts and molds.

The method to determine coliform bacteria involves insemination in BBLV, with fermentation tube and incubating at 37°C, 24-48 hours. Brilliant Green Bile Broth (BBLV) is a liquid medium used for the detection or confirmation of coliform bacteria in water and wastewater, foods, dairy products and other materials of sanitary importance. The development of some bacterial cultures with gas production in the fermentation tubes, consisting exclusively of Gram bacilli or coccobacilli, were considered positive reactions for coliform bacteria. The results were interpreted and the analysis continues with the determination of *E.coli* species. From the tubes tested positive for coliform bacteria, there were done inseminations in Petri dishes with Levine medium. The confirmation was performed in BBLV, tryptone water and slanted agar with incubation at 45°C, for 24 hours.

The work technique for *Salmonella* sp. isolation and identification, by horizontal method, assume the following steps:

- Stage I - Pre-enrichment in unselective liquid mediums.

The *Salmonella* bacteria may be in low number and often accompanied by a large number of

Enterobacteriaceae or other bacteria species. Pre-enrichment is necessary to discover the low number of *Salmonella* sp. or modified *Salmonella* sp.
Buffered peptone water is inoculated along with sample, then incubated at 37°C ± 10°C for 18 h ± 2 h.
- Stage II - Enriching in selective liquid mediums.
The Rappaport-Vassiliadis soybean medium (RVS broth) and tetrathionate/novobiocin Muller-Kauffmann broth (MKTTn broth) are inoculated with bacteria culture medium obtained in buffered peptone water. The RVS broth is incubated at 41.5°C ± 1°C for 24 hours ± 3 hours and the MKTTn broth at 37°C ± 1°C for 24 hours ± 3 hours.
- Stage III - Isolation and identification.
From the culture mediums obtained, two solid selective mediums are inoculated: xylose-lysine-dezoxycholate agar (XLD agar - Figure 1) and Rambach agar (Figure 2). They are incubated at 37°C ± 1°C and examined after 24 h ± 3 h.
- Stage IV - Identity confirmation.
Isolated colonies, presumed as *Salmonella* sp., are confirmed by biochemical and serological tests.
The detection of a large number of yeasts and molds in food products indicates the existence of inadequate hygienic conditions during food products obtaining and storage. (Apostu, 2006). The determination method involves performing decimal dilutions and inseminations on suitable mediums (Sabouraud agar with glucose and chloramphenicol). After 4-5 days of incubation, the formed colonies are counted.
Coagulase-positive staphylococci are microorganisms that form typical colonies on the surface of selective culture mediums. These microorganisms show a positive coagulase reaction, the coagulase production being a main index for enterotoxicity evaluation (Bărzoi, 2002). The determination method involves performing several steps: decimal dilutions and inseminations in Petri dishes with Chapman agar, incubation and developed colonies counting and performing the coagulase test.

RESULTS AND DISCUSSIONS

In Table 1, there are presented the results obtained after microbiologic examination of finite product, in P3 location, in warm season.

Table 1. Results obtained after microbiologic examination of finite product, in P3 - warm season.

No.	Coliform bacteria/g	Coagulase-positive staphylococci/g	Yeasts and molds/g
1	15	10	864
2	9.5	10	769
3	7.5	9	753
4	6.5	7	851
5	7.5	8	982
6	7.5	8	971
7	9.5	6	860
8	3	5	764
9	4	9	853
10	6.5	9	874
11	7.5	8	930
12	9.5	10	926
13	7.5	9	897
14	9.5	8	925
15	9.5	6	760
16	3	7	824
17	4	5	930
18	6.5	10	885
19	9.5	9	760
20	9.5	9	774
21	7.5	8	768
22	6.5	7	528
23	4.5	6	560
24	7.5	9	589
25	4	8	974
26	6.5	10	831
27	9.5	10	658
28	7.5	8	706
29	9.5	7	821
30	4.5	3	930

It can be observed that coliform bacteria had values between 3 and 15 germs/g, Coagulase-positive staphylococci had values from 3 to 10 germs/g and contamination with *E. coli* and *Salmonella* was absent. For yeasts and mold there was determined a number between 528 to 982 germs/g.
In Figure 1 there is presented the degree of contamination variation with coliform bacteria, for Telemea cheese, in warm season, in P3 location. This parameter it is a line which has a descending trend.

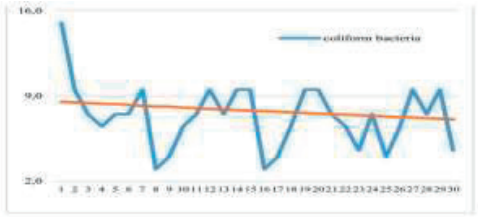

Figure 1. Dynamic of coliform bacteria contamination in Telemea cheese, in warm season, in P3 location

In Figure 2 there is presented the Coagulase-positive staphylococci fluctuation, observed in finite product during warm season. This parameter is also represented throw a line which has a descending trend.

Figure 2. Dynamic of Coagulase-positive staphylococci contamination in Telemea cheese, in warm season, in P3 location

In Figure 3 there is presented the Yeasts and mold fluctuation, observed in finite product during warm season. This parameter is represented also throw a line with a descendent trend, which means an improvement of the hygienic parameters provided in the legislation of dairy products

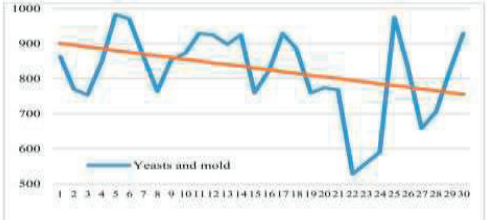

Figure 3. Dynamic of Yeasts and molds contamination in Telemea cheese, in warm season, in P3 location

In Table 2 there are presented the statistic analyses of main level and dispersion parameters for the analysed sample taken in P3 location, in warm season.

Table 2. Analyses of main level and dispersion parameters for the analysed sample taken in P3 location, in warm season

Parameter	n	$\bar{x}$	S^2	S	CV%
Coliform bacteria	30	7.33±0.46	6.60	2.57	35.05
Coagulase-positive staphylococci	30	7.93±0.32	3.10	1.76	22.18
Yeasts and molds	30	817.23±21.90	14399.90	120	14.68

The variability coefficient registered for coliform bacteria has the highest values (35.05%)

In Table 3, there are presented the results obtained after microbiologic examination of finite product, in P5 location, in warm season.

Table 3. Results obtained after microbiologic examination of finite product, in P5, warm season

No.	Coliform bacteria/g	Coagulase-positive staphylococci/g	Yeasts and molds/g
1	6.5	2	654
2	7.5	6	589
3	7.5	5	573
4	6.5	5	569
5	6.5	3	681
6	3.5	1	536
7	4	7	490
8	2.5	6	486
9	6.5	5	512
10	4	8	607
11	4	6	617
12	7.5	7	530
13	7.5	5	589
14	6.5	3	573
15	7.5	4	494
16	6.5	4	602
17	7.5	8	612
18	3.0	8	634
19	2.5	7	656
20	6.5	1	594
21	2.5	3	586
22	4	6	703
23	6.5	2	684
24	6.5	8	690
25	3.5	7	681
26	2.5	6	594
27	7.5	5	659
28	4.5	8	549
29	6.5	5	638
30	4	3	679

It can be observed that coliform bacteria had values between 2.5 and 7.5 germs/g, Coagulase-positive staphylococci had values from 1 to 8 germs/g and contamination with E.coli and Salmonella was absent. For yeasts

and mold there was determined a number between 486 to 690 germs/g.
In Figure 4 there is presented the degree of contamination variation with coliform bacteria, for Telemea cheese, in warm season, in P5 location. This parameter is represented by a line which has a descending trend.

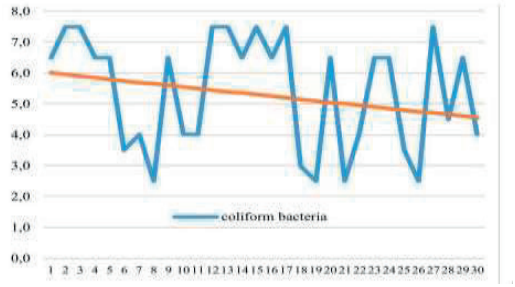

Figure 4. Dynamic of coliform bacteria contained in Telemea chese, in warm season, in P5 location

In Figure 5 there is presented the degree of contamination variation with Coagulase-positive staphylococci, for Telemea cheese, in warm season, in P5 location. This parameter is represented by a line which has an ascending trend.

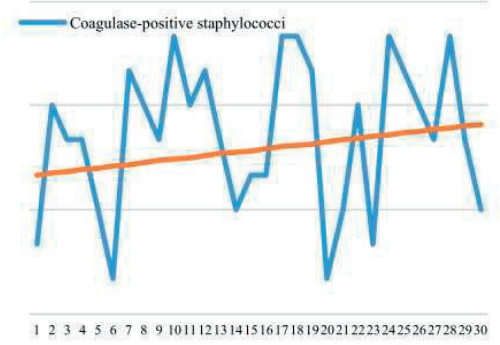

Figure 5. Dynamic of Coagulase-positive staphylococci contamination in Telemea cheese, in warm season, in P5 location

In Figure 6 there is presented the Yeasts and mold fluctuation, observed in finite product during warm season, in P5 location. This parameter is represented throw a line which has a slightly ascendent trend.

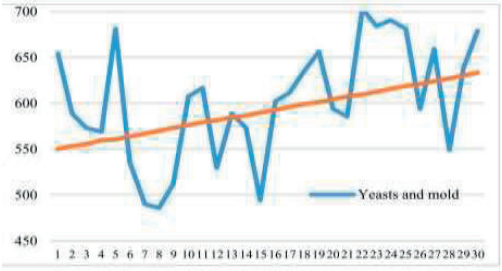

Figure 6. Dynamic of Yeasts and molds contamination in Telemea cheese, in warm season, in P5 location

In Table 4 there are presented the statistical analyses of main level and dispersion parameters for the analysed sample taken in P5 location, in warm season.

Table 4. Analyses of main level and dispersion parameters for the analysed sample taken in P5 location, in warm season

Parameter	n	$\bar{x}$	S^2	S	CV%
Coliform bacteria	30	5.4±0.33	3.45	1.85	34.42
Coagulase-positive staphylococci	30	5.13±0.39	4.62	2.14	41.79
Yeasts and molds	30	602.03±11.55	4007.76	63.30	10.51

The variability coefficient registered for Coagulase-positive staphylococci in warm season, P5 location, has the highest value (41.79%)
Testing the significance of the differences for Coliform bacteria in Telemea cheese, in warm season, it was found that between P3 and P5 locations, the differences are distinctly significant (Table 5). This is due to fact that in these locations the HCCP plan (Hazard analysis Critical Control Point Read) is implemented.

Table 5. Analyses of main level and dispersion parameters for the analysed sample taken in P5 location, in warm season

Parameter	**Calculated t**	**table t (t α)**			
		$t_{0.05}$	**$t_{0.01}$**	**$t_{0.001}$**	**$t_{0.1}$**
Coliform bacteria	3.33 **	2.00	**2.66**	3.46	1.29
Coagulase-positive staphylococci	5.52***				
Yeasts and molds	8.68***				

After testing the significance of the differences between P3 and P5 locations, in warm season, it was found that for Coagulase-positive staphylococci and for yeast and mold parameters, the differences are very significant.
In Table 6, there are presented the results obtained after microbiologic examination of finite product, in P3 location, in cold season.

Table 6. Results obtained after microbiologic examination of finite product, in P3 - cold season

No.	Coliform bacteria/g	Coagulase-positive staphylococci/g	Yeasts and molds/g
1	4	4	861
2	3	9	763
3	3	9	654
4	2.5	2	817
5	3.5	8	980
6	6.5	8	873
7	3.5	6	845
8	4	3	762
9	3.5	9	814
10	7.5	8	861
11	7.5	8	918
12	2.5	8	935
13	9.5	5	863
14	7.5	6	918
15	6.5	5	734
16	4	4	721
17	4.5	7	818
18	3	9	862
19	3	3	715
20	6.5	4	761
21	2.5	9	753
22	7.5	6	524
23	4	8	540
24	3	6	579
25	6.5	8	871
26	7.5	8	739
27	6.5	7	698
28	4	6	725
29	3	2	886
30	5.5	5	694

It can be observed that coliform bacteria had values between 2.5 and 9.5 germs/g, Coagulase-positive staphylococci had values from 2 to 9 germs/g and contamination with E.coli and Salmonella was absent. For yeasts and mold there was determined a number between 524 to 980 germs/g.
In figure 7 there is presented the degree of contamination variation with coliform bacteria, for Telemea cheese, in cold season, in P3 location. This parameter it is a line which has an ascending trend.

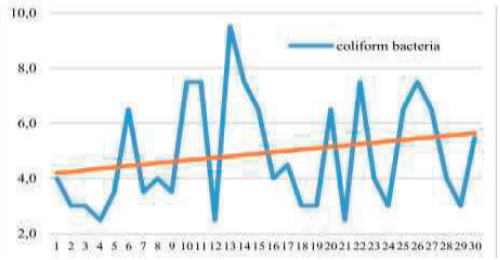

Figure 7. Dynamic of coliform bacteria contamination in Telemea cheese, in cold season, in P3 location

In Figure 8 there is presented the Coagulase-positive staphylococci fluctuation, observed in finite product during cold season. This parameter is represented throw a line which has a descending trend.

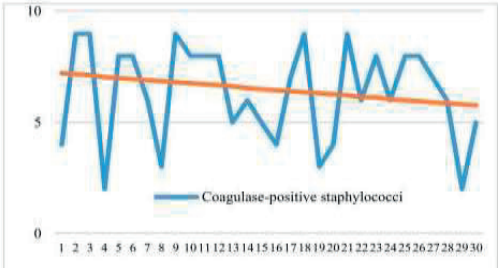

Figure 8. Dynamic of Coagulase-positive staphylococci contamination in Telemea cheese, in cold season, in P3 location

In Figure 9 there is presented the Yeasts and mold fluctuation, observed in finite product during cold season. This parameter is represented also throw a line with a descendent trend, which means an improvement of the hygienic parameters provided in the legislation of dairy products

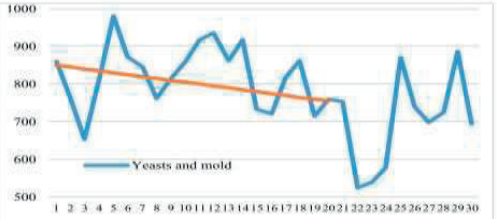

Figure 9. Dynamic of Yeasts and molds contamination in Telemea cheese, in cold season, in P3 location

In Table 7 there are presented the statistical analyses of main level and dispersion

parameters for the analyzed sample taken in P3 location, in cold season.

Table 7. Analyses of main level and dispersion parameters for the analyzed sample taken in P3 location, in cold season

Parameter	n	$\bar{x}$	S^2	S	CV%
Coliform bacteria	30	4.85±0.36	4.03	2.00	41.42
Coagulase-positive staphylococci	30	6.33±0.40	4.85	2.20	34.77
Yeasts and molds	30	782.8±20.75	12921.96	113.67	14.52

The variability coefficient registered for coliform bacteria has the highest values (41.42%).

In table 8, there are presented the results obtained after microbiologic examination of finite product, in P5 location, in cold season.

Table 8. Results obtained after microbiologic examination of finite product, in P5, cold season

No.	Coliform bacteria/g	Coagulase-positive staphylococci/g	Yeasts and molds/g
1	4	3	653
2	4.5	4	586
3	3.5	6	537
4	6.5	3	558
5	7.5	3	680
6	6.5	2	530
7	6.5	8	489
8	4	2	468
9	3.5	7	570
10	4	8	603
11	6.5	2	607
12	7.5	4	508
13	4	5	568
14	3.5	6	537
15	2.5	6	518
16	4	3	620
17	6.5	4	621
18	6.5	8	624
19	4	6	646
20	3.5	5	549
21	4	3	568
22	2.5	7	603
23	4	8	584
24	3	2	609
25	4	5	549
26	3.5	4	638
27	4	3	522
28	2.5	3	518
29	7.5	5	658
30	4	3	539

Coliform bacteria had values between 2.5 and 7.5 germs/g, Coagulase-positive staphylococci had values from 2 to 8 germs/g and contamination with E.coli and Salmonella was absent. For yeasts and mold there was determined a number between 468 to 680 germs/g.

In Figure 10 there is presented the degree of contamination variation with coliform bacteria, for Telemea cheese, in cold season, in P5 location. This parameter is represented by a line which has a descending trend, which means that this parameter was better monitored.

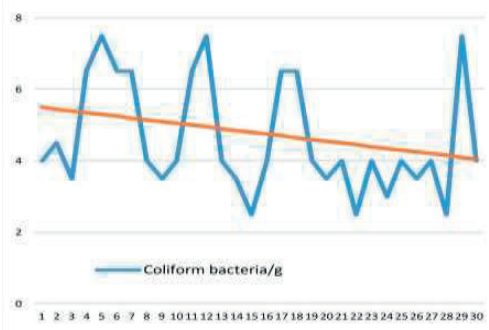

Figure 10. Dynamic of coliform bacteria contamination in Telemea chese, in cold season, in P5 location

In Figure 11 there is presented the degree of contamination variation with Coagulase-positive staphylococci, for Telemea cheese, in cold season, in P5 location. This parameter is represented by a line which present a relative constant values

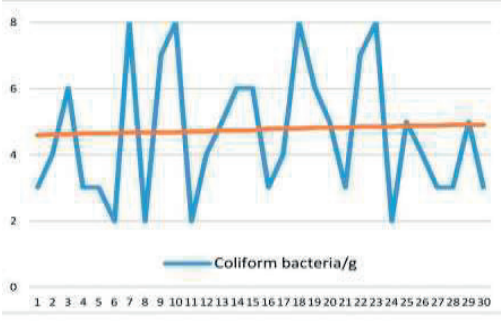

Figure 11. Dynamic of Coagulase-positive staphylococci contamination in Telemea cheese, in cold season, in P5 location

In Figure 12 there is presented the Yeasts and mold fluctuation, observed in finite product during cold season, in P5 location. This parameter is represented throw a line which also present a relative constant values.

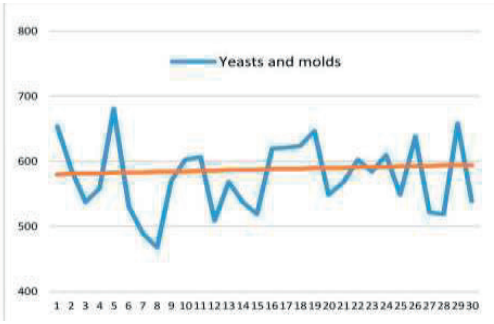

Figure 12. Dynamic of Yeasts and molds contamination in Telemea cheese, in cold season, in P5 location

In Table 9 there are presented the statistical analyses of main level and dispersion parameters for the analysed sample taken in P5 location, in cold season.

Table 9. Analyses of main level and dispersion parameters for the analysed sample taken in P5 location, in cold season

Parameter	n	$\bar{x}$	S^2	S	CV%
Coliform bacteria	30	4.6±0.28	2.5	1.58	34.42
Coagulase-positive staphylococci	30	4.6±0.36	3.97	1.99	43.32
Yeasts and molds	30	575.33±9.83	2900.37	53.85	9.36

The variability coefficient registered for Coagulase-positive staphylococci in P5 location, in cold season, has the highest value (43.32%)

Table 10 shows the differences between the values obtained in the locations where the Telemea cheese was analysed.

Table 10. Analyses of main level and dispersion parameters for the analysed sample taken in P5 location, in cold season

Parameter	**Calculated t**	**table t (t α)**			
		$t_{0.05}$	**$t_{0.01}$**	**$t_{0.001}$**	**$t_{0.1}$**
Coliform bacteria	0.53^{NS}	2.00	**2.66**	3.46	1.29
Coagulase-positive staphylococci	3.19**				
Yeasts and molds	9.03***				

After testing the significance of the differences between P3 and P5 locations, in cold season, it was found that for Coliform bacteria, differences are insignificant, for Coagulase-positive staphylococci, the differences are distinctly significant and for yeast and mold parameter the differences are very significant.

CONCLUSIONS

After testing the significance of the differences between P3 and P5 locations, in the summer season, a distinctly significant difference was found for coliform bacteria, and for Coagulase-positive staphylococci and Yeasts and molds the differences were very significant.

The determination of coliform bacteria in food provides information on the hygienic conditions in which the products were obtained, which is why it is recommended to review the existing regulations in the researched locations.

In cold season the differences between P3 and P5 locations, for coliform bacteria are insignificant.

Distinctly and very significant differences found between P3 and P5 locations, for Coagulase-positive staphylococci, and yeast and mold parameter underline the need to use the HACCP system, in order to eliminate the sources of contamination, in order to obtain healthy and safe products for consumption.

Following the obtained results, it can be said that location P3 has poorer hygiene conditions than location P5 where the HACCP system is implemented, which makes the dairy product, Telemea cheese, less wholesome.

For this reason, it is mandatory to urgently implement food quality assurance systems.

It is also necessary a training and qualification of the staff, through specialized courses, for the awareness and understanding of the quality concept and the importance of ensuring it for the health of consumers.

Food processing technologies will be less efficient if no increased attention is paid to laboratory analysis and expertise, inspection methods, staff training and improving the performance of investigative equipment according to current requirements. (Enache, 2005).

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