UDC: 637.1/.2:579.67]:614.31(497.7)
Professional paper

MICROBIOLOGICAL SAFETY OF DAIRY PRODUCTS OBTAINED FROM A DAIRY STORE

Lina DIMOVSKA[*], Biljana GAGACHEVA[2], Nina DIMOVSKA[3], Gordana DIMITROVSKA[4], Tatjana BLAZHEVSKA[5]

[1]PhD student, University "St. Kliment Ohridski"- Bitola, Faculty of Biotechnical Sciences - Bitola, North Macedonia*
[2]Anima-Vet Laboratory, Bitola, North Macedonia
[3]University "St. Kliment Ohridski"- Bitola, Faculty of Veterinary medicine - Bitola, North Macedonia
[4]University "St. Kliment Ohridski"- Bitola, Faculty of Biotechnical Sciences - Bitola, North Macedonia
[5]University "St. Kliment Ohridski"- Bitola, Faculty of Technology and Technical sciences - Veles, North Macedonia
**Corresponding Author: e-mail: dimovskalina5@gmail.com*

Abstract

Dairy product consumption holds significant importance in the diet of the population in North Macedonia. While a majority of these dairy products are available in packaged form, a considerable portion can also be acquired from specialty stores that sell dairy products in bulk. Foodborne illnesses can be triggered by various types of pathogens, and their presence can be traced back to direct contact with contaminated sources during farming, milk and dairy production, and the handling of products in these specialty dairy stores.

This paper aims to shed light on the microbiological safety of dairy products obtained from specialized dairy stores in Bitola, North Macedonia. The primary objectives are to identify the main contaminants in dairy products sold in these stores and to propose appropriate precautionary and control measures. A total of 85 dairy product samples were collected and tested for the presence of four different pathogens: Escherichia coli, Coagulase-positive Staphylococcus, Listeria spp. (as specified in Official Gazette No. 100 from 2013), and Salmonella spp. Among these samples, 50 were acquired from bulk bins in dairy stores, while 35 of the same products were obtained in packaged form.

The analysis revealed that Escherichia coli and Coagulase-positive Staphylococcus were commonly found in most of the samples. However, neither Salmonella spp. nor L. monocytogenes was detected in any of the products, and Listeria spp. were only found in ten dairy products

Keywords: microbiological safety, dairy products, dairy store, contamination

1. Introduction

Milk and milk products have played a crucial role in human nutrition since ancient times (Pal, 2014). They are rich sources of essential nutrients, such as protein, vitamins, calcium, phosphorus, magnesium, zinc, etc., which are essential for a healthy life for people of all age groups and both sexes. Microbiological contamination is one of the leading causes of food spoilage worldwide (Pal, 2014), and can occur at any stage of the food chain (Vrdoljak et al., 2016). The high nutritional value of dairy products makes them particularly conducive to the growth of microorganisms (Ledenbah and Marshall, 2009). Cheese, often considered a ready-to-eat-food, should be regarded as potential source of foodborne pathogens, particularly L. monocytogenes, a foodborne pathogen in cheese, mainly at the post-processing stage (Vrdoljak et al., 2016). During the production of hard cheeses, some bacteria such as L. monocytogenes, S. aureus and Salmonella spp. can survive without subsequent growth. The number of E. coli may increase during cheese production, which is due to a combination of a concentration effect during curd formation and potential growth of the pathogen. During the ripening, Escherichia coli, L. monocytogenes and Salmonella spp. may

decrease in number, but they can still be detected even after extended periods of ripening. Butter, prepared by churning fresh or fermented cream and milk, contains fat, protein and water (Pal et al., 2014a). Contemporary hygienic production practices have substantially reduced bacterial spoilage in butter. However, occasional microbial defects can still occur. The main microbiological hazards of butter, prepared from raw milk are L. monocytogenes, Escherichia coli and S. aureus, as the most frequently detected pathogens in butter. All three pathogens are known to cause foodborne diseases in humans (Pal, 2007). However, the risk of infection after consuming raw milk butter is estimated to be relatively lower compared to other dairy products (Verraes et al., 2015). Previously, dairy products were classified under "safe food", but after the 1980s, infections and intoxications related to the consumption of cheese contaminated with pathogenic microorganisms and/or their toxins during the production stages have been reported. This poses a threat to public health and also causes defects in cheese quality, leading to large economic losses (Lindqvist et al., 2002). Dairy products sold in bulk can pose a major risk to public health, due to unsanitary and unhygienic storage conditions, including poor infrastructure, inadequate storage temperature, and poor hygiene among handlers during commercialization (Rodrigues et al., 2016).

2. Materials and methods

2.1. Sampling: The sampling process adhered to the guidelines specified in the Regulation on special requirements concerning microbiological criteria for food, as outlined in the Official Gazette of the Republic of Macedonia no. 100/2013 and subsequent amendments and additions (Official Gazette no. 145/2014, 37/2017, 173/2018, 229/2020, 287/2022). Specifically, five samples were collected for each product in accordance with these regulations. In total, 80 samples were taken, from ten well-established dairies in Macedonian, of which 50 samples were from dairy shops, sold in bulk and 35 samples were packaged, obtained from supermarkets. Seven products were taken in bulk and packaged from the same dairy, while for three products no packaged samples were found. The majority of samples represented cow's white cheese, while three represented butter, curd and "bieno" cheese (bieno sirenje).

2.2. Microbiological analysis: All analyzes were performed according to standard methods (Table 1).

Table 1: Standard methods used for the detection of pathogenic bacteria

Analyte	Standard method
Escherichia coli	MKC EN ISO 16649-2:2008 - Horizontal method for counting β-glucuronidase positive *Escherichia coli* - Part 2: Colony counting technique at 44ºC using 6-bromo-4-chloro-3-indolyl β-D-glucuronide
Staphylococcus aureus	MKC EN ISO 6888-1:2022 – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird Parker agar (ISO 6888-1:2021)
Listeria monocytogenes	MKC EN ISO 11290-1: 2018 Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria spp.* - Part 1: Detection method
Salmonella spp.	MKC EN ISO 6579-1: 2017 – Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella spp.*

3. Results

In this research, we focused on identifying the presence of the four most common pathogenic bacteria typically found in dairy products. The detailed results can be found in Table 2 and Table 3.

Table 2: Presence of bacteria in bulk products

Sample	Product	*E. coli* (CFU/g)	*S. aureus* (CFU/g)	*L. monocytogenes/Listeria spp.*	*Salmonella spp.*
RA-1	Curd	14200	120	Not detected	Not detected
RA-2	Curd	14000	100	Not detected	Not detected
RA-3	Curd	15000	150	Not detected	Not detected
RA-4	Curd	14900	110	Not detected	Not detected
RA-5	Curd	13900	120	Not detected	Not detected
RB-1	Butter	0	20	Not detected	Not detected
RB-2	Butter	0	20	Not detected	Not detected
RB-3	Butter	0	20	Not detected	Not detected
RB-4	Butter	0	20	Not detected	Not detected
RB-5	Butter	0	20	Not detected	Not detected
RC-1	"Bieno" cheese	350	0	Not detected/Detected (*L.innocua*)	Not detected
RC-2	"Bieno" cheese	400	0	Not detected/Detected (*L.innocua*)	Not detected
RC-3	"Bieno" cheese	300	0	Not detected/Detected (*L.innocua*)	Not detected
RC-4	"Bieno" cheese	280	0	Not detected/Detected (*L.innocua*)	Not detected
RC-5	"Bieno" cheese	370	0	Not detected/Detected (*L.innocua*)	Not detected
RD-1	Cow's cheese	0	10	Not detected	Not detected
RD-2	Cow's cheese	0	0	Not detected	Not detected
RD-3	Cow's cheese	0	0	Not detected	Not detected
RD-4	Cow's cheese	0	10	Not detected	Not detected
RD-5	Cow's cheese	0	10	Not detected	Not detected
RE-1	Cow's cheese	1400	200	Not detected	Not detected
RE-2	Cow's cheese	1550	250	Not detected	Not detected
RE-3	Cow's cheese	1200	190	Not detected	Not detected
RE-4	Cow's cheese	1000	230	Not detected	Not detected
RE-5	Cow's cheese	1400	220	Not detected	Not detected
RF-1	Cow's cheese	0	10	Not detected	Not detected
RF-2	Cow's cheese	0	20	Not detected	Not detected
RF-3	Cow's cheese	0	10	Not detected	Not detected
RF-4	Cow's cheese	0	0	Not detected	Not detected
RF-5	Cow's cheese	0	20	Not detected	Not detected
RG-1	Cow's cheese	10	50	Not detected	Not detected

RG-2	Cow's cheese	20	60	Not detected	Not detected
RG-3	Cow's cheese	20	40	Not detected	Not detected
RG-4	Cow's cheese	40	40	Not detected	Not detected
RG-5	Cow's cheese	10	40	Not detected	Not detected
RH-1	Cow's cheese	110	250	Not detected	Not detected
RH-2	Cow's cheese	110	300	Not detected	Not detected
RH-3	Cow's cheese	140	310	Not detected	Not detected
RH-4	Cow's cheese	190	270	Not detected	Not detected
RH-5	Cow's cheese	150	280	Not detected	Not detected
RI-1	Cow's cheese	17200	400	Not detected	Not detected
RI-2	Cow's cheese	17200	450	Not detected	Not detected
RI-3	Cow's cheese	16900	420	Not detected	Not detected
RI-4	Cow's cheese	16500	400	Not detected	Not detected
RI-5	Cow's cheese	17000	420	Not detected	Not detected
RJ-1	Cow's cheese	50	0	Not detected/Detected (*L.innocua*)	Not detected
RJ-2	Cow's cheese	60	0	Not detected/Detected (*L.innocua*)	Not detected
RJ-3	Cow's cheese	30	10	Not detected/Detected (*L.innocua*)	Not detected
RJ-4	Cow's cheese	40	10	Not detected/Detected (*L.innocua*)	Not detected
RJ-5	Cow's cheese	20	10	Not detected/Detected (*L.innocua*)	Not detected

The obtained results as presented in Table 2 indicate the presence of the bacteria *E.coli* in 70% of the samples. The mean value of *E.coli* ranged from $1x10^1$ to 17 $x10^3$ CFU/g. The highest value of *E.coli* appeared in cow's white cheese, labeled RI. *S.aureus* was detected in all dairy samples, and the average value ranged from $1x10^1$ to $4.5x10^2$ CFU/g. The highest value of *S.aureus* appeared in cow's white cheese, labeled RI. *L.monocytogenes* and *Salmonella spp.* were not detected in any sample, while *Listeria spp*. was detected in 20% of the samples, i.e. in cow's white cheese marked RJ and "bieno" cheese marked RS. With further analysis for confirmation of *Listeria spp.*, we proved that it was *L.innocua.*

Table 3: Presence of bacteria in packaged products

Sample	**Product**	***E. coli* (CFU/g)**	***S. aureus* (CFU/g)**	***L. monocytogenes/Listeria spp.***	***Salmonella spp.***
PA-1	Curd	9300	120	Not detected	Not detected
PA-2	Curd	9400	130	Not detected	Not detected
PA-3	Curd	9500	120	Not detected	Not detected
PA-4	Curd	8800	100	Not detected	Not detected
PA-5	Curd	9000	100	Not detected	Not detected
PB-1	Butter	0	0	Not detected	Not detected
PB-2	Butter	0	0	Not detected	Not detected
PB-3	Butter	0	0	Not detected	Not detected

PB-4	Butter	0	0	Not detected	Not detected
PB-5	Butter	0	0	Not detected	Not detected
PC-1	"Bieno" cheese	0	0	Not detected	Not detected
PC-2	"Bieno" cheese	0	0	Not detected	Not detected
PC-3	"Bieno" cheese	0	0	Not detected	Not detected
PC-4	"Bieno" cheese	0	0	Not detected	Not detected
PC-5	"Bieno" cheese	0	0	Not detected	Not detected
PD-1	Cow's cheese	10	0	Not detected	Not detected
PD-2	Cow's cheese	20	0	Not detected	Not detected
PD-3	Cow's cheese	30	0	Not detected	Not detected
PD-4	Cow's cheese	10	0	Not detected	Not detected
PD-5	Cow's cheese	30	0	Not detected	Not detected
PG-1	Cow's cheese	0	0	Not detected	Not detected
PG-2	Cow's cheese	0	0	Not detected	Not detected
PG-3	Cow's cheese	0	0	Not detected	Not detected
PG-4	Cow's cheese	0	0	Not detected	Not detected
PG-5	Cow's cheese	0	0	Not detected	Not detected
PH-1	Cow's cheese	0	0	Not detected	Not detected
PH-2	Cow's cheese	0	0	Not detected	Not detected
PH-3	Cow's cheese	0	0	Not detected	Not detected
PH-4	Cow's cheese	0	0	Not detected	Not detected
PH-5	Cow's cheese	0	0	Not detected	Not detected
PJ-1	Cow's cheese	0	0	Not detected	Not detected
PJ-2	Cow's cheese	0	0	Not detected	Not detected
PJ-3	Cow's cheese	0	0	Not detected	Not detected
PJ-4	Cow's cheese	0	0	Not detected	Not detected
PJ-5	Cow's cheese	0	0	Not detected	Not detected

The results presented in Table 3 indicate the presence of *E.coli* in 28.6% of the samples,with *E.coli* levels ranging from $1x10^1$ to 95 $x10^2$ CFU/g. The highest value of *E.coli* appeared in curd, labeled PA. *S.aureus* was detected in just one sample accounting for 14.3% of the total samples. This bacterium was found in curd labeled RA, with a values ranging from of 10 to $13x10^1$ CFU/g.
Salmonella spp., L.monocytogenes and *Listeria spp*. were not detected in any sample.

4. Discussion

Table 4: Presence of bacteria in bulk and packaged products

	Products in bulk		**Packaged products**	
Bacteria	**CFU/g**	**%**	**CFU/g**	**%**
E.coli	$1x10^1$ - 17 $x10^3$	70	$1x10^1$ - 95 $x10^2$	28,6 %
S.aureus	$1x10^1$ - 4,5 $x10^2$	100	10 $x10^1$ - $13x10^1$	14,3%
Listeria spp. (L.innocua)	/	20%	/	0%
L.monocytogenes	/	0%	/	0%
Salmonella spp.	/	0%	/	0%

During the ripening and storage of cheese, bacterial levels typically decrease, *but Salmonella spp., E.coli* and *S.aureus* can still be detected. *E.coli* and *L.monocytogenes* levels may even increase during these processes (Verraes et al., 2015). Recently, *Salmonella spp., L.monocytogenes* and *E.coli* have been associated with outbreaks of foodborne diseases and are more commonly associated with cheese produced from pasteurized milk. On the other hand, *S.aureus* has not been associated with outbreaks of foodborne diseases associated with cheese, although this pathogen is generally associated with foods, such as cheese, that are often handled by hand during processing and packaging (Leong et al., 2014). Table 4 shows the comparative results for all samples, for each pathogenic bacterium separately. From the obtained results, it can be concluded that the bulk products indicate a higher number of detected bacteria, i.e. in 70% of the bulk products *E. coli* was detected, and in a higher concentration, compared to the packaged products where it was detected in 28.6% of the products, with a lower concentration in each of the samples. High levels of *E. coli* exceeding the standard limits define the product as unsuitable for commercialization and consequently for human consumption due to faecal contamination (Silva et al., 2006). Trmcic et al. (2016), in their study where they examined the presence of coliform bacteria in different types of cheese, bought in bulk and packaged, determined the presence of all coliform bacteria, with a higher prevalence of four times in fresh cheese from raw milk, than cheese produced from pasteurized milk. *S.aureus* was detected in all bulk products, in a higher concentration, while in 14.3% of packaged products, in a lower concentration. High values of *S.aureus* in bulk products, which do not meet the criteria of the Regulation, may be related to contamination of the base material during production. This fact may also be related to the handlers, as pathogens have common mechanisms of interaction with the host and they can often be found in the skin and mucus (Heikens et al., 2005). *S.aureus* is usually transmitted to food via personnel handling the products (hands, coughing, sneezing, etc.). *Listeria spp. (L.innocua)* was detected only in 20% of bulk products, while it was not detected in the packaged products. Similar results were obtained in the research of Silva et al. (2003), where they found the presence of this bacteria on the surfaces in the cheese storage room, mostly associated with unhygienic post-production processes. Although several studies (Mena et al., 2004; Manfreda et al., 2005; Colak et al., 2007) have proven that *L.monocytogenes* is often present in packaged dairy products, in our study we did not prove its presence.

According to the Regulation on special requirements relating to microbiological criteria for food, Official Gazette of the Republic of Macedonia no. 100/2013 and amendments and additions Off. Gazette no. 145/2014, 37/2017, 173/2018, 229/2020, 287/2022, all products meet the criteria for food safety, from chapter 1 point 1.2., i.e. the absence of *L.monocytogenes* in a 25 gram sample. If we consider the results of the bulk products, we can conclude that 50% of the samples of the dairy products, examined for the presence of the bacteria *E.coli* and *S.aureus*, meet the criteria of the Regulation on special requirements relating to microbiological criteria for food, Official Gazette of the Republic of Macedonia no. 100/2013 and amendments and additions Off. Gazette no. 145/2014, 37/2017, 173/2018, 229/2020, 287/2022, from chapter 2 point 2.2.4 (for the products: "bieno" cheese, curd and cow's white cheese) and point 2.2.6. (for the product RB-butter). On the other hand, of the packaged products, only the curd showed the presence of *E.coli* and *S.aureus* in higher concentrations than allowed, which does not meet the requirements of the Regulation on special requirements relating to microbiological criteria for food, Official Gazette of the Republic of Macedonia no. 100/2013 and amendments and additions Off. Gazette no. 145/2014, 37/2017, 173/2018, 229/2020, 287/2022 according to chapter 2 point 2.2.4. Although the presence of *Listeria spp.* is not specified in the Regulation, their presence is an indicator of hygiene in the handling of dairy products during production, processing and packaging. Listeria is inactivated under normal pasteurization conditions (Schaack and Marth, 1988), but problems can arise from contamination after pasteurization.

5. Conclusion

In our research on the microbiological safety of dairy products obtained from local dairy stores, we demonstrated that these products presented for consumption, fail to meet the essential food safety and production process hygiene criteria. The microbiological results highlight that they can be directly related to inadequate hygiene and sanitation conditions and prove that these aspects are the main causes of contamination. Notably, a concerning observation was the simultaneous handling of money and food by all food vendors without proper handwashing, compromising the fundamental principles of safe food handling. It is important to introduce stricter controls by inspection bodies in order to protect the public health of consumers, especially in local dairy shops where products in bulk can be obtained. In conclusion, the results show that the hygienic quality of the dairy products sold in the dairy stores in Bitola was low and lacked sufficient assurance in terms of public health.

Reference

[1]. Colak, H., Hampikyan, H., Bingol, E.B. & Ulusoy, B. (2007). Prevalence of L.monocytogenes and Salmonella spp. in Tulum cheese. Food Cotrol 18:576-579.

[2]. Heikens, E., Fleer, A., Paauw, A., Florijn, A. & Fluit, A. C. (2009). Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. Journal of clinical microbiology, 43(5):2286-90

[3]. Ledenbach, L.H. & Marshall, R.T. (2009). Microbiological spoilage of dairy products. In: Sperber, W., Doyle, M. (eds) Compendium of the Microbiological Spoilage of Foods and Beverages. Food Microbiology and Food Safety. Springer

[4]. Leong, W.M., Feier, R., Engstrom, S., Ingham, S., Ingham, B. & Smukowski, M. (2014). Growth of Listeria monocytogenes, Salmonella spp., Escherichia coli O157:H7, and Staphylococcus aureus on Cheese during Extended Storage at 25oC. Journal of Food Protection, Vol. 77, Np.8, 1275-1288

[5]. Lindqvist, R., Sylvén, S. & Vagsholm, I. (2002). Quantitative microbial risk assessment exemplified by Staphylococcus aureus in unripened cheese made from raw milk. International Journal of food Microbiology, 78(1-2):155-70

[6]. Manfreda, G., De Cesare A., Stella, S., Cozzi M. & Cantoni C. (2005). Occurrence and ribotypes of Listeria monocytogenes in Gorgonzola cheeses. International Journal of Food Microbiology. 102:287-293.

[7]. Mena C., Almeida G., Carneiro L., Teixeira P., Hogg T. & Gibbs P.A. (2004). Incidence of Listeria monocytogenes in different food products commercialized in Portugal. Food Microbiology. 21:213-216.

[8]. Pal, M. (2007). Zoonoses. 2nd Edition, Satyam Publishers, Jaipur.

[9]. Pal, M. (2014). Spoilage of dairy products due to fungi. Beverage and Food World, 41:37-38, 40.

[10]. Pal, M. & Awel, H. (2014a). Public health significance of Listeria monocytogenes in milk and milk products. Journal of Veterinary Public Health, 12: 1-5.

[11]. Rodrigues, M.J., Barros, L. S. S., Barreto, N. S. E. & Lima, D. V. (2016). Escherichia coli O157 in curd cheese. African Journal of Agricultural Research, vol. 11, 407–415

[12]. Schaack, M.M. & Marth, E.H. (1988). Behavior of Listeria monocytogenes in skim milk during fermentation with mesophilic lactic starter cultures. Journal of food protection, 51(8):600-606.

[13]. Silva, I.M.M., Almeida, R.C.C., Alves M.A.O. & Almeida, P.F. (2003). Occurrence of Listeria spp. in critical control points and the environment of Minas Frescal cheese processing, International Journal of Food Microbiology 81:241-248.

[14]. Silva, M.P., Cavalli, D.R. & Oliveira, T.C.R.M. (2006). Assesent of standard coliforms at 45oC and comparison of efficiency of multiple tube and petrifilmec techniques to detect total coliforms and Echerichia coli in food. Ciencia e Tecnologia Alimentar, vol. 26, 352-359.

[15]. Verraes, C., Valemynck G., Weyenberg, S.V., De Zutter, L., Daube, G., Sindic, M., Uyttendaele, M. & Herman L., (2015). A review of the microbiological hazards of dairy products made from raw milk. International Dairy Journal, 50:32-44.

[16]. Vrdoljak, J., Dobranic V., Filipovic I. & Zdolec N. (2016). Microbiological quality of soft, semi-hard and hard cheeses during the shelf-life. Journal of Macedonian Veterinary Review, 39:59-64.

[17]. Правилник за посебните барања кои се однесуваат на микробиолошките критериуми за храната, Службен весник на РМ, бр. 100 од 15.07.2013 година

[18]. Trmcic A., Chauhan, K., Kent, D.J., Ralyea R.D., Martin, N.H., Boor, K.J. & Wiedmann, M. (2016). Coliform detection in cheese is associated with specific cheese characteristics, but no association was found with pathogen detection. Journal of Dairy Science, 99:6105-6120.