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COLIFORM BACTERIA CONTAMINATION PROFILING OF RAW MILK CHEESES USING STATISTICAL ASSESSMENT OF TYPE-LINKED CORRELATIONS

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Abstract

In the light of the current trend of increasing consumption of ecological, unprocessed and slow food, hygiene-associated microbiological drawbacks of raw milk cheeses are still an issue. This study was designed for the coliforms contamination assessment of multiple types of raw milk cheeses manufactured by local producers. The paper is focused on searching for possible correlations between the sample type and coliform bacteria contamination, using the chi-squared test of significance for statistical processing of the data. The results for a total sample size of 253 cheeses indicated significant associations (p<0.0005) between salting, smoking, milk origin and cheese recipe, on one hand and coliform bacteria contamination on the other hand. No significant association (p=0.438) was found between coliforms and whey-cheese versus milk-cheese classification parameter.

Keywords*: coliforms, raw milk cheese, type-linked correlations.*

INTRODUCTION

Impersonation of refinement and culinary delicacies, traditional cheeses are not only highly appreciated food commodities, but also gastronomy elements with powerful historical, social, economical and cultural implications in the individuality of Romanian people. Consumers' interest for ecological, organic and traditional food products is continuously rising, while international authorities and organizations encourage research in this field. The need for an accurate scientific evaluation of the safety and quality of these cheeses is still a topic of great interest, aiming to create the adequate legal frame and to prevent the loss of many cultural and social traditions that have otherwise survived for centuries (Ross R.P. et al., 2001). It is a fact that developing countries have poor hygiene standards in traditional milk production, and that is reflected in the poor hygiene quality of traditionally made cheeses. It is well known that in many countries, including Romania, traditional cheese is made in harsh climate conditions. This situation is at times associated with a lack of producers' knowledge for observing adequate hygiene and manufacturing practices all throughout the food chain, from milking, to cheese marketing (Fox P.F. et al., 2000; Rodriguez Eva et al., 2001). All these drawbacks may bring damage to traditional cheese reputation.

This study aimed to search for possible correlations between the hygiene conditions of various raw milk cheeses, expressed by the coliforms' contamination level and different processing or recipe parameters, in order to highlight the significant factors involved in the occurrence of hygiene deviations for these traditional products.

MATERIALS AND METHODS

The study was performed on 253 raw milk cheese samples obtained in a two years' time frame, from various local producers from three mountain counties (Prahova, Braşov and Argeş) in Romania (Table 1). The microbial hazard laboratory analysis was performed according to the national standard for coliforms' enumeration method (SR ISO 4832:2009). No available references for maximum contamination levels are available: Regulation 2073/2005 indicates maximum levels only for coagulase-positive staphylococci within the process hygiene criteria, while Ord. 27/2011 does not make reference to raw milk cheeses. Therefore, we compared the coliforms' contamination level in analyzed samples against maximum limits listed by the former Romanian regulations (Ord. 975/1998): 10^3CFU/g cheese for fresh cheeses and 10^2CFU/g cheese for the other raw milk cheeses subjected to analysis. The associations between coliform bacteria contamination and various processing or recipe parameters were statistically analyzed using univariate (Chi-square test) statistical tests (De Coster, 2006). We considered $p<0.05$ to be statistically significant. SPSS statistical software (SPSS, Inc., Chicago, IL) was used for data analysis.

RESULTS AND DISCUSSIONS

Considering the maximum limits of former Romanian legislation (Ord. 975/1998), the coliforms' contamination rate was important, with 107 exceeding samples (42.29% of the analyzed samples) (Table 1). No coliform exceeding samples were found for salted, smoked, cow's and ewe's milk cas cheese (a type of scalded cheese), while the highest contamination levels were seen in ewe's milk telemea cheese (a type of brined, ripened soft cheese). The average level of coliforms' contamination of various sample types was greatly heterogenous, as illustrated in Figure 1.

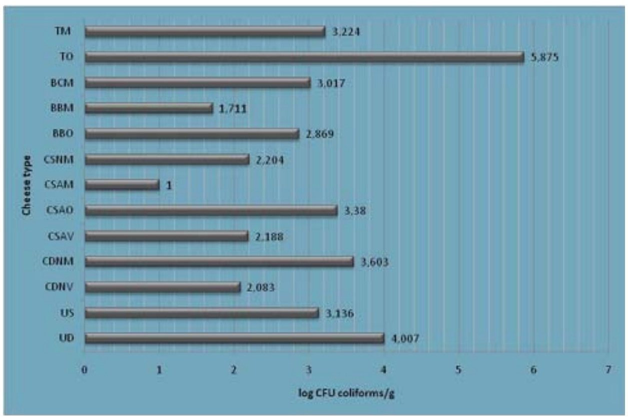

Figure 1. Averge coliform contamination levels of analyzed cheese samples

Simply analyzing the coliform counts of the samples investigated is not useful in drawing a certain conclusion related to the factors associated with the highest or the lowest contamination levels (Figure 1).

In addition, the analysis of the average coliform counts may be misleading in that direction. Another drawback in the attempt of making correlations between the coliform count and various parameters of cheese type is the important difference in the number of samples investigated of each type of cheese. While burduf cheese in fir bark (BCM) participates in the study with 62 samples, brined cheese made of ewe's milk (TO), or salted smoke ewe's milk caş (CSAO), were available for analysis with only two samples (Table 1).

In order to achieve an adequate analysis of coliform count results for the investigated cheese samples, we classified the analyzed cheeses into various categories using the following processing and recipe parameters, as criteria:

- Salting
- Smoking
- Curding
- Cheese recipe (scalded, fresh, kneaded, brined)
- Milk origin.

For milk origin, we compared cheeses made of cow's milk against mixture cheeses, on one hand and mixture cheeses against cow's and against ewe's milk, on the other hand, in order to obtain the most adequate correlation result with the contamination level (Table 2).

We used the Chi-square statistical test to compare observed data (in terms of samples exceeding the reference coliforms' count limit) with the expected data, for each criterion, which would be obtained according to a specific hypothesis. The null hypothesis in this case is that the specific processing and recipe parameters considered as classification criteria are not associated with the coliforms' count. We considered that for $p<0.05$, the null hypothesis can be rejected.

Based on the total number of each cheese type and the associated numer of samples with exceeding of coliforms' count limits considered, we calculated the Chi-square values and p-values, as seen in Table 2.

In order to prove the significance of the Chi-square test, the unpaired T-test was used for pairs of values representing the cheese categories compared in the study. Results indicated that the compared means are considered to be statistically significant (p values ranging from <0.0001, to 0.03) for salting, smoking, curding, cheese recipe (almost all pair combinations considered), and for mil origin, when comparing cow's milk and ewe's milk cheeses and also when comparing ewe's milk cheeses with mixture milk cheeses (Table 3). Yet, the difference between the average coliform contamination levels of urda and telemea cheeses was considered to be not statistically significant (p=0.9560). Also, as sees in table 3, a similar result was obtained through the unpaired T-test for cow's milk cheeses compared with mixture milk cheeses in terms of coliform contamination (p=0.1560)

Results indicated (Table 2) statistically significant associations between the number of samples with exceeding coliforms' count and the following parameters:

- Salting: Chi-square=14.351, DF=1, p=0.0001517,
- Smoking: Chi-square=16.83, DF=1, p=0.00004,
- Cheese recipe: Chi-square=37.89, DF=3, p=0,
- Milk origin (cow vs. mixture): Chi-square=9.93, DF=1, p=0.00162,
- Milk origin (cow vs. ewe vs. mixture): Chi-square=15.43, DF=2, p=0.00044.

Since chi-square tests are generally considered inappropriate if any expected frequency is below 1 or if the expected frequency is less than 5 in more than 20% of the cells used for Chi Square calculation tables, we concluded that this would be the case for milk origin parameters (both cow's milk versus mixture milk and cow's, versus ewe's, versus mixture milk correlation schemes).

However, expected frequencies less than 5 may be used for interpretation if Yates' correction is employed (Preacher K.J., 2001). Therefore, using the Yates correction for milk origin, we obtain the following Chi Square and p values:

1 Milk origin (cow vs. mixture milk)
   - Yates' Chi-square = 9.087
   - Yates' p-value = 0.00257

2 Milk origin (cow vs. ewe vs. mixture)
   - Yates' Chi-square = 12.939
   - Yates' p-value = 0.00155.

Table 1. Raw milk, traditional cheese samples subjected to analysis

No	Cheese type (samples)	Total no.	Average coliform level (log CFU/g)	Standard deviation	Maximum admitted level (log CFU/g)*	No. exceedings
1	Un-salted urda – UD	14	4.007	1.376449	3	8
2	Salted urda – US	21	3.136	0.903556	3	9
3	Un-salted, un-smoked, cow's milk caş – CDNV	16	2.083	0.312648	2	16
4	Un-salted, un-smoked, cow's and ewe's milk caş – CDNM	20	3.603	2.20078	2	9
5	Salted, smoked, cow's milk caş – CSAV	24	2.188	0.820595	2	9
6	Salted, smoked, ewe's milk caş – CSAO	2	3.38	1.889389	2	2
7	Salted, smoked, ewe's and cow's milk caş – CSAM	32	1	0	2	0
8	Salted, un-smoked, ewe's and cow's milk caş – CSNM	5	2.204	0.495812	2	4
9	Burduf cheese in natural membrane, ewe's milk – BBO	3	2.869	1.213656	2	2
10	Burduf cheese in natural membrane, ewe's and cow's milk – BBM	32	1.711	0.343484	2	2
11	Burduf cheese in fir bark, cow's and ewe's milk – BCM	62	3.017	1.920569	2	24
12	Brined cheese, ewe's milk – TO	2	5.875	0.425678	2	2
13	Brined cheese, cow's and ewe's milk - TM	20	3.224	1.218318	2	20
	TOTAL	253				107

**According to former Romanian legislation (Ord. 975/1998). There are no available upper limits of coliform colonies in traditional raw milk cheeses in current legislation*

Table 2. Associations between processing parameters and coliform levels exceedings

Processing/recipe parameters	*No. of samples*	*No. of exceeding samples*	*% of exceedings*	*Degrees of freedom*	*Chi-square*	*p value*
Salting				**1**	**14.351**	**p – 0.0001517**
salted	203	74	36.45%			
un-salted	50	33	62.26%			
Smoking				**1**	**16.83**	**p – 0.00004**
smoked	58	11	18.96%			
Un-smoked	195	96	49.23%			
Curding				**1**	**0.656**	**p - 0.4179**
Whey curding (whey cheese)	35	17	48.57%			
Milk curding (regular cheese)	218	90	41.28%			
Cheese recipe				**3**	**37.89**	**p – $3e^{-8}$**
Caş (scalded cheese)	99	40	40.4%			
Urda (fresh, whey cheese)	35	17	48.57%			
Burduf (kneaded cheese)	97	28	28.86%			
Telemea (brined cheese)	22	22	100%			
Milk origin (cow vs. mixture)				**1**	**9.936**	**p – 0.00162**
Cow's milk	75	42	56%			
Mixture of cow's and ewe's milk	171	59	34.5%			
Milk origin (cow vs. ewe vs. mixture)				**2**	**15.433**	**p – 0.00044**
Cow's milk	75	42	56%			
Ewe's milk	7	6	85.71%			

Table 3. Unpaired T-test results used to verify the significance of the Chi-square test

Processing/recipe parameters	*No. of samples (N)*	*Average coliform contamination (log CFU/g)*	*Standard deviation (SD)*	*Standard error of the mean (SEM)*	*t value*	*p value*
Salting					t = 3.2425	p = 0.0013
salted	203	2.43776	1.49357	0.10483		
un-salted	50	3.2299	1.75193	0.24776		
Smoking					t = 5.9912	p< 0.0001
smoked	58	1.57397	0.89044	0.11692		
Un-smoked	195	2.89779	1.60963	0.11527		
Curding					t = 3.6896	p = 0.0003
Whey curding (whey cheese)	35	3.48486	1.17977	0.19942		
Milk curding (regular cheese)	218	2.45133	1.58716	0.10750		
Cheese recipe					t = 5.1421	p = 0.0001
Caş (scalded cheese)	99	2.09803	1.43198	0.14392		
Urda (fresh, whey cheese)	35	3.48486	1.17977	0.19942		
Cheese recipe					t = 2.3031	p = 0.0230
Burduf (kneaded cheese)	97	2.58185	1.67046	0.16961		
Telemea (brined cheese)	22	3.46573	1.39988	0.29846		
Cheese recipe					t = 2.1784	p = 0.0306
Caş (scalded cheese)	99	2.09803	1.43198	0.14392		
Burduf (kneaded cheese)	97	2.58185	1.67046	0.16961		
Cheese recipe					t = 4.0681	p < 0.0001
Caş (scalded cheese)	99	2.09803	1.43198	0.14392		
Telemea (brined cheese)	22	3.46573	1.39988	0.29846		
Cheese recipe					t = 2.9410	p = 0.0039
Urda (fresh, whey cheese)	35	3.48486	1.17977	0.19942		
Burduf (kneaded cheese)	97	2.58185	1.67046	0.16961		
Cheese recipe					**t = 0.0554**	**p = 0.9560**
Urda (fresh, whey	35	3.48486	1.17977	0.19942		

Processing/recipe parameters	*No. of samples (N)*	*Average coliform contamination (log CFU/g)*	*Standard deviation (SD)*	*Standard error of the mean (SEM)*	*t value*	*p value*
cheese)						
Telemea (brined cheese)	22	3.46573	1.39988	0.29846		
Milk origin (cow vs. mixture)					**t = 1.4229**	**p = 0.1560**
Cow's milk	75	2.77113	1.14969	0.13276		
Mixture of cow's and ewe's milk	171	2.46436	1.70371	0.13029		
Milk origin (cow vs. ewe)					15.433	p = 0.0230
Cow's milk	75	2.77113	1.14969	0.13276		
Ewe's milk	7	3.87414	1.74256	0.65862		
Milk origin (ewe vs. mixture)					t = 2.1441	p = 0.0334
Ewe's milk	7	3.87414	1.74256	0.65862		
Mixture cow's and ewe's milk	171	2.46436	1.70371	0.13029		

Therefore, according to these results and based on the cheese samples analyzed, we may consider that there is a statistically significant association between salting and higher coliforms' contamination, unsalted cheeses revealing higher coliforms' contamination. Also, there is a statistically significant association between un-smoked raw milk cheeses and a higher coliforms' count. Brined cheese telemea is statistically significant associated with the highest coliforms' exceeding sample percent, while burduf cheese is statistically significant associated with the lowest coliforms' exceeding rate, among the cheese types sampled. Considering milk origin, our results indicated that mixture milk cheeses are statistically significant associated with a lower coliform contamination than ewe's milk cheeses. Moreover, ewe's milk cheeses a statistically significant more contaminated with coliforms than cow's milk cheeses.

However, no statistically significant association was found between the curding technology (cheeses made of whey were compared with cheeses made of milk, in terms of coliforms' count) and the rate of coliforms' contamination level ($p>0.05$, DF=1, Chi-square=0.6).

CONCLUSIONS

The results for a total sample size of 253 raw milk, traditional cheeses, indicated significant associations ($p<0.05$) between salting, smoking, milk origin and cheese recipe, on one hand and coliform bacteria contamination, on the other hand.

A higher number of samples exceeding the considered coliforms' count limit, was statistically significant associated with un-salted, un-smoked cheeses, telemea cheese and cheese made of only one type of milk regarding its origin: ewe's milk cheeses were significantly associated with a higher number of coliforms, than mixture milk and cow's milk cheeses.

No significant association ($p=0.438$, Chi-square=0.656) was found between coliforms and whey-cheese versus milk-cheese classification parameter, even though the difference between the two means of coliform contamination levels was considered statistically significant by the unpaired T-test ($p=0.0003$, $t=3.6896$), indicating a higher coliform contamination level for whey cheeses than for milk curded cheeses.

These type-linked correlations may be helpful in a more accurate focusing on the source of process-related deviations concerning the hygiene practices and manufacturing practices that help proving the desired level of food safety for such traditional commodities.

REFERENCES

De Coster J., 2006. Testing Group Differences using T-tests, ANOVA, and Nonparametric Measures. retrieved 10.03.2014 from http://www.stat-help.com/notes.html.

Fox P.F., Guinee T.P., Cogan T.M., McSweeney P.L.H., 2000. Fundamentals of cheese science, Aspen

Publication Inc., Gaithersburg, Maryland, USA, ISBN 0-8324-1260-9, p. 484-500.

Romanian National Sanitary Veterinary Authority for Food Safety Ord. 27/2011 Regarding the approval of microbiological and hygiene criterions for food products, other than the ones mentioned in Regulation CE no. 2073/2005 of the Commission from November 15, 2005 concerning microbiological criterions for foods.

Ord. 975/1998 of the Romanian Health Minister, regarding the approval of the hygiene and sanitary norms for foods, chapter VI, art. 97, published in Romanian Official Monitor Journal no. 268, 11.06.1999.

Preacher K.J., 2001. Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence [Computer software]. Available from http://quantpsy.org.

Regulation (CE) 2073/2005 consolidated version, concerning the microbiological criterions for foods.

Rodriguez Eva, Arques J.L., Gaya P., Nunez M., Medina Margarita, 2001. Control of *Listeria monocytogenes* by bacteriocins and monitoring of bacteriocin-producing lactic acid bacteria by colony hybridization in semi-hard raw milk cheese. Journal of Dairy Research, Cambridge University Press, 68: p. 131-137.

Ross R.P., Hill C., O'Keeffe T., McAuliffe O., Ryan M., O'Connor P., Freyne T., 2001. Control of Cheese Microflora using Bacteriocins. The Dairy Products Research Center, Moorepark, Fermoy, Co. Cork., ISBN 1 84170 174 2.

SR ISO 44832:2009. Microbiology of foods and feeds. Horizontal method for coliform enumeration. Colonies counting method.